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PREFACE

The Institute of Cytology & Preventive Oncology (ICMR) is honored to host the 24th Annual Convention of the Indian Association for Cancer Research (IACR) and an International Symposium on "Human Papillomavirus and Cervical Cancer" in the ICPO's new premises at Noida, India. Cervical cancer being the major cancer in Indian women, the Symposium is to focus mainly on cervical cancer and its principal causative agent, human papillomavirus (HPV) while IACR Convention will cover all aspects of human cancer. The meeting will have a special emphasis on clinical cancer research including early detection, pharmacological interventions, genomics and proteomics, prevention and management of cancer.

The highlights of this four day meeting include sessions on molecular epidemiology, molecular mechanisms of carcinogenesis, bioinformatics, HPV Vaccine and a special session of panel discussion on early detection of cervical cancer, an interface between basic scientists and clinicians in oncology that will be moderated by Prof. Usha Luthra and Dr. John Sellors. Besides, there will be several plenary talks by renowned experts from India and abroad and special public talks by Prof. Harald zur Hausen, Prof. Inder M. Verma, Dr. P.B. Desai and Prof. Indraneel Mittra. Besides, there will be product exhibition by industries under a banner "Technology Against Cancer - 2005".

The Symposium on HPV and Cervical Cancer which is focused mainly on early detection of cervical cancer and HPV Vaccine will be a meeting of first of its kind in India. This meeting has a special significance as it is being organized at a critical juncture when Indian Government has agreed to start trial of the HPV Vaccine against cervical cancer in India and ICPO has been recognized as a national co-coordinating centre for this vaccine programme.

In this Conference about 250 abstracts will be presented. There will be ample opportunity for young scientists to win awards both in oral and poster sessions. The Conference Secretariat has created additional Ten Special Appreciation Awards for good poster presentations.

We hope that this abstract book would serve as a knowledge house which will enthuse many young researchers to present their work in the future IACR meetings. The future of cancer research in India would set for an encouraging growth both in quality as well as quantity.

I thank all young research students and the staff of Molecular Oncology Division and other staff of ICPO who worked tirelessly to categorize, edit and organize these abstracts. Special thanks are also to Prof. Neeta Singh of AIIMS and Dr. K. Satyanarayana, Editor of IJMR, New Delhi for their unstinted support and help in printing this book of abstracts.

B.C. Das
Organizing Secretary
24th Annual Convention of IACR &
International Symposium on "HPV and Cervical Cancer"

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PRESIDENTIAL ORATION

POL-1
Molecular Biomarkers in Oral Cancers

Dhananjaya Saranath*

Reliance Life Sciences Pvt. Ltd., Sir H.N. Hospital & Research Centre, New Tower Building, Raja Ram Mohan Roy Road, Mumbai, 400 004, India. *dhananjaya_saranath@relbio.com

Globally, over a quarter million new cases of cancers of the oral cavity were diagnosed in the year 2000, with 80,000 (30%) being Indian oral cancer patients and 47,000 oral cancer related deaths in India. The poor survival and high mortality rates are due to presentation in advanced stages, recurrence of the primary and development of second primary tumors. Hence, efforts to understand the mechanism of oral carcinogenesis and the biology of oral cancer are imperative, and may define the pathology and processes involved in initiation and progression of the disease, as also indicate biological behaviour of the cancer cells. Development of predictive biomarkers of high oral cancer risk individuals, as well as biomarkers of radio-chemo resistance, may result in better patient management. Hence, we investigated molecular genetic and epigenetic events in oral carcinogenesis. In our studies, transcriptional silencing of multiple regulatory genes – p16, MGMT, DAPK and GSTP1, by hypermethylation at the promoter regions, was observed as an early event occurring in 88% of the tumor tissue samples and 75% corresponding tumor adjacent mucosa. Further, multiple genes were hypermethylated in 56% malignant cancers and 46% tumor adjacent mucosa. It was interesting to note that a majority of buccal scrapings from premalignant oral lesions and long term tobacco users were also hypermethylated in the genes. On the other hand, hypermethylation was not observed in buccal scrapings of normal healthy individuals with no tobacco/alcohol habits. Additional biomarkers including p53 and H.ras mutations and consequent over-expression, EGF-R amplification and overexpression, microsatellite instability and loss of heterozygosity on chromosomes 3 and 9, were observed as early events occurring in a certain proportion of premalignant oral lesions. Telomerase activity was observed in 88% oral cancer samples and 25% premalignant oral lesions. Our data indicates that a judicious selection of a panel of biomarkers may indicate useful predictive biomarkers in individuals with higher risk of progressing to oral cancers. These would constitute important objective markers in selection of high risk individuals for preventive chemotherapeutic trials, and objectives assessment of the efficacy of the chemo- and radio- therapy. Molecular aberrations including activation of oncogenes by amplification, point mutations and restriction fragment length polymorphisms of c.myc/N.myc, K.ras/N.ras, Bcl-2/Bax, erbB-1 and ERK3, were observed in oral cancer tissues. Several of these biomarkers viz p53, H.ras and BclXL, may indicate inherent resistance of the cancer cells to chemo-radioresistance The specific alterations in an individual would also be useful in objective monitoring of efficacy of therapy, using buccal scrapings of the individual, post therapy, and in aggressive follow-up protocols. The aberrant expression of specific genes in oral cancers and premalignant oral lesions, would be useful in determining novel therapeutic targets in prevention and treatment of oral cancers.

KEYNOTE / PUBLIC LECTURES

KPL-1
Papillomaviruses in Human Cancers

Harald zur Hausen*

Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany. *zurhausen@dkfz.de

KPL-2
Gene Therapy: Medicine of 21st Century

Inder M. Verma*

Laboratory of Genetics, The Salk Institute, La Jolla, CA 92037, USA. *verma@salk.edu

At the beginning of the third millennium, man has an opportunity to fulfill the cherished goal of improving the lot of humankind. Newer modalities of medicine are being practiced and daily new breakthroughs are being reported. I would like to talk about gene therapy, a form of molecular medicine, which will have a major impact on human health. At present, gene therapy is being contemplated for both genetic and acquired diseases. These include hemophilia, cystic fibrosis, diabetes, cancer, Parkinson's, Alzheimer's, etc. In the former case, a wide variety of somatic tissues are being explored for the Introduction of foreign genes with a view towards gene therapy. A prime requirement for successful gene therapy is the sustained expression of the therapeutic gene without any adverse effect on the recipient. A highly desirable delivery vehicle will be the one that can be generated at high amounts, integrate in non-dividing cells and have little or no associated immune problems. We have recently generated vectors based on the AIDS virus that have the ability to introduce genes into both dividing and non-dividing cells. The vectors (lentiviral) also have a very expanded host range and can introduce genes in a variety of cells. We have recently generated third generation of lentiviral packaging constructs that contain only the gag/pol, VSV G envelope and the sin vector. Thus our current lentiviral vectors are devoid of six viral genes and therefore we consider them to be safe vectors. Using third generation lentiviral vectors we can introduce genes directly into brain, liver, muscle, hematopoietic stem cells, and more recently retina and a number of tumor cells. Our data shows that lentiviral vectors can not only efficiently deliver genes, but also have long term sustained production of the foreign protein. We have not observed any untoward immunological consequences due to the vector. My talk will discuss in detail the use of vectors for a wide variety of genetic and acquired diseases. Additionally I will discuss the use of lentiviral vectors for transgenesis, and uses in studying complex biological systems. I will also discuss the social and ethical implications of genetic approaches to human health.

KPL-3
Cancer Causes and Prevention

I Mittra*

Bhopal Memorial Hospital & Resaerch Centre (BHMRC), Bhopal. *imittra@vsnl.com

KPL-4
Is Cancer Curable? – Future of cancer therapy

P.B. Desai*

307, Samuder Mahal, Dr. Ani Basent Road, Worli Mumbai-400018. *praful@mtnl.net.in

KPL-5
An Overview on Cancers in India

K.T. Dinshaw*

Tata Memorial Centre, Department of Radiation Oncology, Dr. Ernest Borges Marg, Parel, Mumbai – 400012. *tmhdir@tmc.ernet.in

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PLENARY ORAL PRESENTATIONS

PO-1
Convergence of Information Technology and Cancer Surveillance in the Descriptive epidemiology of cancer cervix in India

A. Nandakumar*

National Cancer Registry Programme (ICMR), India. *ank@blr.vsnl.net.in

The Indian Council of Medical Research initiated a network of cancer registries under the National Cancer Registry Programme (NCRP) in 1981 and data collection commenced in these registries from January 1982. Since then, the registries have provided information on incidence and patterns of cancer that in terms of quality and validity meet international standards. Thus, in India, for cancer, and perhaps for only this disease, we have a systematic programme of data collation so as to have reliable incidence and mortality rates, thereby laying a foundation for scientific research - whether that research be epidemiological, basic, clinical or in cancer control. However, India being a vast country, setting up of new registries throughout the country as in some Western countries would involve enormous cost in establishing and maintaining the same. Therefore, under a project, on 'Development of an Atlas of Cancer in India' a cost-effective design and plan using advances in modern electronic information technology, was conceived, to collate and process relevant data on cancer so as to fulfill the objective of obtaining an overview of patterns of cancer in different parts of the country; and, calculating estimates of cancer incidence wherever feasible. The Results presented are based on the data from both the cancer registries of the NCRP and that from the project on cancer atlas. Cancer of the cervix has been and continues to be the most important cancer in women in India. This is despite the decline in the incidence of this cancer, and in the absence of any organised screening programme. In the past two decades since the commencement of the NCRP, the Population Based Cancer Registry (PBCR) at Chennai (Madras) has shown cancer cervix as the leading site of cancer in women and continues to do so. Among the cancer registries in India, Chennai PBCR has always recorded the highest incidence rate. (Age Adjusted Incidence Rate (AAR): 28.8/100,000). This figure is somewhat lower than the highest incidence rates in the world. The most recent data from the report of the above project on 'cancer atlas' show that at least five districts have even higher incidence rates than that recorded at Chennai. Four of these five districts are concentrated in the north eastern region of Tamil Nadu state and Pondicherry. The atlas has further revealed that this area has also some of the highest incidence rates of penile cancer. There are reports in the literature that the prevalence of Human Papilloma Virus (HPV) is not only high among cancer cervix patients, but also high among patients with penile cancer. This part of Tamil Nadu state has also a high prevalence of Human Immunodeficiency Virus. Thus, this part of India is worthy of undertaking several research studies and control measures in cancer cervix.

PO-2
Targeting Transcription Factors for Prevention and Therapy of Cancer

Bharat B. Aggarwal*

Cytokine Research Laboratory, Department of Experimental Therapeutics, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030, U.S.A. *aggarwal@mdanderson.org

NF-kB, a transcription factor, is present normally in the cytoplasm as an inactive heterotrimer consisting of p50, p65 and IkBa subunits. When activated, NF-kB translocates to the as a p50-p65 heterodimer. This

factor regulates the expression of various genes that control apoptosis, viral replication, tumorigenesis, various autoimmune diseases, and inflammation. NF-kB has been linked to the development of carcinogenesis for several reasons. First, various carcinogens and tumor promoters have been shown to activate NF-kB. Second, activation of NF-kB has been shown to block apoptosis and promote proliferation. Third, the tumor microenvironment can induce NF-kB activation. Fourth, constitutive expression of NF-kB is frequently found in tumor cells. Fifth, NF-kB activation induces resistance to chemotherapeutic agents. Fifth, several genes involved in tumor initiation, promotion, and metastasis are regulated by NF-kB. Sixth, various chemopreventive agents have been found to downregulate the NF-kB activation. All these observation suggest that NF-kB could mediate tumorigenesis and thus can be used as a target for chemoprevention and for the treatment of cancer. Besides NF-kB, we have also targeted AP-1 and STAT3, other transcription factors that mediate tumorigenesis. We will present the data which shows that phyochemicals are important inhibitors of NF-kB, AP-1 and STAT3 activation, and can suppress the expression of genes involved in carcinogenesis and tumorigenesis in vivo.

PO-3
Understanding the De-Regulation of Cell Cycle in Tobacco Chewing Mediated Oral Cancer

Bibhu. R. Das*

Research & Development Division, SRL- Ranbaxy, 113, MIDC, 15th Street, Andheri (E), Mumbai - 400 093, India. *brdas@srlranbaxy.com

The present abstract summarizes the salient observations made during our studies to comprehend the key molecular events leading towards cell cycle deregulation during the process of oral carcinogenesis due to tobacco chewing habit. The phase I of the study involved investigating the expression levels of different cell cycle regulatory proteins in oral squamous cell carcinoma (OSCC) as compared to premalignant lesions and normal oral mucosa. Cyclins A, B1, B2, D1, D2, D3 and E were screened at both RNA and protein levels (IHC as well as western blot analysis). Besides the screening of cell cycle regulators, the important cell death regulators like p53 and Bcl2 were also analyzed. It was observed that among cyclins, Cyclin A and D1 showed the over expression with cancer progression. Among the cyclin dependent kinases (Cdk's) CDK4 (catalytic part of Cyclin D1) showed significant overexpression in oral tumors. Since CDK4 is inhibited be the INK4 family members like p14(ARF), p15(INK4B) and p16(INK4A), hence there expression levels was also evaluated. The expression levels of all three CDKI's showed the gradual decrease with progression from normal epithelium to premalignant lesions to OSCC. A high p53 immunoreactivity was observed. Surprisingly there was absence of mutation in p53 gene whereas frequent genomic rearrangement was seen in coding region of p53 as well as its promoter. Bcl-2 protein expression was observed more with higher-grade samples and oral cancer progression. In phase II, detailed promoter analysis of Cyclin D1 and CDK4 showed binding of STAT5 to CyclinD1 and binding of a novel transcription factor (named CDK4 Regulating Factor) to CDK4 promoter sequence. The present study shows that upregulation of CyclinD1 and CDK4 as well as down regulation of p16 contributes in a significant way towards the tobacco chewing mediated oral carcinogenesis. Thus our studies led us to several first hand findings having implications in early and sensitive diagnosis, effective treatment planning and in predicting prognosis.

PO-4
Involvement of Bax in the Regulation of Curcumin-induced Apoptosis

Devarajan Karunagaran*, Ramachandran Rashmi and Santhosh Kumar

Cancer Biology Laboratory, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala-695 014, India. *dkarunagaran@hotmail.com

Deregulated expression of pro and antiapoptotic proteins and their regulators often has an important role in chemoresistance. Curcumin, a dietary compound from turmeric, is known to induce apoptosis in a variety of cancer cells. To understand the role of Bax, a proapoptotic protein, in curcumin-induced apoptosis we used HCT116 human colon cancer cells with one allele of Bax gene (Bax+/-) and Bax knockout HCT116 (Bax-/-) cells in which Bax gene is inactivated by homologous recombination. Cell viability decreased in a concentration-dependent manner in Bax+/- cells treated with curcumin (0-50 M) whereas only minimal changes in viability were observed in Bax-/- cells upon curcumin treatment. In Bax-/- cells curcumin induced activation of caspases 9 and 3 was blocked and that of caspase 8 remained unaltered. Curcumin-induced release of cytochrome c, Smac and AIF was also blocked in Bax-/- cells and reintroduction of Bax, downregulation of the antiapoptotic protein Bcl-XL by anti sense DNA as well as overexpression of Smac highly sensitized the Bax-/- cells towards curcumin-induced apoptosis. There was no considerable difference in the percentage of apoptotic cells in Bak RNAi transfected Bax+/- or Bax-/- cells treated with curcumin when compared with their corresponding vector transfected cells treated with curcumin. The present study demonstrates the role of Bax but not Bak as a critical regulator of curcumin-induced apoptosis and implies the potential of targeting antiapoptotic proteins like Bcl-XL or over expression of proapoptotic proteins like Smac as interventional approaches to deal with Bax-deficient chemoresistant cancers for curcumin-based therapy.

PO-5

The Interaction between the E6 Oncoproteins of High-Risk Human Papillomaviruses and PDZ Protein Targets; Contribution to Cervical Cancer

David C. Pim**, Miranda Thomas, Helena Sterlinko-Grm, Paola Massimi, Noor Gammoh, Nisha Naryan and Lawrence Banks

International Centre for Genetic Engineering and Biotechnology, Padriciano, 34012 Trieste, Italy. *pim@icgeb.org

The E6 and E7 oncoproteins from the high-risk group of mucosal Human Papillomaviruses target the cellular tumour-suppressor proteins p53 and pRB respectively. The levels of both proteins are strongly reduced in cell lines derived from cervical tumours, due to the E6 and E7 recruitment of cellular ubiquitin ligases and degradation of the target proteins at the 26S proteasome. Despite the importance of these proteins, a great deal of evidence suggests that other cellular targets play critical roles in the path towards tumourigenesis. One group of cellular E6 targets are proteins containing PDZ domains, including Membrane Associated Guanylate Kinase homologues (MAGUKs) that are involved in the organisation of cell junctions and mediate cell polarity. The prototype PDZ protein is the mammalian homologue of *Drosophila* Discs Large - Dlg. This has been shown to bind to the carboxyl terminus of oncogenic E6 proteins, the last four amino acids of which conform to a consensus PDZ-binding domain, and which is not found on the E6 proteins from low-risk HPVs. We have shown that E6 binding to Dlg Results in its degradation at the 26S proteasome, but via a cellular ubiquitin ligase that appears to be different from E6AP, the ligase involved in p53 degradation. Other PDZ domain-containing targets include MAGI 1, MAGI 2, MAGI 3, MUPP1 and hScrib, the human homologue of *Drosophila* Scribble. We have shown that the relative efficiencies with which HPV-16 and 18 E6 oncoproteins bind and induce the degradation of PDZ targets such as Dlg and Scrib is determined by the last amino acid in their PDZ-binding domains and may correlate with the prognosis of patients with HPV-16 or HPV-18 derived cervical cancer. The importance of these target proteins in HPV-induced hyperplasia has been demonstrated by recent studies in transgenic mice. We have shown that in response to growth arrest signals, a significant portion of cellular

Dlg translocates to the cell nucleus and that it is this fraction that is preferentially targeted by the E6 protein. Studies on the E6-dependent and independent regulation of Dlg stability demonstrate a complex pattern of phosphorylation that is induced by a series of converging kinase signalling pathways.

PO-6
Recent Developments in HPV Prophylactic Vaccines

David Jenkins*

STD/ Viral Vaccines, GlaxoSmithKline Biologicals, Rue de L'Institut, Rixensart, Belgium. *david.jenkins@gskbio.com

PO-7
Modulation of Mitogenic Signaling Cascades in Mouse Liver by the Hepatitis B Virus X Protein

Debi P. Sarkar*

Dept. of Biochemistry, University of Delhi South Campus, Benito Juarez Road, New Delhi-110021. *dpsarkar59@rediffmail.com or debi_365@hotmail.com

Transcriptional activation of diverse cellular genes by the X protein (HBx) of hepatitis B virus (HBV) has been suggested as one of the mechanisms for HBV-associated hepatocellular carcinoma (HCC). However, such functions of HBx have been studied using transformed cells in culture and have not been examined in the normal adult hepatocytes, a natural host of HBV. Using an efficient hepatocyte-specific viral-based gene delivery system developed in our laboratory earlier, we studied the mechanisms of HBx action in vivo. We show that HBx induces a significant increase in the activity of extracellular signal-regulated kinases (ERKs) in the liver of experimental mice. Inhibition of HBx-induced ERK activation following intravenous administration of PD98059, a MEK inhibitor, confirmed the requirement of MEK in the activation of ERKs by HBx. HBx induction of ERK activity was sustained up to 30 days. Interestingly, sustained activation of c-Jun N-terminal kinases (JNKs) up to 30 days was also noted. Such constitutive ERK and JNK activation by HBx also led to sustained stimulation of further downstream events such as increased levels of c-Jun and c-Fos proteins along with the persistent induction of AP-1 binding activity. The minimum domain of HBx responsible for such activation has been identified. Taken together, our data suggest a critical role of these molecules in HBx-mediated cell transformation.

PO-8
Presence of Papillomavirus Sequences in Condylomatous Lesions of the Mamillae and in Invasive Carcinoma of the Breast

Ethel-Michele de Villiers*, Robert E Sandstrom, Harald zur Hausen, Charles E Buck

Referenzzentrum fur humanpathogene papillomviren, Abteilung Tumorvirus-Charakterisierung, F0700 Deutsches Krebsforschungszentrum, Im Neuenheimer Feld, 242 D-69120, Heidelberg, Germany. *e.devilliers@dkfz-heidelberg.de

Background: Viruses including Epstein-Barr virus (EBV), a human equivalent of murine mammary tumour virus (MMTV) and human papillomavirus (HPV) have been implicated in the aetiology of human breast cancer. We report the presence of HPV DNA sequences in areolar tissue and tumour tissue samples from female patients with breast carcinoma. The presence of virus in the areolar-nipple complex suggests to us a potential pathogenic mechanism. **Methods:** Polymerase chain reaction (PCR) was undertaken to amplify HPV types in areolar and tumour tissue from breast cancer cases. *In situ* hybridisation supported the PCR findings and localised the virus in nipple, areolar and tumour tissue. **Results:** Papillomavirus DNA was present in 25 of 29 samples of breast carcinoma and in 20 of 29 samples from the corresponding mamilla. The most prevalent type in both carcinomas and nipples was HPV 11, followed by HPV 6. Other types detected were HPV 16, 23, 27 and 57 (nipples and carcinomas), HPV 20, 21, 32, 37, 38, 66 and GA3-1 (nipples only) and HPV 3, 15, 24, 87 and DL473 (carcinomas only). Multiple types were demonstrated in seven carcinomas and ten nipple samples. **Conclusions:** The data demonstrate the occurrence of HPV in nipple and areolar tissues in patients with breast carcinoma. The authors postulate a retrograde ductular pattern of viral spread that may have pathogenic significance.

PO-9

Chromatin Meets Cancer - Histone Deacetylases as Targets in Cervical Cancer Therapy

Frank Rösl*

Angewandte Tumorvirologie; Abteilung Virale Transformationsmechanismen, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 242, 69120 Heidelberg, F.R.G. *F.Roesl@DKFZ.de

To elucidate the molecular effects of histone deacetylase (HDAC) inhibition in the context of HPV 16/18-induced carcinogenesis, we used the HPV 18-positive cervical carcinoma cells as well as primary human foreskin keratinocytes, which were separately immortalized with amphotropic retroviruses carrying the open reading frames of HPV 16 E6, E7 or E6/E7. Here we show that HDAC inhibition strongly inhibit G1 to S transition in HPV-positive cells, which was paralleled by an up-regulation of the cyclin-dependent kinase inhibitors (CKIs) $p21^{CIP1}$ and $p27^{KIP1}$ as well as the complete loss of cdk2 activity. Although HPV expression was hitherto thought to be required to maintain a proliferative phenotype, cdk2 suppression was achieved even in the presence of ongoing viral gene expression. HDAC inhibition also triggered an E7-dependent degradation of pRb and other pocket proteins, while the levels of E2F remained unaffected. The presence of free intracellular E2F and the concomitant up-regulation of CKIs during G1 arrest Results in a classical "conflicting growth situation", which finally renders the cells to undergo type II apoptosis through the mitochondrial pathway. Programmed cell death is mediated both by suppression of NF-kB and a strong activation of the E2F target gene p73. These data provide novel molecular insights into how the transforming potential of HPV can be circumvented. Furthermore, pretreatment with HDAC inhibitors highly sensitize formerly resistant HPV-positive cells to undergo TNF-a and TRAIL mediated apoptosis. This may determine future therapeutic strategies in which HDAC inhibitors can effectively eliminate HPV-positive cells by an apoptotic route that does not rely on the reactivation of the "classical" p53 pathway through a preceding shut-off of viral gene expression.

PO-10

Osteopontin, a Chemokine like Protein Regulates Tumor Growth and uPA-dependent MMP-9 Activation through NIK/PI 3-kinase/MAPK Signaling Pathways

Hema Rangaswami, Riku Das, Anuradha Bulbule, Ganapati H. Mahabeleshwar and Gopal C. Kundu*

National Centre for Cell Science, NCCS Complex, Pune 411 007, India. *kundu@nccs.res.in

Cancer progression depends on an accumulation of metastasis supporting cell signaling molecules that target signal transduction pathways and ultimately gene expression. Osteopontin (OPN) is one such chemokine like metastasis gene which plays key role in regulating the oncogenic potential of various cancers by controlling cell motility, invasiveness and tumor growth. We have recently reported that OPN stimulates NFkB-mediated pro-MMP-2 activation through IkBa/IKK signaling pathways. The molecular mechanism by which various upstream kinases regulate OPN-induced NF-kB/AP-1 activation and uPA secretion in human breast cancer cells is not well defined. We have shown that OPN induces $a_v b_3$ integrin-mediated PI 3'-kinase activity and Akt phosphorylation in both low and highly invasive breast cancer cells. OPN enhances NF B activation through phosphorylation and degradation of I B by inducing the IKK activity. OPN also enhances uPA secretion, cell motility and ECM-invasion. Moreover, the data also revealed that OPN stimulates v 3 integrin-mediated c-Src kinase activity and c-Src-dependent EGF receptor transactivation in these cells. OPN also induces c-Src and EGF receptor-dependent ERK phosphorylation and AP-1 activation. Interestingly, dn c-Src also suppressed OPN-induced PI 3'-kinase activity in these cells indicating that c-Src acts as master switch in regulating OPN-induced MAPK and PI 3'-kinase signaling pathways. Furthermore, OPN induces NIK activation and NIK-dependent MAPK/I -mediated NF-kB activation in melanoma cells. OPN also enhances uPA secretion and uPA-mediated pro-MMP-9 activation in these cells. Taken together, these data demonstrated that OPN regulates NF-kB/AP-1-mediated uPA secretion and uPA dependent pro-MMP-9 activation through NIK/PI 3'-kinase/ MAPK signaling pathways and all of these ultimately control the breast and melanoma cell motility, invasiveness and tumor growth.

PO-11
Cancer Causation by Viruses

Harald zur Hausen*

Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany. *zurhausen@dkfz.de

Several different virus families contain members involved in human carcinogenesis. Epstein-Barr virus (EBV) and human herpes virus type 8 (HHV-8), various types of papillomaviruses, hepatitis B virus (HBV), hepatitis C virus and human T-lymphotropic retrovirus type 1 (HTLV-1) , all belong into different virus families. Whereas EBV, HHV-8, high risk human papillomaviruses (HPV), and HTLV-1 are considered as direct carcinogens, where the malignant phenotype of virus-positive tumors depends on persistence of viral genomes within the tumor cells, other infections contribute indirectly to human cancer development. Human immunodeficiency virus (HIV) infections frequently result in B cell lymphomas and Kaposi sarcomas as a consequence of prolonged immunosuppression. Prevention of apoptosis in skin exposed to intensive solar exposure emerges as a possible mechanism by which several cutaneous papillomavirus types seem to contribute indirectly to the development of squamous cell carcinomas of the skin. Amplification of persisting papillomavirus or polyomavirus DNA sequences by incurrent infections with herpes simplex of cytomegaloviruses may also contribute to the emergence of malignant tumors. Several virus infections cause specific and/or random chromosomal aberrations within infected host cells. Specific changes have been reported for adenovirus type 12, herpes simplex and human cytomegalovirus infections. In view of multiple steps required for cancer development, it is difficult to assess the role of these modifications for subsequent proliferative events. A recently newly discovered virus family, *Anelloviridae*, contains a large number of different genotypes of TT viruses. These viruses are ubiquitous, present in the majority of human adults and persist in the human host probably for life time after primary infection. Although these viruses have yet been established as human pathogens, their remarkable variability, resulting to the isolation of up to 24 different genotypes from one single

tumor biopsy, should encourage the search for potential "high risk" types. The identification of viruses as causative agents for specific human cancers permits novel approaches for the prevention, diagnosis and therapy of virus-linked cancers. Clinical trials to prevent hepatitis B-linked hepatocellular carcinomas by hepatitis B vaccines, but also by preventing precursor lesions of cervical cancer by HPV vaccination provided evidence for the cancer-preventive potential of this approach. The global application of HBV and the respective HPV vaccines could significantly reduce the world-wide cancer burden. Attempts to develop therapeutic vaccines against virus-linked cancers or their precursor lesions, in contrast, thus far yielded mainly disappointing results.

PO-12
Immunotherapy for Cancer and Precancer Lesions from Cervical Cancer Trials in the Lab and the Clinic

Ian Frazer*

Centre for Immunology and Cancer Research, The University of Queensland, 4th Floor Research Extension, Building 1, Princess Alexandra Hospital, Ipswich Road, Woolloongabba Q'land 4102 Australia. * ifrazer@cicr.uq.edu.au

Vaccines to prevent human papillomavirus (HPV) infection, based on HPV virus like particles (VLPs), are in late stage clinical trials, having been shown "100% effective" at preventing persisting infection with HPV16 in at least two recent major phase II clinical trials. These HPV VLP vaccines are conventional vaccines, which work by inducing neutralizing antibody to the virus. They will therefore have to be given before infection with HPV has occurred. The critical question for such vaccines is to work out how long protection against HPV infection might last following immunization, and hence to work out the optimal target group for vaccination, and vaccine delivery strategy for preventing HPV associated disease including cervical cancer. Vaccines currently available include only two oncogenic HPV types (HPV16 and HPV18) and will therefore likely prevent about 70% of the HPV infections associated with cancer. They will therefore NOT replace conventional screening programs for HPV. Vaccines to treat existing HPV infection including HPV associated cancer are at a much earlier stage of development. Several are in trial but none have yet proven effective at eradicating HPV infection any more rapidly than would occur through natural processes. The reasons for this are many: optimal vaccine material and adjuvants are unknown, and there are no surrogate markers of efficacy to speed up clinical trials. There are underlying problems with poor presentation of HPV antigens by skin cells, such that even if good therapeutic vaccines are developed, the induced effector cells may not be able to find their target cells. More basic research will be required to sort out the key elements of an effective therapeutic vaccine, while ongoing early phase clinical trials will test possible candidate vaccines for efficacy and for induction of cytotoxic T cells with the likely characteristics for success.

PO-13
Chronic Inflammation, Stress Response Enzymes and DNA Damage

Jagadeesan Nair ***and Helmut Bartsch***

Division of Toxicology and Cancer Risk Factors, German Cancer Research Center (DKFZ), Heidelberg, Germany. *J.Nair@dkfz-heidelberg.de

Common pathways of chronic degenerative diseases involve biologically relevant reactive oxygen species (ROS) and reactive nitrogen species (RNS). These can be generated by biochemical redox reactions, phagocytes and up-regulation of response enzymes like cyclooxygenase-2 (COX-2), lipoxygenases (LOX) and inducible nitric oxide synthase (iNOS). The resulting oxidative stress is implicated in several human cancers where chronic inflammation and persistent infections are involved. We have developed ultrasensitve methods for measuring DNA damage induced by 4-hydroxynonenal (HNE) and malondialdehyde (MDA) primarily formed by lipid peroxidation of N-6 polyunsaturated fatty acids (N-6 PUFAs) such as arachidonic acid and linoleic acid. These are also metabolized by enzymes overexpressed during chronic inflammation such as COX-2, LOX and iNOS to form the above reactive aldehydes. We have reported an increased DNA damage caused by reactive aldehydes to occur in colonic polyps of familial adenomatous polyposis patients where COX-2 is overexpressed. In the DMBA-TPA multistage mouse skin carcinogenesis model, a strong positive correlation was observed between the formation of HNE-derived DNA adducts and the LOX-catalysed arachidonic acid metabolites, hydroxyeicosatetraenoic acids (HETE). In the SJL mouse model and in p53 knock-out mice, HNE-derived DNA adducts in affected tissues were elevated due to an increased iNOS and nitric oxide over-production. Taken together our results clearly demonstrate an increased DNA damage to occur during chronic inflammation as a result of overexpressed stress response enzymes; the ensuing mutations and genetic instability may drive cells towards malignancy.

PO-14
Clinical Applications of HPV-DNA Testing in Cervical Cancer Control

Jeffrey Tan*

Royal Women's Hospital, Melbourne, Australia. *jeff.tan@rwh.org.au

Human papillomavirus (HPV) DNA testing are increasingly being reported in clinical applications for the following conditions: (a) Triage of women with cytological determinations of atypical squamous cells of undetermined significance (ASC-US), (b) As a marker for test of cure post-treatment and, (c) Adjunct to cytology in routine cervical cancer screening programs. HPV-Testing methods were either the Hybrid Capture 2 (HC2) test or the polymerase chain reaction (PCR) test. a) Using The Bethesda System (TBS) 1991, HPV-DNA detection rate was reported in Negative 32%, ASCUS 49%, LSIL/HSIL 93%. On review using TBS 2001, HPV-DNA was detected in ASC-US 56% and ASC-H 71%. The major findings of the ASCUS-LSIL Triage Study (ALTS) was summarised by Schiffman et al. The prevalence of oncogenic HPV was too high to permit effective triage of LSIL using HPV DNA testing by HPV HC2. HPV triage is at least as sensitive as immediate colposcopy for detecting CIN 3 among women with ASCUS. A program of repeat cytology is also sensitive if an ASCUS threshold is maintained and loss to follow-up is minimal. The immediate colposcopy strategy is certainly the least specific, referring 100% of women to colposcopy. b) Systematic review of studies (1985 to March 2002) by Paraskevaidis et al indicates the sensitivity of HPV DNA testing in detecting treatment failures was quite good in most studies, reaching 100% in four of them whereas the specificity of the test differed across the studies, ranging from 44% to 95%. Among women in whom the treatment was considered to be successful, 84.2% had a negative postoperative HPV DNA test and 15.8% a positive one. The corresponding rates for cases with treatment failures were 17.2% and 82.8% respectively. In meta-analysis of 11 studies (published between 1996 and 2003) by Zielinski et al, the negative predictive value (NPV) for recurrent/residual disease of HPV-DNA testing was 98% and that of cervical cytology 93%. When HPV-DNA testing was performed in conjunction with cytology, the sensitivity was 96%, specificity 81%, the associated positive predictive value (PPV) 46% and NPV 99%. c) HPV-DNA testing is reported as more sensitive than

cytology in predicting high grade abnormality of cervix (HSIL). A combination of HPV-DNA and Papanicolaou testing had almost 100% sensitivity and negative predictive value. Women with persistent positive HPV-DNA tests and normal cytology are at risk of developing HSIL. There is suggestion that HPV-DNA test could be used for primary screening in women older than 30 years, with cytology used to triage HPV-DNA positive women. Research continues into approaches for improving the performance and cost-effectiveness of HPV detection methods via improved HPV typing capabilities and Rapid Capture machine allowing increased throughput. Combining this test with expression levels of other markers such as proliferative and cell cycle regulatory proteins will allow subdivision of HPV-DNA positive women into those who are at greater risk of cancer and those who can be safely followed by screening at longer intervals.

PO-15
Prophylactic HPV Vaccines to Prevent Cervical Cancer

John T. Schiller*

Lab. of Cellular Oncology, National Cancer Institute, Bethesda, MD. *schillej@dc37a.nci.nih.gov

The establishment of sexually transmitted HPV infections as the central cause of cervical cancer provides an exceptional opportunity for cervical cancer prevention through vaccination. Prophylactic vaccines are based primarily on induction of virion neutralizing antibodies by non-infectious virus-like particles (VLPs) composed of assemblages of the L1 major capsid protein. VLP vaccines for the major oncogenic type HPV16 have consistently induced high titer of neutralizing antibodies with minimal side effects and induced 100% protection from persistent HPV16 infection in proof of concept efficacy trials. The ability of the VLPs to avidly bind and induce a variety of innate immune responses in systemic antigen presenting cells likely contributes to their induction of potent B and T cell responses after injection, even in the absence of adjuvant. Three large phase III prophylactic HPV VLP trials are now in progress and there is wide spread optimism for the prospects of regulatory approval for general marketing of a VLP vaccine in 2-4 years. A licensed prophylactic HPV vaccine would raise a number of implementation issues. These include the general acceptance of a vaccine targeting a sexually transmitted infection, the logistics of administering a series of three injections to adolescents or preadolescent girls, and the relative benefits of also vaccinating males. The relatively high cost of VLP vaccine production and distribution, the expected type-specificity of their protection, and the unlikely prospects for therapeutic efficacy will be impediments of particular concern for vaccine implementation in developing countries, where 80% of cervical cancer occurs. Second generation vaccines that address the limitations of the current VLP vaccines are under development. Examples include mucosal deliver of VLPs, chimeric VLPs containing E7 polypeptides to function as combined prophylactic/therapeutic vaccines, and L2 minor capsid protein-based vaccines to induce protection against more HPV types. However, none of these strategies is sufficiently advanced to warrant large-scale efficacy trials at the present time.

PO-16
Cervical Cancer Prevention-Emerging Options for Developing Countries

John W. Sellors[1]*, Youlin Qiao[2], Rengaswamy Sankaranarayanan[3], Roshni Chinoy[4], B. M. Nene[5], K. Jayant[5], Sylla Malvi[4], Roger B. Peck[1], Bernhard H. Weigl[1]

[1]PATH, 1455 NW Leary Way, Seattle, WA USA 98107. [2]Cancer Institute Chinese Academy of Medical Sciences,

17 S. Pan Jian Yuan Lane, PO Box 2258, Beijing 100021, PR China; [3]International Agency for Research on Cancer, 150 cours Albert Thomas, Lyon Cedex 08 F-69372, France; [4]Tata Memorial Centre, Parel, Mumbai 400 012, India; [5]Nargis Dutt Memorial Cancer Hospital, Barshi, Solapur, (Maharashtra) 413 401, India. *jsellors@path.org

Introduction: Of the 490,000 cervical cancer cases and 270,000 deaths in women worldwide each year, over 85% are in the developing world. The majority of these cancers would be prevented in developing countries by once or twice in a lifetime screening of women between 30 to 50 years of age, followed by treatment of precancerous lesions, if the testing and treatment were effective. **Objectives**: To assess alternative screening methods to cervical cytology: visual methods and rapid tests for HPV and outpatient treatment with cryotherapy. **Methods**: Data were compared from methodologically rigorous studies in low-resource settings on the performance of visual inspection with the naked eye after washing the cervix with dilute acetic acid (VIA) or Lugol's iodine (VILI) with cytology; and the effectiveness of cryotherapy. Progress on the development of two rapid, accurate, simple, and affordable HPV screening tests was also assessed. **Results**: Studies from India, China, and African countries have shown that the sensitivity of VIA and VILI exceed that of cytology, the specificity of cytology is better, and the cost-effectiveness of VIA and VILI dominate cytology, especially when cryotherapy is used without prior confirmation by colposcopy and biopsy. Development is progressing on two HPV tests for low-resource settings: a batch-based, DNA detection assay based on hybrid capture 2 (Digene Corporation, Gaithersburg, MD) and a viral protein activity marker lateral flow strip assay (Arbor Vita Corporation, Sunnyvale, CA). **Conclusion**: VIA and VILI are promising alternatives to cytology, cryotherapy is effective, and progress is being made on development of HPV tests for screening in low-resource settings.

PO-17

Activator Protein 2 Alpha (AP-2) Status Determines the Chemosensitivity: Implications in Cancer Chemotherapy

Kumaravel Somasundaram*

Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore 560 012, India. *skumar@mcbl.iisc.ernet.in

Inactivation of proapoptotic genes or activation of survival signaling leads to chemoresistance. Activator protein 2 , a developmentally regulated sequence-specific DNA-binding transcription factor, has been shown to function like a tumor suppressor. While genetic alterations in AP-2a gene in cancer cells have not been reported, progressive loss of AP-2a expression with tumor progression has been reported in breast, colon, prostate cancer and melanoma. The loss of AP-2a expression in invasive breast cancer has been correlated with hypermethylation (of CpG island in the promoter of AP-2a gene) mediated silencing of AP-2a gene We have shown previously that overexpression of AP-2 inhibits the growth of cancer cells by inducing cell cycle arrest and apoptosis. Here we show that controlled expression of AP-2a , using tetracyline inducible system, increased the chemosensitivity of cancer cells by several fold. Under these conditions, neither AP-2a expression nor drug treatment resulted in apoptosis induction while in combination, the cancer cells underwent massive apoptosis. We found endogenous AP-2a is induced by a variety of chemotherapeutic drugs. Blocking endogenous AP-2a by siRNA lead to chemoresistance of human cancer cells irrespective of their p53 status. This suggests that AP-2a induction by chemotherapeutic drugs plays a major role in determining the chemosensitivity. We further show that 5-aza-2'deoxycytidine (5aza2dC) induced re-expression of AP-2a in MDA-MB-231 breast cancer cells, wherein AP-2a expression is silenced by hypermethylation, resulted in

massive apoptosis induction, increased chemosensitivity and loss of tumorigenesis upon chemotherapy. However, in MDA-MB-231 cells transfected with AP-2a siRNA, 5aza2dC treatment failed to increase apoptosis and chemosensitivity upon chemotherapy. Considering the fact that 75% of invasive breast cancers have epigenetically silenced AP-2a, our approach of combined treatment of breast cancer with 5-aza-2'deoxycytidine and chemotherapy provides a novel way of modifying the chemosensitivity of breast cancer. These results establish an important role for AP-2a in cancer cell chemosensitivity and provide new insights for modifying the chemosensitivty of cancer cells by activating apoptotic pathways. Overall, our data provides both in vitro and in vivo validation for a strategy to reverse chemoresistance in human cancers, in particular breast cancer and underscores the value of tailoring cancer therapy on the basis of tumor genotype.

PO-18
HPV and Cancers at Non-Genital Sites

Keerti V. Shah*

Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe Street, Baltimore, MD 21205, USA. *kvshah@jhsph.edu

The etiologic role of HPV infections in cancers of the lower genital tract is well established. The virus is responsible for almost all cases of squamous cell carcinoma and adenocarcinoma of the cervix and for significant fractions of vulvar, vaginal, perineal and penile cancers. While HPV sequences have been reported to be present in cancers at many sites other than the lower genital tract (e.g., body of uterus, ovary, esophagus, oral cavity, colon, lung), the evidence for an etiologic role of the virus is most compelling for some cancers of the oropharynx. The HPV-associated oropharyngeal cancers are differentiated from oropharyngeal cancers not associated with HPVs by virological, molecular and clinical criteria as follows. The HPV-associated cancers have the viral genome localized to the tumor cells, a lesser frequency of p53 mutations, more frequent basaloid pathology, and a better prognosis. The presence of HPV sequences (derived from the lysis of tumor cells) in the plasma of some of these patients may be indicative of more extensive disease and of the risk of recurrence. HPV type 16 accounts for an overwhelming majority of the HPV-associated cancers. The precursor lesion of these cancers has not yet been identified. Prophylactic HPV-based immunization against cervical cancer may be expected to also prevent HPV-associated oropharyngeal cancers.

PO-19
Recent Advances in Diagnosis and Management of Cancers - a Genetic Approach

Kiran Kucheria ***and Rashmi Talwar***

Department of Anatomy, All India Institute of Medical Sciences, New Delhi. *kkucheria@hotmail.com

Introduction: The diagnosis, assessment of prognosis and minimal residual disease (MRD) of cancers depends on understanding of chromosomal and gene alterations using highly sensitive molecular techniques. **Objective:** The aim of the study was to evaluate cytogenetic and molecular anomalies at diagnosis and sequential follow-ups in leukemias and cancers. **Methods:** Conventional cytogenetic and Fluorescence In Situ Hybridization (FISH) analysis were done in 420 cases of leukemias of which 195 cases were follow-ups. Ten samples of urinary bladder cancer and 4 of breast cancer were analyzed using FISH to assess aneusomies and HER-2/neu

amplification status respectively. Status of other genes like p53 and N-myc were also evaluated in various cancers. Seventy cases of Retinoblastoma were analyzed using conventional cytogenetics and mutational analysis was conducted in 14 cases using denaturation high-performance liquid chromatography (DHPLC). **Results:** Sequential cytogenetic and FISH analysis in leukemias revealed appearance of additional anomalies that were later correlated with hematological and clinical findings. Most of these cases revealed disease progression and relapse in subsequent follow-ups that was preceded by cytogenetic/molecular relapse. In some cases of leukemias and other cancers, FISH analysis revealed molecular changes and MRD that were not evident using conventional cytogenetics. FISH analysis in urinary bladder and breast cancers reveals prognostic significance of gene alterations and recurrence up to 6 months sooner than cytoscopy and cytology. Novel mutations were identified in some Retinoblastoma cases using DHPLC. **Conclusion:** Importance of analysis of chromosomal and gene alterations using highly sensitive molecular techniques in management of cancers is highlighted

PO-20
Haemopoietic Stem Cell Transplantation (HSCT): Newer Advances

Lalit Kumar**, K Ganessan, M Sengar, N Khatry, K Mehdi, S Mohanty and V Kochupillai

Department of Medical Oncology, Institute Rotary Cancer Hospital, All India Institute of Medical Sciences, New Delhi 110029, India. *lalitaiims@yahoo.com

High dose chemotherapy (HDCT) followed by haemopoietic stem cell transplantation (HSCT) is now an established therapy for treatment of a no of non malignant and malignant conditions. Severe aplastic anemia, haemoglobinopathies (beta-thalassemia, sickle cell anemia), immunodeficiency diseases (SCID, WAS etc), genetic metabolic disorders (mucopolysaccharidosis) are common non- malignant conditions. Among malignant conditions, acute leukemia, chronic myeloid leukemia, myelodysplastic syndrome, multiple myeloma, Hodgkins and non Hodgkin's lymphoma and high risk neuroblastoma are important indications for HSCT. HDCT with HSCT is also being considered in the treatment of poor risk germ cell tumors, child hood tumors and auto-immune disease (rheumatoid arthritis, systemic lupus erythematosus, scleroderma) especially for the patients who have failed after standard therapy. Haemopoietic stem cells can be obtained either from a genetically identical twin (syngeneic) or from an HLA-identical matched sibling or unrelated donor (allogeneic) or from patient's own (autologous) BM or peripheral blood (PB). : Accurate HLA typing is essential for patients receiving allogeneic transplants. In addition to standard serology currently, DNA based techniques such as as PCR- with sequence specific oligonucleotide probes are used (for class II regions) for HLA typing. The probability of finding a HLA match in the family is about 25-35%. For the remaining, either family members other than HLA-identical siblings or matched voluntary unrelated donors (MUD) could be alternative donors. The later can be identified through the help of bone marrow donor registry programme which are already in place in the developed countries. In India, a beginning has been made but the progress is very slow. Traditionally, BM has been used as a source of stem cells for the purpose of transplantation but during the past 15 years, peripheral blood (PB) & umbilical cord has become an important source. PB stem cells are mobilized using inj G-CSF with or without chemotherapy with the help of apheresis machine. Practically today, all the autologous stem cell transplantations are being done using PB stem cells. Even for the allogeneic HSCT, use of PB stem cells have increased over the past decade. PB stem cells have an advantage of early recovery (engraftment), with no increased risk of acute GVHD. However, there is slight higher risk of chronic GVHD. Umbilical cord (UC) blood is a rich source of most primitive (stem) cells that are able to produce 'in vivo' long term repopulating haemopoietic stem cells compared to adult stem cells. Since, the total yield of stem cells from a single cord blood is limited, presently, UC blood is being used for children weighing up to 25 Kg. More than 6000 transplants have been done worldwide using UC blood. With improved supportive care and experience , more and more

allogeneic HSCT are being done now for patients of higher age (>45 years) which was earlier limited to younger patients (<40 to 45 years). Another development in the past few years is the use of more immunosuppressive regimens (called non - myeloablative or less intensive) for conditioning rather than myeloablative chemotherapy. Drugs such as Fludarabine, antithymocyte globulin (ATG) or 2 CDA are commonly used for this purpose. Use of non myeloablative regimens is associated with decreased risk of infectious complications and reduced frequency & intensity of acute GVHD. Currently such transplants are being done for patients with low grade lymphomas, myelodysplastic syndrome, chronic lymphocytic leukemia etc. Recent understanding of the phenomenon of 'stem cell plasticity' has led to exploration of use of embryonic stem cells / BM stem cells in the repair of myocardium post myocardial infarction, treatment of neurological disorders such as stroke, Parkinson's disease, spinal cord injury, duchene muscular dystrophy etc. Most of this work is in experimental stage at present but it appears that during coming years, stem cell therapy will be used for the treatment of many of these diseases.

PO-21
The Nutrigenomics of Cervical Cancer

M. Radhakrishna Pillai and Asok C. Antony*

Department of Molecular Medicine, Regional Cancer Centre, Thiruvananthapuram, India and Indiana University School of Medicne, Indianapolis, USA. *mrpillai@vsnl.com

India has one third of the world's cervical cancer burden. This, together with continuing unacceptably low treatment response rates begs for novel and innovative forms of diagnosis and therapy for both prevention and treatment of locally advanced and metastatic disease. Human papillomavirus (HPV) has been shown to be the principle etiological factor in the pathogenesis of this cancer although studies by others and us demonstrate the requirement for additional co-factors. Nutrigenomics encompasses the fields of biotechnology, genomics, molecular medicine and human nutrition, enabling examination of diet and nutrition in a whole new light. The aim of nutrigenomics is to scrutinize and comprehend an individual's response to micro and macronutrients through the analysis of their unique genomic make up, which can consequently lay the foundation for the cultivation of safe and effective dietary treatments for the individual. Recent data for Indian women have shown a high incidence of folate deficiency. Our preliminary epidemiological studies have shown an association between HPV infection and low folate levels. It was observed that folate deficiency co-existing with HPV, increased the risk of developing Cervical Intraepithelial Neoplasia (CIN) by seven times. Hence folic acid deficiency may be a precursor or transmission stage of HPV in cervical cells and enhance its progression. Folate metabolism is also influenced by Single Nucleotide Polymorphisms (SNPs) of methylene tetrahydrofolate reductase (MTHFR) gene, observed at 677 (A-C) and 1298 (C-T) nucleotides in the gene sequence. Both these polymorphisms greatly impair folate metabolism. Folate deficiency either due to a genetic reason or dietary deficiency will result in accumulation of homocysteine. This accumulation will be compounded when there is deficiency of Vitamin B12 and B6. We will present data and a working hypothesis to explain how accumulation of homocysteine can influence tumor progression in HPV initiated cells. This involves the modulation of the NF kappa B inhibitor I kappa B by homocysteine and consequent activation of NF kappa B system. A second important consequence of folate and homocysteine involves the up-regulation of folate receptors (FR). Work on the regulation of FR in cervical cancer cells has led to an intimate study of hnRNP-E1, which can specifically bind to the sense strand of the HPV-16 L2 viral capsid protein mRNA to inhibit its synthesis in vitro. There is substantial experimental evidence to support a model for translational up-regulation of the FR in folate deficiency. This is based on a critical role for accumulated intracellular homocysteine which promotes the interaction of hnRNP E1 with an

18-base cis-element located in the 5'-untranslated region of FR mRNA which leads to increased biosynthesis of FR at the translational level. Therefore information on hnRNP-E1 expression in the cervix is valuable in assessing its possible functional role in FR synthesis in vivo. In addition, because hnRNP-E1 expression can potentially modulate HPV viral proliferation in the cervix, knowledge of hnRNP-E1 expression patterns could also provide insights into the possible biological constraint(s) exerted by these proteins on HPV proliferation. This would be particularly informative in women with HPV-mediated transformation of cervical tissue to cancer.

PO-22
p16INK4a, A Novel Biomarker for Early Detection and Prevention of Cervical Cancer

Magnus von Knebel Doeberitz*

Institute of Molecular Pathology, University of Heidelberg, Germany. *knebel@med.uni-heidelberg.de

Extensive research over the past 20 years provided strong evidence that persistent infections with high risk type human papillomaviruses (HR-HPVs) cause cervical cancer. However, depending on their age, more than 20% of normal women are infected with these viruses and only very few develop clinically relevant dysplastic lesions or even cancer. During an acute HPV infection, expression of viral genes, in particular the viral E6 and E7 oncogenes is restricted to differentiated epithelial cells, which lost the capability to replicate their genomes and are therefore at no further risk for acquiring functionally relevant mutations upon genotoxic damage. High grade cervical dysplasia, however, is initiated by deregulated expression of viral oncogenes in replicating basal or parabasal cells, where the E6-E7 genes submerge control of the cell cycle and mitotic spindle pole formation and induce severe chromosomal instability. Expression of HR-HPV E7 oncogene products in basal or parabasal cells uniformly results in strong over-expression of the cyclin dependent kinase inhibitor $p16^{ink4a}$. This can be used to identify dysplastic cells in histological slides, cytological smears or samples taken for biochemical analyses with very high sensitivity and specificity and suggests that novel, more precise and thus less costly cervical cancer screening algorithms will be established soon. Clinical studies, that confirm this concept will be reviewed in detail at the conference. Moreover, HPV-mediated over-expression of $p16^{INK4a}$ also elicits an immune response against cervical cancer cells and their precursors that may play an important role in the immune surveillance of HPV-transformed cells.

PO-23
HPV Vaccines: Prospects for Eradicating Ano-genital Neoplasia

Margaret A Stanley*

Department of Pathology University of Cambridge UK. *mas@mole.bio.cam.ac.uk, mas1001@cam.ac.uk

The ability to generate human papillomavirus (HPV) virus like particles (VLPs) by the synthesis and self-assembly in vitro of the major virus capsid protein L1 has transformed our prospects for preventing cervical carcinoma in women. Immunisation with L1 VLPs provides type specific protection in all the animal infections so far tested. In Phase I trials in humans HPV L1 VLP vaccines are safe and highly immunogenic stimulating robust B and T cell responses and generating high titres of neutralising antibody. Phase II and Phase III trials are in progress with preliminary data suggesting that antibody levels persist at measurable levels for at least 30

months post vaccination. Phase II proof of principle efficacy trials are immensely encouraging with evidence that these vaccines protect against infection. However it must be recognised that VLP vaccines are type specific, likely to be expensive, require a cold chain and medical or para medical personnel for delivery. Furthermore L1 vaccines must be delivered before the sexual debut to prepubertal females (or males) and social and cultural issues may be important in determining vaccine take up. L1 based vaccines are not protective after exposure and in post exposure HPV infection, cell mediated immunity is critical. Studies in animal papillomavirus infections suggest that immunisation with specific early proteins, particularly E1 and E2, could be effective and immunotherapies for established lesions such as ano-genital warts and low-grade intra-epithelial lesions are realistic. Such vaccines are likely to be combined with immunomodulators such as cytokines in order to maximise the response. Prime/boost strategies combining DNA and/or protein and/or recombinant viruses look to have significant potential as immunotherapies for benign or low grade HPV induced disease. Immunotherapies for HPV associated high-grade pre-cancers and invasive cancers are problematic. Almost every vaccine delivery system known has been used to deliver HPV E6 and E7 oncoantigens in transplantable tumour models in rodents. HPV specific cytotoxic T cells are generated and antigen specific killing of HPV expressing tumour cells can be demonstrated. Clinical trials with any of these modalities have been minimal and to date no regression of any cervical cancer in response to the various immunotherapies has been shown but partial responses in high grade pre-cancers have been demonstrated. Tumour evasion mechanisms, such as down regulation of MHC Class I, remain a tough barrier for successful cancer immunotherapies.

PO-24
Screening with HPV-test for Cervical Cancer

Matti Hakama**, Laura Kotaniemi, Pekka Nieminen, Ahti Anttila

Finnish Cancer Registry, Helsinki, and University of Tampere, Finland. *matti.hakama@uta.fi

Several studies have addressed on the problem of low sensitivity of the traditional screening test for cervical cancer. The most promising new technology to improve the test is based on the etiology of cervical cancer resulting in HPV-test. Evidence, so far stems mainly from the developed countries and with a design where the same woman is subjected to the two tests compared for sensitivity. This is a valid approach if there is no overdiagnosis, i.e. if all the lesions confirmed will progress to cancer. This is not true for the preinvasive lesions detected by screening for cervical cancer. In India there are going on one of the very few randomised trials where each woman is randomly allocated for one screening test only. In Finland the same approach is applied in the routine screening that is run as a public health policy since 1960's. In Finland about 200 000 women are annually invited and 150 000 women attend the routine organised programme of screening for cervical cancer. Individual municipalities decide on joining and financing the programme but the policy is regulated by a national by-law. There is a mass screening registry within the Finnish Cancer Registry that designs, collects data and analyses the performance and outcome of the screening programme. Municipalities have their own screening laboratory or make an agreement with an external one. Within this infrastructure the HPV-test was introduced in 2003. Several municipalities served by the Cancer Society of Finland laboratory joined an individually randomised trial with one third screened with HPV-test one third screened with automation assisted test and one third with traditional papsmear. The objective is to assess the sensitivity of each test and to evaluate the effectiveness of routine screening with each test compared to the two other tests. The activity falls in the area of health services research as routine public health policy is evaluated but the design is that of an scientific experiment. In 2003 altogether 15 000 women were randomised and the target population will get increased year by year. In fact, the automation assisted screening was run since 1999 with more than 500 000 tests at present. As the screening interval is 5 years in Finland the final evaluation for the HPV-test takes place in 2008 earliest. The cost of each test is the same for the municipality and the difference to the automation assisted screening

and HPV-test screening is covered by the Cancer Society of Finland and by research grants. The cost can be directly evaluated only for the same infrastructure without allowing for variation as to ages of target population and for screening interval. Automation assisted screening seems to result in equal detection of cervical cancer and precancerous lesions as the conventional screening (Nieminen et al. Int J Cancer 2003; 103:422-426), data on interval cancer rates are not yet available. Preliminary experience on HPV-screening in a pilot study predicts HPV-test to be more sensitive by detecting severe and moderate dysplasias (Nieminen et al. BJOG 2004; in press) at higher rate than the conventional pap test.

PO-25

Molecular Epidemiology, Prevention and Therapeutic strategies in the Management of Hepatocellular Carcinoma

P. Kar*

Department of Medicine, Maulana Azad Medical College, New Delhi-110002, India. *pkar@vsnl.co.in

Hepatocellular Carcinoma (HCC) is the most frequent primary tumor of the liver in adults. It ranks as the fifth most common cancer in the world and the third most common cause of cancer related deaths. Most of the HCC develop on underlying cirrhotic liver. The major etiologic risk factors for HCC development include HBV and HCV infection, toxins (alcohol, Aflatoxin B1) and various inherited metabolic liver diseases, such as Hemochromatosis and alpha-1-antitrypsin deficiency. The most frequently altered human genes in HCC are tumor suppressor genes such as p53, RB1, p16, and IGF2R genes in which Loss of Heterozygocity has been reported, with a frequency higher than 20%. Other genes, like GST and CYP450 (detoxifying genes) are also altered, but the frequency of individual gene mutations is low. Early detection of HCC remains the best strategy for reducing tumor related mortality. FNAC or biopsy is the gold standard for HCC diagnosis. Ultrasound and AFP assessment every six month is the recommended procedure for surveillance in patients with cirrhosis. A multidisciplinary approach comprising of interventional radiologists, medical oncologists, radiation oncologist and surgeons are required for optimal care of patients with HCC. The options includes resection of liver segment, tumor ablation by injecting with absolute alcohol or acetic acid or by using radiofrequency probe, liver transplantation, chemoembolisation, systemic chemotherapy, certain experimental drugs, hormones and cytokines. The ineffectiveness of conventional chemotherapic strategies in this condition has prompted studies of novel systemic strategies, including Antioestrogen therapy, Thalidomide, long acting Somatostatin, Interferon and Interleukin 2 therapy. Preventive measures should have a major impact on the incidence of HCC. Further, the prevention of a local recurrence or the development of new HCC lesions in patients after successful surgical or non-surgical HCC treatment (secondary prevention) is of paramount importance. Based on rapid scientific advances, molecular diagnosis, gene therapy and molecular prevention are becoming increasingly part of our patient management and will eventually complement and in part replace existing diagnostic, therapeutic and preventive strategies. Overall, this should result in reduction of the incidence of HCC, one of the most devastating malignancies worldwide.

PO-26

Host immune Response to Human Papilloma Virus (HPV) as Predictive Marker for Persistence or Progression of Cervical Neoplasia

Partha Basu*, Robert P Edwards[#], Sherry Willer[#], Bennett Jenson[#], Shin-je Ghim[#]

Chittaranjan National Cancer Institute, Kolkata, India. #James Graham Brown Cancer Center, Louisville, US. *ceds@vsnl.com

The immune response to HPV and HPV-induced cervical neoplasia appears to be best understood in the context of T helper cells 1 and 2 (Th-1 and Th-2) subsets that can determine susceptibility or resistance to the disease. The Th-1 (inflammatory) response is associated with cell-mediated immunity and an IgG2 isotype antibody response. The Th-2 (anti-inflammatory) response is associated with humoral immunity and an IgG1 isotype antibody response. Deviation from a balanced Th-1:Th-2 response to a Th-2 predominant response may reflect the capacity of Th-2 cytokines to down-regulate or cross-regulate cytokines associated with Th-1. Alternatively, pre-existing Th-2 cytokines can redirect a Th-1 response to a Th-2 response by immune deviation. Most cancers appear during a Th-2 response, ineffective for viral clearance. HPV onco-proteins E6 and E7 are continually expressed in cervical cancers and high-grade intraepithelial neoplasias. Detection of IgG isotype reactivity with the E7 oncoproteins of HPV appears to reflect the effectiveness of the immune response against cervical cancers and their precursors. Till date there is no efficient marker to differentiate the women with progressive disease from the women whose CIN lesions will regress or remain stationary. From immuno-biologic point of view, women having predominant Th1 response (high serum IgG2 level) are more likely to clear the infection/lesion compared to those women having predominant Th2 response (high serum IgG1 level). Estimation of titers of IgG1, IgG2 and their relative proportion in the serum can serve as a predictive marker for progression of CIN lesions to higher grades and invasive disease. Serial estimation of IgG1 and IgG2 levels during follow up of cervical cancer patients may help to diagnose the recurrent disease earlier as a predominant humoral response is likely to be associated with reappearance of neoplasia. An ELISA test has been developed and optimized at James Graham Brown Cancer Center to estimate the serum IgG1 and IgG2 against E7 protein of HPV 16 and 18. The assay is being done in the sera collected from women having normal cervix, precancer of cervix and cancer of cervix. The updated results will be presented.

PO-27

The Role of HPV Testing in the Early Detection of Cervical Neoplasia in Low-Resource Settings

R. Sankaranarayanan*

Screening Group, International Agency for Research on Cancer, Lyon, France. *narayan@iacr.fr or sankar@iarc.fr

The knowledge that cervical neoplasia are caused by persistent infection with high-risk types of human papillomaviruses (HPV) has led to the evaluation of vaccination and the detection high-risk HPV types as a cervical screening strategy. The accuracy of HPV testing in primary screening for cervical neoplasia has been evaluated in several cross-sectional studies. The second-generation Hybrid Capture II (HC II) probe B (which is a pool of full-length RNA probes for HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 microtitre assay) has been widely used for HPV testing in a number of studies. Polymerase chain reaction (PCR) assays have also been evaluated. The sensitivity of HPV testing by HC II in detecting CIN 2,3 lesions and invasive cancer varied from 62-100% and the specificity varied from 41-96% in different developed and developing country settings. The sensitivity of HPV testing when specimens have been taken and/or analyzed in developing country settings has generally been lower than that where the entire specimen chain (collection/testing) was completed in a developed country. In studies from China, Mexico, South Africa and Zimbabwe, the sensitivity and specificity varied from 62 to 97% and 41 to 92%. In a pooled analysis of 4 cross-sectional studies with a common protocol in India, involving 18 085 women aged 25-65 years, the sensitivity and specificity to detect

CIN 2.3 lesions and invasive cancer were 68% (95% CI: 61-74%), and 94% (95% CI: 61-74%) respectively. The sensitivity varied from 50 to 80% in the individual studies. Results from a large randomized intervention trial Maharashtra, India, comparing HPV testing, cytology and visual screening with acetic acid (VIA), indicate that all the tests had similar detection rates of CIN 2-3 lesions. The sensitivity of HPV testing in vaginal self-sampling studies was generally lower than that of cervical direct sampling by clinicians or nurses. The lower sensitivity in self-sampling studies as compared to clinician sampled studies indicate that adequacy of specimen collection is an important determinant of the success of HPV testing. Recently an international working group of the International Agency for Research on Cancer (IARC) concluded that there is sufficient evidence that HPV testing can reduce mortality from cervical cancer. HPV testing is a promising approach with the highest reproducibility among all cervical screening tests. However, it is costlier (20-30 US$) than other screening tests and requires sophisticated laboratory infrastructure including testing equipment, trained technicians and storage facilities for samples. Future developments such as less expensive, and faster testing are essential for HPV testing to be feasible in low-resource settings.

PO-28

Genomic Gains and Losses in Diffuse Large B-Cell Lymphoma by Array Comparative Genomic Hybridization: Clinical Outcome Correlations and Target Gene Discovery

R. S. K. Chaganti**, Seeta R. Chaganti, Weiyi Chen

Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10021, USA. *chagantr@mskcc.org

Conventional karyotype and chromosomal comparative genomic hybridization (CGH) studies of diffuse large B-cell lymphoma (DLBCL) have revealed few chromosomal abnormalities associated with outcome. In order to evaluate the association between genomic copy number changes in DLBCL and clinical outcome at a higher resolution, we assayed a panel of 64 newly-diagnosed DLBCL specimens by array-CGH. For each specimen, the respective patient had a known response to anthracycline-based therapy, and the median follow-up was 5-years. Forward and reverse hybridizations were performed for all specimens to avoid dye-bias, using BAC/PAC arrays with 1-4 Mb resolution coverage of the genome (Spectral Genomics). After customized normalization, the circular binary segmentation (CBS) algorithm was used to identify regional copy number changes along each chromosome in each specimen. Of the 64 specimens, 60 (93.7%) displayed regional copy number changes. The frequency of gain/loss of each clone for all 64 specimens was then calculated based on the CBS results, and, additionally, based on singleton clone changes if they were outside of CBS-defined regions. Clones displaying change in 10% of specimens for at least two contiguous clones were further considered as recurrent sites for clinical correlations. 54 sites of recurrent gain and 38 sites of recurrent loss were identified. Association between loss or gain of sites and International Prognostic Index (IPI) were evaluated using Fisher s exact test, and associations between loss or gain of sites and time to treatment failure (TTF) and overall survival (OS) were tested using the log-rank test. In a univariate analysis, 16 chromosomal regions (indicated in parentheses as Mb intervals from the p telomeric end) showed significant ($p \leq 0.05$) correlation with clinical outcome. Among these, 4 predicted adverse outcome whereas 12 predicted favorable outcome. Gain of chromosome 13 (85-91.9) and loss of chromosome 16 (33.8-35.6) were associated with both adverse TTF and OS. Gain of chromosome 6 (0.1-5.9) and loss of chromosome 2 (2.4-4.1) were associated with adverse TTF and OS, respectively. Favorable TTF was associate with gain of chromosomes 3 (138.4-188.7), 9 (122.1-132.8), and 19 (43.1-63.7), and loss of chromosomes 1 (78.2-79.1), 4 (24.9-34.7), 6 (62.2-170.5), 7 (18.8-19.2), 9 (8.3-12.5), 10 (107-120), and 15 (41.2-45.5). Favorable OS was associated with gain of chromosomes 3 (0.2-204.6), 9 (122.1-132.8), 19 (43.1-63.7), and 20 (22.5-63.6), and loss of chromosomes 1 (78.2-79.1) and 4 (24.9-

34.7). Gain of chromosome 3 (138.4-188.7) was the only marker significantly associated with lower IPI. In a multivariate analysis after stratifying by IPI, using the stratified log-rank test, loss of chromosomes 7 (18.8-19.2), 1 (78.2-79.1), 2 (2.4-4.1), and 16 (33.8-35.6) and gain of chromosome 9 (122.1-132.8) provided significant extra contributions in predicting clinical outcome. Notably, 7 of the sites were £5 Mb, facilitating the identification of target genes. In summary, array-CGH has lead to the identification of gain or loss at several novel chromosomal regions with prognostic significance in DLBCL, which in some cases are of a size amenable for target gene identification.

PO-29
Molecular Stratagies for Cancer Drug Development

Rama Mukherjee*

Dabur Research Foundation, Site 22, Sahibabad, India. *ramam@dabur.com

PO-30
Understanding Human Gliomas by Proteomics Approach

Ravi Sirdeshmukh*

Centre for Cellular and Molecular Biology, Hyderabad 500 007, India. *ravi@ccmb.res.in

Gliomas are the most common primary brain tumors. We have studied protein profiles of astrocytomas - a histological sub type, by 2-DE/MS approach and examined differentially expressed proteins as useful molecular indicators to understand these tumors. We identified 72 distinct, differentially expressed proteins belonging to various functional groups, 29 of which were short listed for consistent differential expression and may have a role in their pathology. Some were found to be differentially expressed in both Gr III and IV astrocytomas, while others were associated with a particular grade. These proteins can be further explored as individual markers or as a set of markers for astrocytoma. Some notable observations were, under expression of Prohibitin - a potential tumor suppressor protein, Rho-GDP dissociation inhibitor, (Rho-GDI) - a regulator of Rho GTPases and heat shock proteins (HSPs) as well as destabilization of glial fibrillary acidic protein, GFAP - a major protein of the glial filaments, in Gr these malignant tumors. We attempt to explain glioma malignancy and progression in terms of their combined role. The molecular species pattern of GFAP can be considered as a useful indicator for differentiating the types of gliomas and grades of astrocytomas. Further, destabilization of GFAP may be associated with its phosphorylation at hitherto unknown Thr sites and we implicate the role for a specific kinase and a protease in generating GFAP molecular forms in these tumors.

PO-31
Preliminary Investigations on HPV and its Association with Esophageal Cancer

Robin Mukhopadhyaya*

Virology Lab, Tata Memorial Centre-Advanced Centre for Treatment, Research & Education in Cancer (ACTREC), Kharghar, Navi Mumbai - 410 208, India. *rmukhopadhyaya@actrec.res.in

HPV association has been documented in esophageal squamous cell carcinoma [EC] by several studies in high-risk areas for the occurrence of the said malignancy. In this study we are trying to evaluate i] incidence of high-risk oncogenic variants of HPV in EC biopsies, ii] the anti-HPV status in those patients, specifically high-risk oncogenic variant HPV-16 antibody, and correlate the serology with molecular studies to profile HPV association with EC in India. In the preliminary investigations using multiple PCR on endoscopic EC biopsies, a significant prevalence of the highly oncogenic HPV-16 was documented. To simultaneously assay the viral DNA and anti-HPV-16 antibody in a larger sample size, an in house ELISA is being developed. Towards this end, immunoreactive rHPV-16 L1 major capsid protein has been expressed in yeast, VLP made and ELISA carried out. We also expressed immunoreactive His-tagged rHPV-16 L1 protein derived capsomeres and carried out ELISA using the same. Significance of this study, on small sample number, will be discussed.

PO-32

E-cadherin/b-catenin: Their Role in Intercellular Adhesion of Metastatic Prostate Cancer Cells in Bone

Baisakhi Saha[1], Ahren Arase[1], Sarah S. Imam[1], Denice Tsao-We[2], Susan Groshen[2], Wesley Y. Naritoku[3], Ram H. Datar[3], Lawrence W. Jones[1], S. Ashraf Imam[1]*

[1]Huntington Medical Research Institutes, Pasadena, CA, and Depts of [2]Preventive Medicine and [3]Pathology, USC Keck School of Medicine, Los Angeles, CA, USA. *imam@hmri.org

Background: The development of strategies for the treatment of patients with metastatic prostate cancer requires the understanding of mechanisms of cellular adhesion and growth of cancer cells in bone, which is the most prevalent site of prostate metastasis. In normal prostate, the cytoplasmic domain of a critical intercellular adhesion molecule, E-cadherin, is linked to the actin cytoskeleton via its interaction with a-, b-catenins and p120 for the maintenance of cellular polarity and differentiation of normal epithelial cells. In contrast, lost or reduced membranous expression of E-cad./ b-cat. protein with concurrent hypermethylation of E-cad. gene has been reported to be associated with invasive prostate cancer cells, thereby allowing their detachment and migration from the primary site in prostate. However, the role of these interrelated cellular adhesion proteins in cellular adhesion of metastatic cancer cells in bone remains poorly understood. **Aims and Methods:** The aim of this study was to evaluate the E-cad./ b-cat. protein expression by immunohistochemical (IHC) staining method and the methylation status of E-cad. gene by methylation specific-PCR in prostate cancer cells at the primary site in prostate and metastatic site in bone or lymph node. **Results:** In benign prostate hyperplasia (BPH), the high percent (>50%) of cells with membranous expression of E-cad. and -cat. protein was observed in 91% and 82% of cases respectively, whereas the rest of the cases exhibited low percent (6-50%) of expression of both proteins. In contrast, the frequency of high percent of membranous expression of E-cad. and b-cat. protein reduced to 18% and 27%, respectively, while a low percent of expression was found in the remaining cases of patients with primary prostate cancer. There were statistically significant differences between BPH and primary prostate cancer in terms of the E-cad./ b-cat.-positive cells (Fischer's Exact $p<0.0001$ for E-cad. and $p=0.008$ for b-cat.). Moreover, a statistically significant association was observed between low membranous expression of E-cad. protein and hypermethylated E-cad. gene in the primary prostate cancer (Fischer's Exact $p=0.005$). Surprisingly, metastatic prostate cancer cells in bone exhibited high membranous expression of E-cad. and b-cat. protein in 88% and 82%, respectively, and low membranous expression in the remaining cases. The results showed a statistically significant difference from that of the primary site (Fischer's Exact $p<0.001$ for E-cad. and $p=0.001$ for b-cat.). Furthermore, E-cad. gene was found to be unmethylated in all of the case of metastasis, revealing its significant association with high expression of E-cad. protein (Fischer's Exact

p<0.001). **Conclusions:** The consistent membranous expression of both E-cad. and b-cat. proteins and concurrent unmethylated E-cad. gene in metastatic prostate cancer cells in bone suggest their functional role in the maintenance of cellular adhesion of cancer cells in bone. Indeed, this is a paradoxical observation that challenges the present paradigm that E-cad. protein is a metastatic suppressor gene's protein.

PO-33
Screening in Breast Cancer and Cervical Cancer - Is it Cost Effective?

S. H. Advani*

Medical Oncology, Jaslok Hospital and Research Centre, Mumbai. *shadvani2000@yahoo.com

Breast cancer is the most frequently diagnosed cancer and is the second leading cause of cancer death among women in India. Mammography is perhaps the best screening tool for detecting early breast cancer. Estimates of mammography sensitivity range from 75% to 90% with specificity from 90% to 95%. The positive predictive value of mammography for breast cancer ranges from 20% in women under age 50 to 60% to 80% in women age 50-69. Randomized clinical trials (RCTs) have demonstrated a 30% reduction in breast cancer mortality in women 50-69 years who are screened annually or biennially with mammograms. The data on women under age 50 are less clear. Cost-effectiveness estimates of mammography screening vary widely. Recommendation for women age 40-49 is every 1-2 years and annually after age 50. Though majority of the patients with cervical cancer are young, 25% of the cancers occur in women over age 65. The Papanicolaou (Pap) smear is used to screen for cervical cancer. The lead time to develop invasive cancer is estimated at 8-9 years and early detection could be highly beneficial. Regular triennial screening would achieve 91%-96% of the benefit of annual screening. Screening is more cost-effective for women over age 65 with a history of inadequate screening. Efforts should be made to test women who have not undergone regular testing. No randomized controlled trials to test the effectiveness of Pap smears for prevention of cervical cancer have been conducted, however, case-control studies have clearly demonstrated that women with invasive cervical cancer were less likely to have been screened.

PO-34
Innate Immune Response to Tumors: Role of gdT and NKT Cells

S. V. Chiplunkar*

Immunology, Advanced Centre for Treatment, Research & Education in Cancer, Tata Memorial Centre, Kharghar, Navi Mumbai-410208. *schiplunkar@actrec.res.in

The importance of innate immune mechanisms in controlling viral infections, cancer and autoimmunity is currently an area of intense research. Recently, innate immune lymphocytes NK, natural killer T (NKT) and gdT cells have garnered much attention and their biological significance in the tumor immunity, allergic diseases and infectious diseases is being extensively exploited. These cells help participate in bridging the innate immunity with antigen specific acquired immune responses. gdT cells and NKT cells differ from conventional abT cells with respect to their tissue localization, TCR gene usage and antigen recognition. In addition to intact proteins and peptides, soluble nonpeptidic antigens and glycolipids are recognized by T cells and NKT cells, respectively. Substantial evidence suggests that gd T cells and NKT cells represent important players in the immune system

arsenal of effector cells with potential anti tumor activity. CD1d reactive NKT cells that express the invariant V_{a24}-V_{b11} T cell receptor were enriched from peripheral blood of patients with cervical cancer after stimulation with PBS-14 pulsed dendritic cells. The immunomagnetically purified NKT cells showed potent tumor directed cytotoxicity. Similarly we investigated how Vg_9/Vd_2 T cells enriched from peripheral blood of patients with oral squamous cell carcinoma and stimulated with phosphoantigens can effectively lyse tumor cells. The presentation would focus on dissecting the events leading to activation, regulation, migration and death of these effector cells and their significance in tumor immunity. Potential strategies for immunotherapy of tumors, based on the activation of these effector cells will be discussed.

PO-35

Microarray Analysis of Gene Expression Profiles in Human Papillomavirus-Associated Cervical and Oral Cancers

Saleem A. Khan*, Ivan Martinez, Kenosha Hobson and Robert L. Ferris

Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA. *khan@pitt.edu

A causative role of HPVs in cervical intraepithelial lesions, including cervical carcinoma has been firmly established. DNA of high-risk HPVs such as types 16 and 18 is usually present in an episomal state in benign and premalignant lesions, but is frequently integrated into the genome in cervical carcinomas and in cell lines derived from such lesions. HPVs also appear to be one of the factors that contribute to the development of squamous cell carcinoma of the head and neck (SCCHN) in approximately 25% of the cases, particularly in the oropharynx. The E6 and E7 oncoproteins of high-risk HPVs are involved in cellular transformation. The E6 protein promotes polyubiquitination and proteasomal degradation of the cellular tumor suppressor proteins p53 and DLG. E6 is also known to interact with a number of other cellular proteins and activates telomerase. The E7 protein acts in concert by binding and inactivating the function of the pRB and related p107 and p130 proteins. E7 also interacts with additional cellular proteins such as TBP, histone H1 kinase, cyclin E, etc. The E6 and E7 proteins are also known to significantly alter cellular gene expression and promote chromosomal destabilization, foreign DNA integration and other mutagenic events in the cell. These events, possibly in combination with other cofactors or co-carcinogens, lead to the development of HPV-associated cancers. In order to better understand the early steps in HPV-associated carcinogenesis, we have studied global changes in cellular gene expression profiles in different HPV 16 and 18 positive cell lines (harboring extrachromosomal or integrated viral genomes) using the high density oligonucleotide U133A GeneChip® (Affymetrix) that contains approximately 22,000 human genes. Data analysis using various statistical tests showed that approximately 1600 genes were differentially expressed in all HPV-positive cell lines as compared to the normal cervix, and approximately 1200 genes were differentially expressed in all the HPV-positive cell lines as compared to the HPV-negative cell line C-33A. The above analysis also identified 140 cellular genes whose expression was specifically altered in the presence of HPVs. The results of the microarray analysis were validated by quantitative RT-PCR analysis of a representative number of differentially expressed genes. Using microarray analysis, we also identified 200 up-regulated and 130 down-regulated genes that showed $\geq$ 3-fold change in expression in HPV-positive oropharyngeal tumors as compared to the normal oral mucosa. The up-regulated genes included many that are involved in DNA replication, DNA repair, cell cycle progression and signal transduction. The down-regulated genes included those involved in DNA repair, adhesion, immune response, and apoptosis. The ultimate goal of our studies is to identify tumor markers and classify HPV-positive cancers by molecular profiling that can be of diagnostic and prognostic value. Furthermore, such studies may also identify potential new targets for anti-cancer therapy.

PO-36
The Potential Impact of HPV Prevention Worldwide

Sonia R.Pagliusi* *and Teresa Aguado*

Initiative for Vaccine Research, World Health Organization, Avenue Appia 20, 1211 Geneva 27. *pagliusis@who.int

Human papillomavirus (HPV) infections are associated with a wide spectrum of mucocutaneous diseases ranging from benign skin warts through different grades of pre-cancer to invasive cervical cancer, which is the second most common cancer among women worldwide. While over 90% of genital HPV infections are transient and clear spontaneously without treatment, causing no clinical symptoms, invasive cervical cancer is an uncommon consequence of HPV infection in women. Nevertheless, cancer of the cervix, with an estimated 493,000 new cases and 274,000 deaths in the year 2002 is the major cause of cancer mortality among women in developing countries. Cervical cancer is preventable for two main reasons: First, because it is caused by a infectious agent, which can be averted. Secondly, it develops over many years offering several opportunities for interventions aimed at preventing progression to disease. Thus, effective prevention can be divided into two categories: primary and secondary prevention strategies. Primary prevention strategies are interventions aimed at avoiding or reducing the exposure to the infectious agent(s) and risk factor(s) causing the disease. These are generally based on behavioural, environmental or biological changes, including prophylactic vaccination, that renders the infection innocuous. Secondary prevention strategies are aimed at early detection and timely treatment or removal of recognized early clinical signs of disease, before it comes to a malignant or invasive stage, and these are mostly based on advancements in screening tests, diagnostics and health technology. Combined strategies for primary and secondary prevention are likely to offer the most effective approach for cervical cancer control over the next decade. With the advancements in vaccine development better primary prevention strategies can now be designed and implemented. Defining delivery strategies and finding the best combination of primary and secondary prevention programs will be the challenge.

PO-37
Aurora Kinases in Chromosomal Instability and Cancer

Subrata Sen*

Department of Molecular Pathology, University of Texas M.D. Anderson Cancer Center, Houston 77030 U.S.A. *ssen@mdanderson.org

Aurora kinases representing a novel family of serine/threonine kinases have been identified as key regulators of the mitotic cell division process that are frequently over expressed in many human cancers. The three members of this kinase family, Aurora-A, Aurora-B and Aurora-C kinases, expressed and activated at highest levels during G2-M phase, are known to be involved in the regulation of centrosome function, bipolar spindle assembly and chromosome segregation processes. Elevated expressions of these kinases have been correlated with chromosomal instability and clinically distinct grades and stages of human cancers. Studies from our laboratory and those of others have revealed that while Aurora-A appears to play critical roles in the early as well as late stages of mitosis through its interactions with several key oncogenic and tumor suppressor proteins, Aurora-B and -C, on the other hand, are chromosomal passenger proteins that are involved in the regulation of chromosome congression, segregation and cytokinesis. Additionally, Aurora-A kinase influences DNA damage

response checkpoint pathway proteins while Aurora-B and -C kinases influence the spindle checkpoint pathway proteins, thus revealing that the Aurora kinase family not only regulates mitotic cell proliferation but also the two major cellular damage response pathways. Recent findings further demonstrate that Aurora kinases in addition to modifying their individual substrates also cross talk among themselves to regulate mitosis regulatory pathways. Therefore in addition to elucidating vertical regulatory cascade of each Aurora kinase, it would be important, in the future, to understand how cross talking among Aurora kinases are coordinated during normal cell cycle. This would provide useful knowledge for developing therapeutic strategies for human cancers associated with over expression of Aurora kinases. In this presentation, we will discuss the cellular pathways involving Aurora kinases critical in the development of chromosomal instability and malignant transformation in human cells.

PO-38
Integrating Notch and EGFR/ ErbB2 Signaling in Human Cervical Cancer Progression

Pradip Nair, Bharathi Ramdas, Senthil Kumar and Sudhir Krishna*

National Centre for Biological Sciences, TIFR, GKVK Campus, Bangalore -65. *skrishna@ncbs.res.in

Introduction: We are interested in understanding the relationship between Notch and EGFR pathways in the context of human cervical cancer. **Objectives:** We examine the role of EGFR signaling in mediating the pro-oncogenic phenotypes mediated by Notch signaling in the context of human cervical tumors. **Methods:** This study principally combines an immunocytochemistry of human pre-neoplastic and neoplastic lesions with an analysis of human cervical tumor derived cell lines. In human cervical tumor derived lines, the levels of various EGFR family members was estimated along with the phosphorylation status of EGFR. In parallel, we determined the status of phosphorylated Akt, STAT3 and ERK with and with out specific inhibitors of EGFR, src and ErbB2. Additionally, immunoprecipitates and co-localization experiments of EGFR, src and ErbB2 were undertaken. Following introduction of activated Notch1 alleles (AcN1) in HaCaT cells, these cells were analysed for levels of ErbB2. **Results:** In a limited analysis, human cervical cancers in parallel show upregulated Notch, ErbB2, STAT3 with widespread EGFR expression. Dominant negatives or chemical inhibitors of src block EGFR phosphorylation at tyrosine 84 but not PI3Kinase in CaSki cells. However an ErbB2 inhibitor blocks both STAT3 and PI3kinase. ErbB2 expression is blocked by gamma secretase inhibitors in CaSki cells and induced by AcN1 in HaCaT cells. **Conclusion:** These experiments define a linkage between Notch and EGFR/ErbB2 signaling in human epithelial cells. This information is being integrated with studies undertaken in Apurva Sarin's laboratory on T cells and our data showing a role for deltex in AcN1-P3K activation.

PO-39
Early Onset Breast cancer and Genetic susceptibility in Indian Women

Sunita Saxena*

Institute of Pathology (ICMR), Safdarjang Hospital Campus, New Delhi. *sunita_saxena@yahoo.com

Breast cancer is one of the leading causes of cancer death among Indian women. One in fifty eight women are affected by breast cancer in the age group of 30-70 years and are mainly from urban areas. Although the over all incidence rate of breast cancer in Indian women is not as high as in western countries (23.5 vs 90.7), the incidence

of early onset breast cancer cases (< 40 years) does not show significant variation as compared to population worldwide (12-33 per 100,000 women); suggesting that a greater proportion of all breast cancers is due to early-onset disease in Indian population. Since familial cancer cases often present at an early age in contrast to that of sporadic cancer, genetic factors are considered to be playing far greater role in conferring cancer susceptibility. Following the identification of breast and ovarian cancer susceptibility genes BRCA1 (MIM 113705) and BRCA2 (MIM600185), the frequency and spectrum of disease related mutations have been investigated in North Indian population. We present family history data and molecular analysis from high-risk group of patients for breast cancer. Screening for mutations in coding and intron and exon boundaries of BRCA1 / 2 genes has been studied in 204 breast cancer patients and 50 controls. The study group included 155 (75%) early onset cancer cases (<45 yrs); 48 (23.5%) familial cases, 11 (5.3%) cases with bilateral breast cancer and 8 (3.9%) cases having both breast and ovarian cancer. Total 21 sequence variants were noticed in 25 patients including 3 frame shift (FS), 5 missense (MS), 3 Splice Sites (SS) and 2 Nonsense (NS) in BRCA1 and 1 FS, 5 SS, 1 MS and 1 NS in BRCA2 genes. 66.6% BRCA2 variants were associated with early onset condition while 22.2% with family history. In case of BRCA1 variants again association with the early onset condition was found significantly high (87.5%) as compared to that of family history (18.7%). Genetic susceptibility to cancer is triggered in several ways. The most common mechanism involves uncontrolled cell division due to germline mutations in tumor suppressor genes and DNA repair genes, which ultimately lead to accumulation of mutations in major oncogenes. Breast cancer arising in women with and without a germ line mutation in BRCA1 and BRCA2 gene display different molecular characteristics suggesting unique mechanism of molecular pathogenesis. Molecular pathological analysis of these tumors has been done to define the genetic abnormalities relevant to this specific pathogenesis. Tumor material has been studied from 138 women with breast cancer, 61 having early onset,36 familial cases and 41 having late onset and 15 showing germ line BRCA mutations. The expression of p53, p- glycoprotein ,and E-cadherin revealed statistical significant differences among the various groups of patients. Expression of C-erb2 oncoprotein was also found different in early and late onset Familial cases with Odd ratio of the order of 4.5.

PO-40

Protykin: A Future Medicine for Chemotherapy in Patients with Advanced Breast Cancer

Sushanta K. Banerjee*

Cancer Research Unit, VA Medical Center, Kansas City, MO and Department of Hematology and Oncology, University of Kansas Medical Center, Kansas City, Kansas, USA. *skbanerj@kuhub.cc.ukans.edu or sbanerjee2@kumc.edu

Protykin® is an extract of Polygonum cuspidatum roots, an herb used in traditional Chinese medicine. It is a phytoestrogen (natural plant estrogen) and contains more than 1,000-times the amount of the same antioxidant ingredient in red wine that is believed to promote cardiovascular health and reduce the effects of premature aging. Protykin binds with estrogen receptor- alpha (ER-) and enhances estrogen-like activity in the body, without causing any side effects, which are generally associated with synthetic hormone replacement therapy (HRT). Protykin extract also contains 50% trans-resveratrol, a compound found largely in the skins of red grapes and an oriental medicine used to treat diseases of the blood vessels, heart and liver. Resveratrol may be a powerful cancer-fighting ally. These unique features tempted us to Protykin can be considered as a novel chemopreventive agent in women with high risk of breast cancer and has promising implications for the use of Protykin as a treatment in women with this disease. To test the hypothesis we: 1. determined whether Protykin is able to modulate the breast tumor cell proliferation in vitro, and 2. determined if Protykin-riched diets suppress the tumor growth in nude mice and if so, what molecular events would be involved in this process. BrdU-ELISA assay showed that like other chemopreventive agents Protykin inhibited cellular proliferation both ER-positive (MCF-7) and ER-negative (MDA-MB-231) breast

tumor cell with a maximum inhibitory effect at concentrations close to 50 mg/ml. However, the effect was vigorous in ER-negative MDA-MB-231 metastatic cells. These in vitro results substantiate the in vivo studies and indicate that the growth of MDA-MD 231 breast tumor cell xenografted into athymic nude mice can be suppressed by Protykin when fed Protykin-riched (50-100mg/kg body wt) liquid diet. Moreover, the studies also indicate that protykin-induced suppression of tumor growth is mediated through the inhibition of angiogenic switch via modulation of positive and negative regulators of angiogenesis. Together these studies suggest that Protykin can be used as a potent anticancer drug. Further studies are warranted.

PO-41
Transcription Regulation through Chromatin- A New Target for Anti- Cancer Therapeutics

Tapas K. Kundu*

Transcription and Disease Laboratory, Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, Bangalore- 560064, India. *tapas@jncasr.ac.in

Human genes are organized into a highly compact and dynamic nucleoprotein complex called chromatin, which consists of histones and associated non- histone proteins. Though apparently repressive, the precise organization of chromatin is essential for all the DNA- templated phenomenon inside the cell. Alteration in chromatin organization modulates the expression of underlying genes. The dynamic changes in chromatin structure are brought about by post- translational modifications of chromatin proteins (both histones and non- histones), ATP- dependent chromatin remodeling and histone chaperones. Dysfunction of any of these machineries are causally linked to several diseases, predominantly cancer. Therefore, chromatin- regulated/ modulated gene expression is a new target for anti- neoplastic therapeutics. We focus on understanding the mechanism of chromatin transcription in humans and also searching for the small molecule modulators of histone modifying enzymes (HAT, HDAC and HMTases), which may serve as lead compounds to design novel anti- neoplastic therapeutics. We found that the multi- functional human transcriptional coactivator PC4 enhances p53 function by enhancing its DNA binding and thereby inducing the expression of Bax, a p53 responsive pro-apoptotic gene. Our recent findings suggest that the human histone chaperone nucleophosmin, whose expression dramatically increases in several cancers and upon DNA damage, enhances the acetylation-dependent chromatin transcription and also p53- driven gene expression in vivo. Interestingly, both PC4 and B23 get acetylated and presumably their function is regulated by this post- translational modification. We have discovered several small molecule modulators (activators and inhibitors) of HATs, HDACs and HMTases and their effect on gene expression is being studied in vitro and in vivo. These modulators are also capable of altering the acetylation of non- histone proteins like PC4 and B23. These molecules may serve as lead compounds in the synthesis of anti-neoplastic therapeutics.

PO-42
ATM, Telomeres and Cancer

Tej K. Pandita*

Washington University School of Medicine, St Louis, MO 63108, USA. *pandita@radonc.wustl.edu

Signal transduction pathways activated by DNA damage are critical determinants of cell survival and cellular transformation. A number of genes encode proteins that may be capable of sensing DNA damage.

One of these genes, ATM (ataxia-telangiectasia mutated), appears to be a major regulator of cellular responses to ionizing radiation (IR). ATM is a protein kinase that is activated by IR and phosphorylates a number of different substrates following activation. We have shown that the ATM gene product influences telomere metabolism. A hypothesis explaining such results is that defective telomere maintenance in A-T cells could be due to altered interactions between the telomeres and the nuclear matrix. Consistent with this hypothesis is that telomere nuclear matrix interactions and nucleosomal periodicity are altered in A-T cells. However, the precise mechanism by which ATM regulates the structure and function of telomeres is not known. Some of the common metabolic abnormalities, such as poor growth and IR sensitivity, have been linked with lack of ATM as well as loss of telomeres. The restoration of the telomere length by ectopic expression of catalytic subunit of telomerase (hTERT) in cells deficient for ATM function does not correct the telomere chromatin defect and other cellular phenotypes of the A-T cells, suggesting that ATM is essential for the signaling of telomere mediated functions. The ATM protein is associated with chromatin and telomeres are packaged in telomere specific chromatin. Since the chromatin defect in A-T cells is well pronounced at telomeres, which are heterochromatic, we, therefore, attempted to search for the ATM interacting proteins that have chromodomain region(s). Chromodomain proteins appear to be structural components of a macromolecular chromatin complex and are also involved in remodeling chromatin structure. We will discuss the role of ATM interacting chromatinmodifying factors in genomic stability, telomere metabolisms and oncogenic transformation.

PO-43
Genetic Pathways in Cervical Cancer Progression

Vundavalli V. Murty*

Department of Pathology, Room P&S 14-460, Institute for Cancer Genetics, College of Physicians & Surgeons of Columbia University, 630 West 168th Street, New York, New York 10032. *vvm2@columbia.edu

Carcinoma of the cervix uteri (Cervical Cancer; CC) exhibits a multitude of complex karyotypic alterations suggesting deregulation of numerous genes critical in the tumor formation and progression. The molecular basis of this genomic instability is poorly understood. Our goal is to characterize these genetic changes in invasive CC and through various stages of precancerous lesions using high-throughput methods. We identified a number of chromosomal and genetic changes including a) dosage alterations of loss, gain, or amplifications; b) specific patterns of gene expression profiles; and c) identification of epigenetic signatures. We have also shown that a number of pathways such as FANC/BRCA, SLIT-ROBO, RARB, and MMP play a role in CC tumorigenesis. The importance of these observations will be discussed in relation to tumor progression and identification of biomarkers in the management of cervical cancer.

PO-44
Induction of Caspase-9 Expression Co-operating with p53-Induced Apoptosis in Human Lung Cancer Cells

[1]Yoshihito Gomyo, [1]Ji-ichiro Sasaki, [1]Cynthia Branch, [1]Jack A Roth and [2]Tapas Mukhopadhyay*

[1]M.D. Anderson, Cancer Center, Houston, Tx -77030. [2]National Center for Human Genmome Studies and Research, Panjab University, Chandigarh 160014. *tmukhopa@hotmail.com

Low-dose of 5-aza-deoxycytidine (DAC) caused an accumulation of procaspase-9 through mRNA up-regulation, but the cells did not undergo apoptosis. However, when cells were treated with DAC and infected with a low dose of a recombinant wild-type p53 adenovirus vector (Ad-p53), a synergistic growth inhibitory effect was observed. Combination treatment induced Apaf-1 and procaspase-9 expression in which cytochrome c releases by Ad-p53 triggered the mitochondrial pathway of apoptosis. DAC sensitized lung cancer cells to cisplatin and paclitaxel. DAC treatment may have clinical implications when combined with chemotherapy or apoptosis-inducing gene therapy.

PO-45
Prognostic and Predictive Markers in Carcinoma Cervix

T. Rajkumar*

Department of Molecular Oncology, Cancer Institute (WIA), Adyar, Chennai 600 020. *caninst@md2.vsnl.net.in

PO-46
Clinical Development of Merck's Quadrivalent

Elaine C. Esber*

Merck Vaccine Division, Merck & Co. Inc. Sumneytown Pike, West Point PA USA *elaine_esber@merck.com

PO-47
Early detection of cancer cervix in resource-poor country

Kusum Verma

Department of Pathology, All India Institute of Medical Sciences, New Delhi, India.

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INVITED ORAL PRESENTATIONS

IO-1
Genetics of Human Hand-Use Preference, Homosexuality, Schizophrenia and Bipolar Traits

Amar J. S. Klar*

NCI-Frederick Cancer Research Facility, Frederick, Maryland 21702-1201 USA.*klar@ncifcrf.gov

The causes of schizophrenia and bipolar human psychiatric disorders are unknown. A novel somatic cell genetic model postulated non-random segregation of differentially and epigenetically modified "Watson" vs. "Crick" DNA chains of both copies of a chromosome to specific daughter cells. The model is based on the inherent non-equivalence of DNA chains according to the Watson and Crick's double helix model. As a consequence, one daughter cell inherits an activated gene in both homologs, and the other inherits both epigenetically silenced "epialleles". Such an oriented asymmetric cell division in embryogenesis causes development of healthy, functionally non-equivalent brain hemispheres. Genetic translocations of the chromosome that can be identified cytologically may cause disease by disrupting the process of biased strand segregation. This way the epialleles will be randomly distributed to sister cells such that symmetrical brain hemispheres should develop in 50% of translocation carriers causing psychosis. Accordingly, 50% of chromosome 1 and 11 translocation carriers developing disease were recently explained as a result consistent with the model. Is chromosome 1 or 11 involved? Is the disease caused by a conventional mutation at the breakpoint? Remarkably, two unrelated chromosome 11 translocations searched from the literature caused disease also in ~50% persons. Moreover, their breakpoints lie at three distinct regions, spanning ~40% of chromosome 11. Thus, chromosome 11 is implicated in psychosis, but the breakpoints themselves are unlikely to cause the disease. The results suggest that the genetically caused disease develops without a mutation, and advance the idea that the main function of epigenetic control is for cellular differentiation. Studies implicating single gene with two alleles for specifying right versus left hand-use preference, homosexuality (Excess of anticlockwise parietal hair whorls in homosexual men, Journal of Genetics. December 2004, in press), schizophrenia and bipolar traits (A genetic mechanism implicates chromosome 11 in schizophrenia and bipolar diseases. Genetics 167: 1833-1840) will be presented. The RGHT1 gene is proposed to cause patterned distribution of chromosome 11 chains.

IO-2
Interplay of CD8 T Lymphocytes and Natural Killer Cells in Immunotherapy of Cancer

***Anil Shanker*[1]*, *Else-Marit Inderberg-Suso*[2], *Anne-Marie Schmitt-Verhulst*[2] *and Thomas J Sayers*[1]**

[1]Basic Research Program, SAIC-Frederick Inc., National Cancer Institute-Frederick, MD 21702-1201 USA. [2]Centre d`Immunologie de Marseille-Luminy, CNRS / INSERM / Université de la Méditerranée, Marseille 13288 France. *shankera@ncifcrf.gov

The relative contribution of CD8 T lymphocytes and natural killer (NK) cells in tumor rejection remains to be fully understood particularly in the case of large tumor burden. We investigated the role of CD8 T lymphocytes

in tumor rejection using a subcutaneous model of the mouse mastocytoma P815 that expressed a "cancer-germline" antigen P1A (P511). The P511 tumor was completely rejected in RAG-$1^{-/-}$B10.D2 mice transgenic for TCR reactive to L^d:P1A (TCRP1A) and their survival was significantly increased as compared to tumor-bearing non-transgenic mice or to a cohort of transgenic mice bearing the P1.204 (P1A-deficient P815) tumor. TCRP1A CD8 T lymphocytes showed antigen-specific migration, proliferation and activation in the tumor-draining lymph nodes. Reconstitution of RAG-$1^{-/-}$ mice with naïve TCRP1A CD8 T cells also afforded efficient resistance to the growth of P1A-expressing tumor. However, upon treatment of these mice with depleting anti-NK1.1 antibody, the rejection of this tumor was abolished, indicating that NK cells contributed to tumor resistance. In addition, NK cell mediated resistance of tumor growth was found to be dependent on antigen-specific CD8 T cells, suggesting cooperativity between NK cells and T cells in successful immunotherapy of this tumor. In further studies, to determine mechanisms of tumor destruction during immunotherapy of solid tumors, we evaluated the subcutaneous growth of a mouse renal carcinoma expressing influenza viral antigen haemagglutinin (Renca-HA) in a battery of wild type (Wt), perforin $(PfP)^{-/-}$, $FasL^{-/-}$ and $Fas^{-/-}$ Balb/c mice. Renca-HA was efficiently rejected in Wt, $Pfp^{-/-}$and $Fas^{-/-}$ mice, but not in $FasL^{-/-}$ mice. This strongly suggests that the FasL-mediated death pathway is crucial for the restriction of Renca-HA *in vivo*. Since death ligand-mediated killing is an important antitumor effector mechanism, we are currently attempting to sensitize tumor cells to death ligand-mediated killing to promote the immuno-destruction of a large tumor burden.

IO-3
Pharmacogenomics and Diagnostics in Human Cervical Cancer

Balasubramanyam Nagarajan*

Cancer Institute, Chennai - 600 020, India. *caninst@md2.vsnl.net.in

We analyzed DNA from cytology specimens with cervicitis /inflammation and biopsy-proven dysplasia and invasive cancer by newly devised 32P-postlabeling/TLC systems that showed DNA adduct spectrum ranging from high polarity to high lipophilicity. Similar DNA adduct profiles were detected in the normal specimens, dysplasia and cancer biopsies, each exhibiting subgroups of polar and lipophilic adducts. However, the burden of each subgroups of adducts varied through pathology. The known oxidative lesion, 8-oxodG, a predominant adduct, decreased progressively from inflammation to dysplasia to invasive cancer. For further characterization of these molecular events, biopsies derived from cervical cancer patients of stage II and III were analyzed by RT-PCR method to detect cytokine mRNA. Different cytokines such as IL-6, IFN_y and TNFa were successfully analyzed and antigen presenting dendritic cells as well. CD4 helper cells are involved in MHC II mediated antigen presentation and divided into Th1 cells that secrete IL-2, IFN-g, TNFa while Th-2 cells release IL-4, IL-5, IL-6, IL-10 and IL-13. To address whether this down regulation of Th1 cytokines is associated with Th2 polarization of immune response or deactivation of intratumoral T cells, we have identified and characterized a group of cervical carcinoma patients with undetectable intratumoral T cell derived cytokine mRNA such as IFN-g, IL-4 and IL-17. Down regulation of IFN-g was observed in 5/ 52 patients and seems to be more frequent in advanced stage II and III tumors than in localized stage I tumors. Decrease of Th1 cytokine was not associated with a Th2 polarization of T cells but rather reflected anergy. By differential staining for dendritic cells (DC) we showed that most tumors contained mature DC located around the tumor, whereas immature DC appeared inside the tumor suggesting a maturation block of dendritic cells Significant correlation between the stromal positivity of IL-6 and IL-8 with macrophages infiltration tend to confirm the macrophages as chief sources of this cytokine. Over expression of tumor IL6 showed poor prognosis.

IO-4

Increased Expression of Insulin like Growth Factor Binding Protein (IGFBP2) in HER2 Overexpressing Breast Cancer Cells: Evidence of a Novel Signaling Crosstalk

Chandan Kumar*

Advanced Center for Treatment, Research and Education in Cancer (ACTREC) - Tata Memorial Center (TMC), Kharghar, Navi Mumbai 410208, India. *ckumar@actrec.res.in

Introduction: HER2 (erbB2/neu) gene amplification and overexpression affects 25-30% of breast cancers and results in aggressive disease which is resistant to hormone therapy and is more likely to recur. HER2 also represents a clinically relevant therapeutic target in HER2 positive tumors. HER2 (erbB2/neu) is a member of erbB family of receptor tyrosine kinases and imparts growth factor independence, colony formation, migration and invasion in overexpressing cells. The gene expression changes mediating the tumorigenic effects of HER2 are relatively less characterized. In an earlier study, genes induced by HER2 overexpression in breast cancer cells were identified using microarrays (Kumar-Sinha C *et al*, Cancer Res. 2003; 63:132-9). A gene associated with HER2 expression and activity in that study is Insulin like Growth Factor Binding Protein 2, IGFBP2, known to be overexpressed in tumors of brain, prostate, ovary and breast etc. Role of IGFBP2 in tumors is implicated through its modulation of IGF signaling as well as speculated to be via novel direct mechanisms, independent of IGF. **Objectives:** We have initiated functional characterization of HER2 mediated IGFBP2 expression in breast cells and the likely physiological implications of this crosstalk in breast cancers. **Methods:** Analysis of relative gene expression by Real-Time PCR and protein levels by Western Blotting and Immunohistochemistry. **Results:** Human mammary epithelial cells stably overexpressing HER2 and HER2-overexpressing breast cancer cell lines were found to be overexpressing IGFBP2 as compared to control cells. **Conclusion:** The likely physiological implications of HER2 over-expression leading to increased expression of IGFBP2 merits further study.

IO-5

Estrogen-induced Carcinogenesis: Importance of Oxidative Stress

Hari K. Bhat*

Department of Environmental Health Sciences, Mailman School of Public Health, 60 Haven Avenue-B1, Columbia University, New York 10032. *hb2009@columbia.edu

Based upon a wide range of animal, epidemiological and clinical data, most investigators agree that estrogens contribute to the development of breast neoplasms but the mechanism of estrogen-induced carcinogenesis is not yet clear. Many studies strongly support the premise that oxidative stress plays an important role in this carcinogenic process. Hydroxy metabolic products of tumorigenic estrogens, such as those of 17b-estradiol (bE2) and diethylstilbestrol (DES), are capable of redox cycling that results in the formation of reactive oxygen species (ROS) and free radicals, and consequently leads to oxidative stress. It has been shown that carcinogenic estrogens are capable of producing more oxidative stress compared with poorly carcinogenic or noncarcinogenic estrogens in vivo as well as in vitro. Data suggest that the oxidant stress potential of estrogens depends on their ability to form catechol estrogens, and that the oxidant potential of estrogens can be correlated with their carcinogenic potential. Oxidative stress plays a key role in estrogen-induced carcinogenesis, as it acts in concert with the estrogen receptor (ER)-mediated signaling pathways, leading to DNA damage, altered expression of

genes critical to the control of cellular proliferation and defense against oxidative stress, and thus contributes to the development of neoplasia. Given the recent inclusion of estrogens in the federal register as known human carcinogens, studies aimed at understanding of the mechanisms involved in estrogen-induced carcinogenesis, have great potential to serve as a beginning to the development of therapeutic measures in treatment of not just estrogen- but in general hormone-induced neoplasia.

IO-6

Increased Recombination and Error-prone End Joining Activities in Cell-free Extracts of Breast Carcinoma

Mercy J. Raman*, Ritu Singh and Anand Kumar*

Department of Zoology, **Department of Surgery, Institute of Medical Sciences; Banaras Hindu University, Varanasi-221005, India. *mercyraman@yahoo.com

Introduction: Mutations in DNA repair genes have been linked with carcinogenesis, establishing their role as 'genome caretaker'. Double-strand break (DSB) repair is the major pathway that maintains genomic integrity and specific roles of BRCA1 and BRCA2 proteins in DSB repair connect DSB repair anomalies and carcinogenesis. However, direct analysis of DSB repair activity in cancerous tissues has not been done so far. **Objectives:** The present study was done to know whether there is any anomaly in DSB repair pathways in cancer/tumor tissues compared with normal tissues. **Methods:** Functional status of DSB repair pathways, homologous recombination and nonhomologous end joining (NHEJ), has been examined performing plasmid based assays in cell free extracts of sporadic breast carcinoma, benign tumor (adenofibroma), and normal breast epithelial tissues from far vicinity. NHEJ junctions were sequenced to find out the molecular mechanism of end joining. Levels of DSB repair proteins and their subcellular distribution were assessed by immunodetection. **Results:** Increase in imprecise NHEJ was observed in carcinoma and the DNA joining sites revealed evidence for long bilateral deletions promoting single-strand annealing of microhomology sequences on either ends. Also, hyper-recombination activity and aberrantly high or low levels of DSB repair proteins were noted in extracts of carcinoma and benign tumors, compared with normal tissues. **Conclusion:** We demonstrate that increased recombination, imprecise joining of DNA ends, and alterations in DSB repair protein levels are associated with breast carcinoma. This is the first direct analysis of DSB repair activity in cancer tissues and it suggests that anomalous DSB repair processes could play a primary role in tumorigenesis and promote further genomic instabilities towards carcinogenesis.

IO-7

The Role of Transforming Growth Factor b (TGF b) in Growth Control and Heritable Disorders of Genitals: a Lesson from Drosophila

Rabin N. Chatterjee* and M. Acharyya-Ari

Genetic Research Unit, Department of Zoology, University of Calcutta, 35, B.C. Road, Kolkata-700 019, India. *rabinchatterjee@redifmail.com

Members of the transforming growth factor and their Drosophila counterpart decapentaplegic (dpp) are potent regulators of cell proliferation, differentiation, migration and apoptosis. Intracellular signaling

cascades triggered by these molecules eventually activate transcription factors of Smad family, which then regulate expression of their respective target genes. We present evidence that sex regulatory mutations, intersex and double sex, can cause tumourous growth by alteration of the TGF b signaling pathway in Drosophila.We find that when intersex gene is mutated all of its target genes for female differentiation are more or less deregulated. Thus, it has been observed that tumor-derived TGF b contributed to tumor growth indirectly by suppressing immune surveillance or stimulating production of angiogenic factors. Tumor cells that have selectively lost their growth inhibitory responsiveness to TGF b but retain another wise functional TGF b signaling pathway may exhibit enhanced migration and invasive behaviour in response to TGF b stimulation.

IO-8
Inositol Hexaphosphate Prevents Prostate Cancer by Modulating Cell Regulators

Rajesh Agarwal*

Department of Pharmaceutical Sciences, School of Pharmacy, University of Colorado Health Sciences Center, Denver, CO 80262, USA. * Rajesh.Agarwal@UCHSC.edu

Deregulation of cell cycle progression is an underlined causal event in the growth and development of every form of cancer including prostate cancer (PCA); the most frequently diagnosed malignancy and the second leading cause of cancer deaths in the males in western population. Since PCA progression is correlated with an increasing age where a vast majority of aged men are considered as "high-risk population", chemoprevention employing dietary agents could be a more successful approach in controlling this deadly malignancy. The prevention of PCA is also highly warranted because none of the currently available treatments is capable of curing PCA. Inositol hexaphosphate (IP6), a dietary constituent, has shown promising efficacy against various cancers, however, limited studies have been done with IP6 against PCA. Together, based on above rationales, in recent years, we focused our attention on identifying the efficacy of IP6 in PCA models, and defining its mechanism of efficacy involving cell cycle regulation. Since PCA growth is initially androgen-dependent that becomes independent following androgen-ablation therapy, studies were done employing both LNCaP and DU145 cells that represent androgen-dependent and -independent PCA models, respectively. Completed studies by us show that IP6 induces cyclin-dependent kinase inhibitor (CDKI) Kip1/p27 and Cip1/p21 levels in human PCA LNCaP and DU145 cells, and that this induction results in their increased interaction with cyclin-dependent kinases (CDKs) and cyclins causing almost complete decrease in the kinase activity of CDKs and cyclins. These effects of IP6 ultimately lead to a decrease in hyperphosphorylated (together with an increase in hypophosphorylated) form of retinoblastoma (Rb) or Rb-related proteins in human PCA cells that causes their increased interaction with E2F leading to a down-regulation of E2F-regulated genes involved in PCA cell cycle progression and growth. Together, a resultant biological affect of these alterations in cell cycle regulators was a G1 arrest and cell growth inhibition by IP6 in both LNCaP and DU145 human PCA cells. In pre-clinical PCA prevention studies, IP6 feeding in drinking water resulted in suppression of hormone-refractory human prostate tumor growth without any adverse effect on body weight gain and diet and water consumption during entire study. Though the underlying molecular events related to cell cycle regulation are yet to be established in these tumor tissues, completed studies show that IP6- treated xenografts have a significant reduction in PCNA (that is one of the E2F target molecules)-positive cell. Based on these completed studies, we suggest that more pre-clinical PCA efficacy studies are highly warranted with IP6 in the models such as TRAMP. Positive outcomes of such studies, in conjunction with those completed by us, are expected to form a strong rationale for a clinical trial with IP6 in prostate cancer patients.

IO-9
Alteration in DNA Adduct Profiles during Human Cervical Cancer Development

Ramesh C. Gupta*, Srivani Ravoori, Manicka V. Vadhanam, and Bala Nagarajan[1]

University of Louisville, Louisville, KY, USA and [1]Cancer Institute, Adyar, India. *rcgupta@louisville.edu

Human papillomavirus (HPV), particularly types 16 and 18, is considered a causative factor for human cervical cancer, but only a small fraction of HPV-infected cases undergo dysplastic changes and invasive cancer. This exemplifies the fact that HPV infection alone may not be sufficient to induce transformation and tumor progression. So the emphasis is on evaluating additional co-factors like inflammation-related oxidative DNA damage. Using new ^{32}P-postlabeling/TLC systems, we have discovered a wide array of novel endogenous DNA adducts in human tissues. This technology, which allows measure changes in adduct profiles during cancer development, is termed "adductomics". Cytology specimens and/or cervix biopsies were collected from normal cervix, and cervix with inflammation, pre-neoplasm and malignancy. Analyses revealed no qualitative differences in the adduct profiles, however, increased body burden of some adducts, including the known oxidative lesion, 8-oxodeoxyguanosine, was observed in cytology specimens with inflammation compared with normal cervix. These data suggest that cervical inflammation is accompanied by induction of DNA damage, and this may lead to gene mutations, and ultimately cancer. We hypothesize that HPV infection, or smoking, or both result in accumulation of DNA damage. Due to susceptibility differences, certain individuals may accumulate higher DNA damage, resulting in higher levels of gene mutations and cancer. Thus, these individuals may be pre-disposed to the disease. Evaluation of early biomarkers of susceptibility, exposure, and effect may, to a greater extent, alter the conventional approaches to risk assessment and pave the way for strategic planning of chemopreventive and therapeutic interventions.

IO-10
Implications of p53 Polymorphism and Cervical HPV Viral Load during Pregnancy

Sarmistha Bandyopadhyay and Ramdas Chatterjee*

Department of Tumor Virology, Chittaranjan National Cancer Institute, 37 S P Mukherjee Road, Kolkata 700026. *ramdas_chatterjee@vsnl.com

Pregnancy may be a risk factor for cervical HPV infection due to associated increased hormonal level and immunosuppression. Influence of P53 polymorphism on such risk was investigated. To assess the risk, presence of HPV and its load was also evaluated in pregnant and non-pregnant women in comparison to cervical cancer cases. Overexpression of Arg/Arg homozygosity was observed in HPV positive cervical cancer as well as pregnant specimens. Prevalence of such homozygosity was independent of physical status of HPV in the samples. However, Pro/pro homozygosity was more frequent in samples with episomal form of HPV. Though high viral load was more frequent in cervical cancers median viral copy number/cell did not vary much between pregnant and non-pregnants. However, pregnants had more of moderate level HPV load unlike the low level among the non-pregnant women. High load was associated with presence of HPV type 16 in cancer cases. Such type specificity with viral load was not observed in pregnant/non-pregnant samples.

IO-11

Tumor Suppressor SMAR1 Acts as a Transcriptional Repressor of Cyclin D1 and Viral Promoters

Samit Chattopadhyay*

National Center for Cell Science, Ganeshkhind, Pune 411 007, India. *samit@nccs.res.in

Objectives: Cyclin D1, a cell cycle regulator of G1 progression has been shown to play an important role in the pathogenesis of several ovarian cancers. We have identified a MAR binding protein SMAR1 that regresses tumors through direct interaction with p53 and also repress Cyclin D1. It acts as a transcriptional silencer for various other promoters through recruitment of HDAC-1. Recently we have shown that SMAR1 recruits another repressor protein Cux/CDP that binds to a silencing element and suppress E6 promoter of HPV. **Methods & Rsesults:** We demonstrated that SMAR1 interacts with HDAC1, SIN3 and pocket Rbs to form a multiprotein repressor complex. It also causes deacetylation of chromatin at the promoter locus. Interestingly, we find that the high induction of Cyclin D1 in breast cancer cell lines can be correlated to decreased levels of SMAR1in most of the metastatic lines. An RS domain o SMAR1 binds to Cux and cause transcriptional repression. **Conclusion:** SMAR1 might act as a regulator of HPV promoter that acts through recruitment of Cux/CDP. Since, CDP is expressed in basal epithelial cells but not in differentiated primary keratinocytesit it is possible that repression through papillomavirus silencing motif (PSM) couples HPV transcription to the stratification of epithelia. We have dissected SMAR1 domains that is critical for both Cyclin D1 promoter repression and also CDP interaction, a small peptide fused with HIV-1 Tat PTD domain of SMAR1 has been designed that can potentially be used to downregulate Cyclin D1 and also repress HPV viral promoter activity through recruitment of Cux.

IO-12

Role of LPA3, TIMP3 and PI3K in LPA Induced Ovarian Cancer Metastasis

Saubhik Sengupta*, Michael P. Berk, Pedro Escober, and Yan Xu

Department of Cancer Biology, Lerner Research Institute, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195, U.S.A. *sengups2@ccf.org

Ovarian cancer has a unique ability to metastasize by direct dissemination to secondary organs in the peritoneal cavity and it is one of the major reasons for its extremely poor prognosis. Our laboratory has identified that lysophosphatidic acid (LPA) is elevated in the blood and ascites of patients with ovarian cancer and not in healthy controls or other gynecological cancers. We also identified an autocrine LPA loop in ovarian cancer cells by which cells produce LPA by beta 1 integrin - laminin interaction, use LPA to trigger LPA receptor specific signaling that leads to cPLA2 activation and cell migration. In this study, we determined if LPA could induce metastasis *in vivo* and also to dissect out the molecular mechanisms that are responsible for LPA-induced metastatic spread. LPA stimulated established and primary cell lines to invade *in vitro*. siRNA inhibitors against LPA3, but not LPA1 and LPA2 and downstream signaling inhibitors of LPA3 receptors like PI3K and p38 inhibited this invasion. A unique LPA activity in ovarian cancer cells is to down-regulate TIMP expression but not stimulate MMP activation. TIMP3 down-regulation leads to reverse its inhibitory effect on p38 MAP kinase phosphorylation, a key step in cPLA2 activation and cell migration. To test if LPA stimulates metastasis *in vivo*, we generated an ovarian orthotopic model in NU/NU mice and demonstrated for the first time that LPA stimulates metastasis *in vivo* that could be inhibited by PI3K specific inhibitor LY294002. LPA inhibition thus can have potential importance for ovarian cancer metastasis management.

IO-13

DNA Polymerase β, a Molecular Target in Human Cancer that Interacts with a Novel Protein, MGC5306 Expressed in Ovarian and Breast Carcinomas

Sipra Banerjee*, Liming Wang and Nandan Bhattacharyya

Department of Cancer Biology, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH 44195, USA. *banerjs@ccf.org

The DNA polymerase β (39-kDa) is essential for the gap-filling synthesis of damaged DNA template in base-excision repair pathway. The expression of a dominant-negative mutant of polβ (polβD) protein (36-kDa) was first identified in breast, ovary, colorectal, lung carcinomas and tumor cell lines. Genomic mutations were detected also in ovarian tumors. A binary complex of polβD and XRCC1 inhibits functions of polβ in a dominant specific manner. Cells expressing polβ are hypersensitive to DNA alkylating agents and express neoplastic phenotype in animal model. A novel gene, MGC (mammalian gene collection) 5306 has been identified interacting with pol ?. Binding to pol ? implicates that MGC5306 most probably has relevance in the dominant-negative function of polβD. The annonated MGC5306 gene mapped to chromosome 11q21 consists of 10 exons spanning 11.3-kb encoding a 32-kDa protein. It transcribes a 2.34-kb mRNA. Imaging analysis of MGC5306-GFP fusion protein and Western blot with a newly developed antibody demonstrate clearly that MGC5306 is constitutively targeted to the nuclei of the cells. Survival of cells using siRNA targeted to MGC5306 is regulated by MGC5306. More importantly, down-regulation of MGC5306 causes apoptosis and arrests cell cycle at S phase with abolition of G2-M phase. These results indicate that the MGC5306 modulates cell cycle events. It is expressed both at mRNA and protein levels in ovarian and breast tumors, MDA468 breast and U373 glial tumor cell lines but not in normal corresponding tissues indicating strongly that MGC5306 has a role in development and progression of cancer. Supported by NIH RO1 Ca83768.

IO-14

Concept of Medium-Term Bioassay for Evaluating Anticarcinogenic Potential of Dietary Constituents

Yogeshwer Shukla*, Neetu Kalra and Y.K.Gupta

Industrial Toxicology Research Centre; P. Box No. 80, M.G. Marg, Lucknow-226001. *yogeshwer_shukla@hotmail.com

Our environment contains a great variety of carcinogenic factors including naturally occurring and synthetic carcinogens, radiation, and viruses, all of which have been speculated to play major roles in the etiology of human cancers. Because humans are exposed concurrently or sequentially to a large variety of environmental carcinogens at only very low individual doses over their lifetime, a strong possibility exists that agents may act in combination to induce cancers. Therefore, in addition to detecting carcinogens in our environment, examination of low-dose combination effects of such agents as well as of their prevention by non-toxic chemopreventive agents is an important area for research to reduce human cancer risk. The concept that cancer can be prevented or its onset by diet-derived agents is currently eliciting considerable interest. Review of the epidemiological data of all cancer sites strongly suggests that several plant foods have preventive potential and consumption of raw and fresh vegetables and fruits are associated with a lower risk of cancers. To bridge the disadvantage of long-term carcinogenicity tests and in vitro mutagenicity assays, medium-term bioassays using preneoplastic lesions as end point markers have been proposed. A medium-term liver bioassay for carcinogens can be used

for detecting the effect of chemical mixtures at low dose levels, as well as detection of the carcinogenic potential of individual test chemicals. To achieve this induction of altered hepatic foci (AHF) was quantitated using certain positive and negative biomarkers of carcinogenesis. These biomarkers include quantitative induction of placental isozyme of glutathione-S-transferase (GST-P) and g-glutamyl transpeptidase (GGT) foci and decrease in adenosine triphosphatase (ATPase), alkaline phosphatase and glucose-6-phosphatase (G6Pase) foci in rat liver. In the present set of investigations the anticarcinogenic potential of dietary constituents including Diallyl sulfide, an organosulfur compound from garlic, Indole-3-Carbinol, an active component of cruciferous vegetables and Curcumin, a major constituent of turmeric were evaluated for their efficacy of modulating AHF. All these agents decreased the diethyl nitrosamine induced GST-P and GGT positive foci whereas; increased the ATPase, Alkpase and G6Pase deficient foci. Thus the present study concluded that a liver medium term bioassay could be used effectively for the detection of anticarcinogenic potential of dietary constituents.

IO-15
Novel Aptamer Therapy for Cancer; From the Bench to the Bedside

Donald Miller*

James Graham Brown Cancer Center, University of Louisville, Louisville, KY 40202. *dmi@ulh.org,

IO-16
Identification of a Cell Senescence Gene SEN6A for Ovarian and Breast Cancer

Vikramjit K. Zhawar, G. Pal Kaur and Raghbir S. Athwal*

Fels Institute for Cancer Research and Department of Pathology, Temple University School of Medicine, Philadelphia, USA. *rathwal@temple.edu

Normal diploid human cells multiply for a finite number of generations and then enter a state of replicative senescence. In contrast cancer cells can proliferate indefinitely. Escape from senescence is an essential early step in tumor progression, implicating cellular senescence as a mechanism of tumor suppression. We have devised a functional approach to clone genes involved in the regulation of cell growth and senescence. Starting with the transfer of intact single normal chromosomes into immortal tumor cells, followed by deletion mapping, we progressively mapped the position of a senescence gene at 6q21 within 1cM genetic interval. Precise localization of the gene allowed us to identify YAC and BAC clones, corresponding to the senescence locus. Functional testing of candidate YAC and BAC clones identified one YAC and two BAC clones that restored normal cell growth and senescence in immortal tumor cells. Partial cDNA clones, located on the complementing YAC and BAC clones, were then identified through human genome database search. Candidate cDNA clones were recognized by loss of expression in Northern bots from tumor cells or by deletions within 1Mb genomic interval carrying SEN6A. Full length cDNA clone, representing SEN6A, was assembled from the sequence of partial cDNAs and cloned in a mammalian cell expression vector. Ectopic expression of SEN6A in cancer cells leads to inhibition of cell proliferation and senescence. SEN6A gene encodes for five different variant isoforms comprised of 13-17 exons and is carried in a 1Mb genomic region. The gene is turned off in many tumor cell lines and its expression is modulated through epigenetic chromatin modification. It appears to play role in cancer as well as other diseases.

IO-17
Creation of an HPV Test for Developing Countries

Lorincz A[1], Van Asten M[1], Eder P[1], Sellors J[2], Nazarenko I[1]

[1]Digene Corp, Gaithersburg, Maryland USA; [2]Program for Appropriate Technology in Health (PATH), Seattle, Washington USA. *mvanasten@compuserve.com

Cervical cancer is among the most common malignancies of women in low-resource countries, such as India. Secondary prevention of cancer by population screening strategies is a successful approach, but implementation of routine cytology is too less evolved, complex and expensive in many of these regions; hence, mortality remains high, accounting for nearly 80% of global load. More than 95% of cervical cancers develop from persistent infections by a subset of carcinogenic HPV types, as a single largest cause and screening by purpose to identify these viruses is effective for the highly sensitive detection and treatment of cervical cancer precursors as demonstrated in numerous large population-based studies world wide including in India. Digene has entered into a partnership with PATH to design and develop a simple, relatively rapid, and affordable batch-based diagnostic HPV DNA test (the dcHPV test) for use in low-resource settings. We have used as a starting point Hybrid Capture 2 (hc2) technology that will be completely reconfigured into a faster, thermostable, and robust assay with new portable equipment capable of running from local power mains or portable batteries. dcHPV has a simple format with easy transfers, thus allowing an unskilled technician with minimal training and average dexterity to perform the assay. Analytical sensitivity of the test is comparable to hc2, and its analytical specificity appears better due to the incorporation of a modified procedural step. A prototype dcHPV batch assay can return results on over 50 clinical specimens in almost 2 hours with fewer steps than the hc2 test. The reduced time of the dcHPV test can allow women to be screened, informed of results, and treated if necessary in one visit. Wide dissemination of this new HPV detection assay should allow millions of women to benefit by a reduction in their risk of cervical cancer and disease mortality.

IO-18
Does Estrogen Protect Against E.coli Invasion in Tumor Cells?

Anil Kaul*[1], Senait Assefa[2], Mark Martens[1], Rashmi Kaul[2]

[1]University of Oklahoma, [2]Oklahoma State University, Oklahoma, USA. *anil-kaul@ouhsc.edu

Escherichia coli (*E.coli*) is a frequent colonizer of human gastrointestinal and urogenital tracts. In women, estrogens are reported to alter susceptibility to infections. *E.coli* expressing Dr fimbriae (Dr) are clinically associated with chronic infections. We have reported Dr+*E.coli* attach to and utilize complement-decay accelerating factor (DAF, CD55) on host cells for internalization and DAF is over-expressed in endometrial carcinoma. Recent studies have established the role of estrogen as an immunomodulator. However, its direct role in intracellular innate antibacterial response has not been established. We believe that estrogen may influence bacterial invasion and in susceptible individuals, this may result in the development of chronic disease and possibly carcinogenesis. In the present study, we investigated the role of estrogen in invasion of HuH-7 cells by uropathogenic Dr+*E.coli* using gentamicin protection assay. Cells were seeded for 24 hours and serum starved for 18 hrs and treated with 0.1, 1.0 and 10 mM 17 estradiol for 24 hours. After Dr+*E.coli* invasion and gentamicin treatment, intracellular bacterial colony forming units (CFU) were determined. Results were expressed as a percent of bacterial CFU counts compared to untreated control cells. Dr+*E.coli* were invasive and all estrogen

doses inhibited this invasion. The highest percentage CFU count reduction was at 1mM treatment (62.87+/-19.23, p<0.0035). Protection to invasion was abolished by pretreatment of estrogen treated cells with ICI 182,780 (anti-estrogen) (P<0.0015). These results suggest novel protective role of estrogen in bacterial invasion. This invitro model system maybe useful in studying estrogen related factors involved in bacterial invasion and possible carcinogenesis.

IO-19

Detection of Human Papillomavirus (HPV) DNA by the Hybrid Capture Method

Rohini Kelkar*

Dept of Microbiology, Tata Memorial Hospital, Mumbai. *ahb@vsnl.com

The two commonly used methods for the detection of HPV DNA in cervical lesions are the Polymerase Chain Reaction (PCR) and the Hybrid Capture System (HC-II). The HC-II system™ from Digene detects 18 types of HPV DNA in cervical specimens. This includes 13 high / intermediate risk and 5 low risk types. The current preventive strategies for cervical cancer include early detection and identification of high risk women with HPV infection. Standardization and identification of diagnostic methods to facilitate this in a developing country is therefore important. The proposed vaccine trials further highlight this need. We have a large experience with the HC-II™ system. Participation in two quality control studies has revealed a high degree of reproducibility in the assay results. The advantages of this detection system are that it is a useful screening tool and is easy to perform, besides being sensitive and highly reproducible. One of the major disadvantages is the high cost of testing. Samples with results in the “gray-zone” are difficult to interpret. However, this occurs in a small number of cases. This being a qualitative assay, quantification of the HPV DNA burden is not possible. Difficulties in standardization of in house PCRs are known. HC-II being a signal amplification assay reduces the chances of extraneous DNA contamination.

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DELEGATE ORAL PRESENTATIONS

DO-1
Detection of Hypermethylated Genes in Women With and Without Cervical Neoplasia

Qinghua Feng[1], Akhila Balasubramanian[1]*, Stephen E. Hawes[1], Pap Toure[2], Pap Salif Sow[2], Ahmadou Dem[2], Birama Dembele[2], Cathy W. Critchlow[1], Longfu Xi[1], Hiep Lu[1], Martin W. McIntosh[3], Alicia M. Young[3], Nancy B. Kiviat[1]

[1]University of Washington, Seattle, USA; [2]University of Dakar, Dakar, Senegal; [3]Fred Hutchinson Cancer Research Center, Seattle, USA. *akhila@u.washington.edu

Introduction: DNA methylation changes are an early event in carcinogenesis and are often present in the precursor lesions of various cancers. **Objectives:** To examine whether DNA methylation changes might be used as markers of cervical intraepithelial neoplasia (CIN) and invasive cervical cancer (ICC). **Methods:** We used methylation-specific polymerase chain reaction (PCR) to analyze promoter hypermethylation of 20 genes in exfoliated cell samples and matched tissue biopsy specimens from 319 Senegalese women (histology negative/atypical squamous cells of undetermined significance=142, CIN-1=39, CIN-2=23, CIN-3/carcinoma in situ [CIS]=23, ICC=92). Logic regression was used to determine the best set of candidate genes to use as disease markers. **Results:** Similar promoter methylation patterns were seen in genes from exfoliated cell samples and corresponding biopsy specimens. The best panel of hypermethylated genes included DAPK1, RARB, or TWIST1. At least one of the three genes was hypermethylated in 57% of CIN-3/CIS and in 74% of ICC but in only 5% of samples with <CIN-1. The estimated specificity of the three-gene panel was 95% and its sensitivity was 74% (95% confidence interval [CI]=73%-75%) for ICC and 52% (95%CI=49%-55%) for CIN-3/CIS. By extrapolation, we estimated that among Senegalese women presenting to community clinics, detection of the DAPK1, RARB, or TWIST1 hypermethylated gene would reveal histologically confirmed ³CIN-3 with a sensitivity of 60% (95%CI=57%-63%) and a specificity of 95% (95%CI=94%-95%). **Conclusions:** Aberrant promoter methylation analysis on exfoliated cell samples is a potential diagnostic tool for cervical cancer screening that may be used alone or in conjunction with cytology and/or HPV testing.

DO-2
Avenues for Potential Therapeutic Use of Anticancer Properties of *Vinca rosea, Withania somnifera* and *Ocimum sanctum*

A Nath*, Priyanka Sinha and Preety Sinha

Cell Biology & Toxicology Lab, Department of Zoology, A N College, Patna University, Patna. *anpgzool@hotmail.com

One of the most dreadful diseases threatening the existence of mankind in the 21th century is cancer. Various kinds of cancer are reported due to pesticides which are very liberally used to increase the yield of crops, leading to their entry into the ecosystem, ultimately causing mutation and genotoxicity in human being. Malathion, parathion, rogor, carbamate, endosultan, DDT, Aldrin a, b & g HCH etc are commonly used as pesticides. In the present investigation LM, EM, enzyme and hormone assessment in Swiss albino mice show that endosulfan, malathion and parathion cause cytotoxic effects in various organs but are not carcinogenic. Rogor produces carcinogenic effects

on liver and testes of test animals. In the fifth and sixth successive generation of rogor treated mice tumors appear on back and near the neck in male. Herbal extracts tend to lower pesticide induced carcinogenicity in liver and testes. *Vinca rosea* and *Withania somnifera* were chosen as probable antidotes against such carcinogenicity. *Vinca rosea* extract has vinblastin, which acts as cell-cycle inhibitor and thus can be a potent anticancer device. *W. Somnifera* extracts contain GABA a and GABA b, which activate neuroendocrine system ultimately leading to hyperactivity of endomembrane system giving smooth biosynthetic pathways to various biomolecules. Root extracts of *W. somnifera* was used to lower subcellular toxicity caused by pesticides in liver and testes. *Ocimum sanctum* leaf extract with its antioxidant effects was also found to be beneficial. Administration of sublathal doses of endosulfan in male mice results in complete arrest of spermatogenesis and spermiogenesis, blebbing on the sperm head and deformities in testicular cells leading to reduced sperm count and testosterone level. Administration of *V. rosea* and *W. somnifera* extracts shows remarkable recovery in the testes. In rogor treated mice several abnormalities like fenestrations, blebs, ruffles, labopodia and deformities occur on surface of hepatocytes, white their nuclei become irregular in shape. In testes there is high incidence of changing of Sertoli cells to macrophage like structure. TEM of liver cells show vacuolizations increase in RER, SER and simultaneous increase in ribosomes as well as vast increase in mitochondria number revealing high protein and energy requirement. Serum SGPT is highly raised raised along with total bilirubin content.The supplementary feed mixed with medicinal plant extracts when fed to the test animals for 2-3 months show remarkable regeneration of organelles, which were in damaged state earlier due to pesticide toxicity. It tends to normalize the cellular and subcellular damages like diffusion of plasma membrane increased lysosomal activity, dilation of mitochondrial cristae disrupted secretory pathways and major carcinogenic effects caused due to pesticide toxicity. *Vinca rosea* along with *Ocimum sanctum* leaf extracts acts syneraistically and provide ameliorating effects against carcinogenicity in mice to some extent. It may be administered intraperitoneally or applied as ointment as a potent antidote against cervical cancer as vinblastin and vincristin in *Vinca rosea* might check the proliferation of cancer cells.

DO-3
N-myristoyltransferase: A Novel Therapeutic Target for Cancer

***Anuraag Shrivastav*[1]*, Jonathan R Dimmock[2], Nand K. Singh[3] and Rajendra K Sharma[1].**

[1]Department of Pathology, College of Medicine, University of Saskatchewan and Health Research Division, Saskatchewan Cancer Agency, Saskatoon, Saskatchewan, S7N 4H4, Canada, [2]College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 5C9, Canada, [3]Department of Chemistry, Faculty of Science, Banaras Hindu University, Varanasi, Uttar Pradesh, 221 005, India. *ashrivastav@scf.sk.ca

Protein N-myristoylation is a lipidic modification which involves the covalent attachment of myristate, a 14 carbon saturated fatty acid, to the N-terminal glycine residue of a number of mammalian, viral and fungal proteins. N-myristoylation ensures the proper function and intracellular trafficking of proteins. Many proteins involved in a wide variety of signaling, including cellular transformation and oncogenesis, are myristoylated. The myristoylation of proteins is catalyzed by N-myristoyltransferase (NMT). Earlier, we have reported that NMT activity is higher in colonic epithelial neoplasms than in normal appearing colonic tissue and that the increase in NMT activity appears at an early stage in colonic carcinogenesis. Furthermore, we observed that NMT expression is elevated in colorectal and gallbladder carcinoma. Results from our laboratory have established NMT as a novel therapeutic target for cancer. Attenuation of NMT activity may prove a novel therapeutic protocol for cancer. A large number of compounds of diverse structures and proteins are capable of inhibiting NMT. On the basis of their effects on enzyme reaction, inhibitors may be broadly classified into two general categories namely: proteins and synthetic organic/inorganic compounds. We have discovered recently that

enolase potently inhibited NMT activity in a dose dependent manner with the half maximal inhibition at 4.5 0.35 nM. Synthetic compounds such as mannich bases of 2-arylidenecyclohexanones and transition metal complexes of thiohydrazides showed inhibition of NMT activity. Further characterization of inhibitor protein and compounds in terms of their mode of action will serve as a new set of treatment protocol for cancer.

DO-4

Dynamic Spectral Imaging for in vivo Diagnostics, Screening and Guided Therapeutics of Cervical Neoplasia.

Costas Balas*[1,2], Christos Simos[2], Yiannis Sciadas[2]

[1] Department of Electronic and Computer Engineering, Technical University of Crete, 73100 Chania, Crete, Greece. [2] FORTH-Photonics, 19-21 Theophonous str, 11523, Athens, Greece. *balas@forth-photonics.gr

Introduction: The visual assessment of the acetowhitening characteristics is not effective, since it is qualitative and subjective and depends on the examiner's visual acuity and training. Objectivrs: To develop an alternative digital imaging technology for diagnosis and screening of cervical neoplasia, relying on the measurement, processing and mapping of the acetowhitening characteristics. **Methods:** The DySIS technology, developed at FORTH-Photonics, measures the acetic acid-induced temporal alterations in the optical properties of the cervix. From the measured data, quantitative parameters expressing the acetowhitening characteristics are calculated and displayed, for every image pixel. Different (pseoudo-) colours are used to represent different parameter values, enabling the direct visualization of the various acetowhitening degrees, over the entire tissue surface. The obtained pseoudocolor kinetic map can be displayed and stored together with the actual tissue image, facilitating lesion's localization, mapping and follow-up. **Results:** The unique advantage DySIS technology over visual examination is that it enables the accurate and standardized assessment of the acetowhitening effect. The provided quantitative data improve both sensitivity and specificity, since the discrimination between CIN and non-CIN lesions as well as between different grades of CIN is facilitated. Moreover, the measured acetowhitening kinetic map provides valuable information for the location, size and grade of the lesion, thus improving both biopsy sampling and treatment accuracy. Furthermore by enabling the direct comparison of kinetic maps, obtained during subsequent examinations, possible recurrences can be detected. **Conclusion:** DySIS technology has the potential to become a stand-alone, cost-effective tool for screening, diagnosis and guided biopsy sampling and treatment of cervical neoplasia in developing countries.

DO-5

Identification of a Spliced Variant of Human Sin3b in Adult Lungs and Placenta

***Harish Batra*[1], *Mashook Ali*[1], *Rakesh Singh*[1], *Vineet Kumar*[1], *Shambhavi Mishra*[2], *Balaji Prakash*[2], *Daman Saluja**[1]**

[1]Medical Biotechnology laboratory, Dr B.R. Ambedkar Center for Biomedical Research, University of Delhi, India. [2]Department of Biological Sciences & Bioengineering, Indian Institute of Technology, Kanpur, India. *dsalujach@yahoo.com

Introduction: Sin3 family of proteins contains highly conserved, multifunctional, nuclear oncoproteins that have been implicated as corepressors utilized by several transcriptional repressors regulating diverse

cellular functions. Although ySin3 is not essential for viability, null mutations of Sin3 are lethal in Drosophila. In yeast and several other organism a single isoform of Sin3 has been identified while mouse and human contains atleast two distinct genes for Sin3 namely Sin3A and Sin3B. The two isoforms are close paralogs. Primarily Sin3 proteins have been shown to act as transcriptional repressors by working as a scaffold for assembly of specific HDAC and Swi/SNF components. In mammals, several AML family members fusion oncoproteins were found in a ternary complex with AML-1 and Sin3A, suggesting that they may acts by recruiting transcriptional corepressors and histone deacetylases. hSin3A associates with N-CoR or SMRT and ETO independently. Other DNA binding transcription factors including Max, Ume6, and nuclear hormone-receptors can also recruit hSin3/HDAC/N-CoR complex. Recently, Mxi1-mediated inhibition of Myc has been shown to require interaction with mSin3A proteins through its PAH2 domain. **Objective:** To identify the structure and function of human Sin3B. Methodology: Using silico analysis primers were designed to amplify hSin3B cDNA and clone in bacterial and mammalian vector. Complete sequencing of hSIN3BcDNA was deciphered by designing internal primers. The exon intron boundaries were predicted using Gene Mark program. Various motifs for the Sin3B protein were searched and aligned at their respective positions using PROSITE and CDART. Structural modeling was done using Jpred, 3D-PSSM and MODELLER6.v2 The homology models thus obtained were examined for significant interactions with MAD1 in Insight. The Connolly surfaces of the homology models were generated using INSIGHTII software package. PAH1 interacting protein region of NCoR was modeled using MAD1 interacting helix as a template. Tissue specific expression was checked using human multiple tissue panels (clonetech). **Results:** We here report the characterization and sequence analysis of two isoforms of hSin3B. The gene for hSin3B is localized on chromosome 19p and spans approximately 50 Kb. An alternate spliced form is differentially expressed in adult lungs and placental tissue. The long isoform contains 20 exons and the short isoform lacks exon X. The exon X codes for 32 amino acids and lies downstream of PAH3 domain and this may hold importance in differential gene regulation. In silico structure analysis of the conserved domains predict an alternate protein-protein interaction domain present in hSin3B. Functional interaction studies of hSin3B with other proteins will be presented.

DO-6

Rx of Cancer: Silencing of a Gene using Novel Liposome Entrapped siRNA

Imran Ahmad*

NeoPharm Incorporation, Lake Forest, Illinois, USA. *iahmad@neophrm.com

DO-7

Interleukin-8 Expression in Urine of Patients with Superficial Bladder Cancer after Bacillus Calmette-Guerin Instillation: A Potential Prognostic Marker

Kishore M. Amin*, [1]Hemant B. Tongoankar, [1]Yuvaraja T.B., Sujata S. Hake, [2]Asha S. Raste., Shubhada V. Chiplunkar

Immunology, Tata Memorial Centre, Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Kharghar, Navi Mumbai, [1]Genito-urinary and Gynecological Oncology Service, [2]Biochemistry Department, Tata Memorial Hospital, Parel, Mumbai, India. *kamin@actrec.res.in

Adjuvant intravesical Bacilli Calmette-Guerin (BCG) is an effective method of treating superficial bladder tumors. The mechanism(s) by which BCG inhibits tumor growth and release of cytokine/s are not known. In the present investigation we propose to study the efficacy of BCG instillation by evaluating urinary cytokine response before and after intravesical BCG instillation in Superficial Bladder Cancer (SBC) patients and investigate if Toll Like Receptors are involved. Urinary cytokines IL-2, IL-8, TNF-a and IFN-g were quantitated in patients (n=45) with SBC using sandwich ELISA kits. Reverse Transcriptase polymerase chain reaction (RT-PCR) was used to study mRNA expression of these cytokines in transurethral resection (TURBT) biopsies and peripheral blood lymphocytes (PBL). Results demonstrate that baseline levels of IL-2 and IL-8 were increased in urine of SBC patients when compared to healthy controls. At four weeks interval and after four hours of BCG instillation a marked increase in urinary IL-8 levels were observed in these patients. TNF-a and IFN-g levels did not show any significant increase after 4 hr of BCG instillation. Tumor biopsies of SBC patients showed an increase level of IL-8 mRNA expression but the other three cytokines (IL-2, TNF-a and IFN-g) were poorly expressed. Results demonstrate that elevated levels of IL-8 in urine of SBC patients after BCG instillation serves as a good prognostic marker and further establishes that BCG mediates its action via TLR-2 and TLR-4. Studies are in progress to investigate if ectopic IL-8 released by tumor cells correlates with tumor progression and metastasis.

DO-8
Gene Therapy: Trials and Tribunals

Narendra Chirmule*

Vaccine and biology Research, Merck and Co. Inc., Wayne, PA, USA. *narendra_chirmule@merck.com

Monumental advances have occurred in molecular biology over the past quarter of a century. The discovery of DNA ligase, reverse transcriptase and restriction endonucleases has laid the ground work for the construction of recombinant DNA molecules. These advances have had a profound impact of understanding gene structure, function and control. The mapping of the human genome is completed and those for various other species and micro-organisms have also been defined. An understanding of genetic basis of various human diseases has also accelerated. In this respect, advances in identifying single and multi-gene defects have pinpointed etiology of diseases. On this landscape of important progress, an entirely new paradigm for the prevention, cure, and treatment of human disease - gene therapy - has evolved. "Human Gene Therapy may be defined as the insertion of a normal or modified gene into somatic cells of patients to correct genetic or acquired diseases, through in vivo synthesis of missing, defective or insufficient gene products". Since treatment of the first human experiment on September 14, 1990, a new era of treatment has begun. Extensive studies in animal models have demonstrated proof-of-concept, and efficacy of utilization of this technology in treatment of genetic disorders. Several clinical trials are in progress for therapeutic application of genetic diseases, such as hemophilia, cystic fibrosis, ornithine transcarbamylase deficiencies. In addition, novel approaches utilizing genetic tools have been utilized for treatment of various types of cancers; these include inducers of apoptosis, immune stimulation, targeting susceptibility to drugs. More recently a clinical trial serious adverse events, namely, a death of a patient and another trial with integration of the vector/transgene has underscored the importance of innate immune responses and molecular analyses in gene therapy. This lecture will cover the "holy grails" of gene therapy which include: long term gene expression, readministration, targeting, regulation, site-specific integration and gene replacement.

DO-9
Molecular Diagnostic Modalities for CML: Molecular Beacons

***Nishigandha R Naik*[1]* and PKR Kumar*[2]**

[1]Cancer Research Institute, Advanced Centre for Treatment, Research and Education in Cancer, TMC, Kharghar, Navi Mumbai - 410208. [2]National Institute of Bioscience and Human Technology, Tsukuba, Japan. *nnaik@actrec.res.in

Molecular diagnostics, that is, the use of diagnostic testing to understand the molecular mechanisms of an individual patient's disease, will be pivotal in the delivery of safe and effective therapy of many diseases in the future. Ideally, techniques used in molecular diagnostics, especially in MRD detection should provide some quantification of the target and be rapid, inexpensive, readily standardized and disease specific. Molecular beacons (MB) are short, hairpin shaped, synthetic oligonucleotides with an internally quenched fluorophore whose fluorescence is restored when they bind to a target nucleic acid. Therefore, they may be useful in molecular diagnostics, especially in situations where it is either not possible or desirable to isolate the probe-target hybrids from an excess of hybridization probe. Use of MB in chronic myeloid leukemia (CML) diagnostics was evaluated. MB were designed against the most common bcr-abl chimera - b3a2 that is consistently associated with CML. After checking specificity in vitro by gel shift assay, intracellular specificity of fluorescent label tagged b3a2 MB was checked flow cytometrically and by laser confocal microscopy, using bcr-abl positive cell lines - K562 and 32DCbcr/abl. Data analysis of b3a2 MB uptake by these cells showed that specific signals are obtained within few minutes and are maintained till few hours. These studies show that MB have tremendous potential to be used as biomolecular recognition probes for CML.

DO-10
Combinatorial Chemoprevention of Hereditary Colon Cancer: Models and Biomarkers

Nitin Telang*, Meena Katdare

Strang Cancer Prevention Center and Department of Surgery, Weill Medical College of Cornell University, New York/USA. *telangn@mail.rockepeller.edu

Mutations in tumor suppressor and DNA-mismatch repair genes represent primary genetic defects in the human hereditary nonpolyposis colon cancer (HNPCC) syndrome. Current 5-Fluorouracil based combination therapy produces modest efficacy and substantial systemic toxicity. Alternate approach using low dose combination of Coxibs and DNA inhibitors may enhance efficacy and minimize toxicity. Unlike the human HNPCC syndrome, preclinical animal models exhibit accelerated small intestinal carcinogenesis, and therefore, limit clinically relevant translation. Reliable preclinical models with relevant genetic defects in appropriate target organ site may reduce the need for clinical extrapolation. Subculturable epithelial cell lines established from histologically normal colon of Apc [+/-] / DNA MMR [+/-] mice represents a novel preclinical model. Status of cell proliferative kinetics, cell cycle progression and anchorage-independent colony formation represent the mechanistic endpoint biomarkers. Selected mechanistically distinct, clinically relevant pharmacological agents represent the test compounds for combinatorial efficacy. Relative to the wild type Apc [+/+] / DNA-MMR [+/+] C57 COL cells, the mutant cells exhibit a 41-56% decrease in the population doubling time, and a 95-217% increase in the saturation density. The mutant cells also exhibit enhanced risk for carcinogenesis as evidenced by a 33-100% increase in anchorage-independent colony formation. Treatment of mutant cells with

low dose combinations of mechanistically distinct sulindac (SUL) + Difluoromethyl ornithine (DFMO) or Celecoxib (CLX) + 5-Fluorouracil (5-FU) produce at least a 2.5 fold and 7.2 fold increase respectively, in efficacy for growth inhibition relative to that obtained by individual test compounds. The enhanced efficacy is predominantly due to arrest of the cells in the S and/or G2/M phase of the cell cycle. These results provide phenomenological leads for future studies on relevant molecular mechanisms for enhanced efficacy of these combinations. The present data therefore, validates a cell culture model for a rapid mechanism-based screening and rational prioritization of combinations of new synthetic or naturally occurring chemopreventive agents for subsequent clinical trials. [Support: The Irving Weinstein Foundation and NCI MAO # CN 75029-63].

DO-11

Ablation of Peripheral Dopaminergic Nerves Stimulates Malignant Tumor Growth by Inducing Vascular Permeability Factor/Vascualr Endothelial Growth Factor Mediated Angiogenesis

Sujit Basu[1,2], Chandrani Sarkar[3], Debanjan Chakroborty[3], Janice Nagy[4], Rita Basu Mitra[5], Partha Sarathi Dasgupta [3]*, Debabrata Mukhopadhya[1]

[1]Mayo Clinic Cancer center, Rochester, USA. [2]Dept of Medical Oncology,CNCI, Calcutta. [3]Signal Transduction &Biogenic Amines Lab, CNCI, Calcutta. [4]Dept of Pathology, BIDMC & Harvard Medical School, Boston, USA. [5]Dept of Pathology, IPGMR, Calcutta. * ceds@vsnl.com

Initiation of angiogenic process has been shown to be an essential early step in the progression of malignant tumors.We report here that the ablation of peripheral dopaminergic nerves markedly increased Angiogenesis, microvessel density , microvascular permeability and growth of malignant tumors in mice. Endogenous peripheral dopamine acted throughD2 receptors as significantly more Angiogenesis and tumor growth was observed in D2 dopamine receptor knock out mice in comparison with controls. The vascular endothelial growth factor receptor 2 phosphorylation which is critical for promoting Angiogenesis, was also significantly more in tumor endothelial cells collected from the dopamine depleted and dopamine D2 receptor knock out mice. These results reveal that peripheral endogenous neurotransmitter dopamine might be an important physiological regulator of vascular endothelial growth factor mediated tumor angiogenesis and growth and suggest a novel link between endogenous dopamine angiogenesis and tumor growth

DO-12

L1 Variants of HPV Types 16, 33, 45 and 58 in Indian Patients with Cervical Neoplasia

Priya Abraham[1]*, Narayanan Sathish[1], Abraham Peedicayil[2], Gopalan Sridharan[1], Subhashini John[3], George Chandy[4]

Departments of Clinical Virology[1], Obstetrics and Gynaecology[2], Radiation Oncology[3], Clinical Gastroenterology and Hepatology[4], Christian Medical College, Vellore, India. *priyaabraham@cmcvellore.ac.in

Background: Studies in south India reveal the presence of high-risk oncogenic HPV types 31, 33, 35, 45, 51, 52, 56, and 58 in addition to 16 and 18. Only few reports of intratypic variations in the L1 gene of these types exist. **Objectives:** To look for nucleotide variations in the L1 gene of these high-risk HPV types among Indian women with cervical neoplasia. **Methodology:** In a cross-sectional study, 118 women with cervical neoplasia were recruited comprising 16 with cervical intraepithelial neoplasia and 102 with invasive carcinoma.

HPV genotypes were identified by PCR followed by restriction fragment length polymorphism (RFLP). Untypeable isolates were analyzed by nucleotide sequencing. **Results:** Of 111 women positive for HPV DNA, 96 women (86.5%) were typed by RFLP while 15 women (13.5%) were untypeable. These 15 samples were resolved by nucleotide sequencing revealing: HPV 16 (n=6), HPV 33 (n=2), HPV 45 (n=3) and HPV 58 (n=4). Untypeable isolates of HPV types 33, 45 and 58 and 5 of 6 isolates of HPV 16 had consistent RFLP digestion patterns with identical nucleotide variations in the L1 region. Some of these L1 nucleotide variations produced a change of the encoded amino acid. **Conclusions:** A proportion of HPV types 16, 33, 45 and 58 exhibited nucleotide variations in the L1 gene. Studying intratypic variants could shed more light on the evolutionary and taxonomic status of HPV types in a given geographical region. Whether L1 variations amounting to amino acid change could have a bearing on future vaccine formulations needs to be further evaluated.

DO-13
Cervical Cytology or HPV Testing or Both What is Practicable in India?

R Nijhawan*and A Rajwanshi

Department of Cytology & Gynae Pathology, Post Graduate Institute for Medical Research, Chandigarh, India.
*rajenijhawan@hotmail.com

Introduction: The West has witnessed a dramatic fall in the rate of cervical cancer, thanks to the well-organized screening programmes. Current approaches vary from cervical cytology alone, to cervical cytology and HPV testing, to HPV testing alone. In India, approximately 10 crore women fall in the age groups (30-60 years) most susceptible to cervical cancer. Current screening programmes are grossly unsatisfactory. Mass HPV testing is a distant dream. Even cervical cytology cannot be offered to all who need it. However, cytology being the cheaper of the two, it is worthwhile to study if there are any cytomorphological features in addition to koilocytosis which may indicate an HPV infection. Aims and **Objectives:** 1) To study cervical smears with koilocytes for additional morphological features which might suggest an HPV infection. 2) To study cervical smears for special morphological features in women who show the presence of high-risk HPV DNA. Materials and **Methods:** 1) 30 consecutive cervical smears which showed evidence of koilocytosis were scanned for additional morphological features. 2) 21 cervical smears from women who tested positive for high-risk HPV DNA by HC2 system were screened for special morphological features. **Results:** 1) Of the 30 smears with koilocytosis, four were classified as inflammatory, one as AGUS, 11 as ASUS, five as LSIL, and nine as HSIL. 19 smears showed dyskeratosis, and 17 showed cytoploasmic clearing. 14 displayed both. Five of the ASUS smears revealed occasional atypical parabasal cells, and two of the LSIL smears revealed such cells. 2) Of the 21 smears from women who tested positive for high-risk HPV DNA, 13 showed koilocytosis, and 11 revealed dyskeratosis. Eight smears showed both. Of the 21 (HPV DNA positive) smears, 10 fell in the ASCUS/LSIL group. Of these six revealed occasional atypical parabasal cells. **Conclusion:** In addition to koilocytosis, features like dyskeratosis and cytoplasmic clearing hold promise as additional morphological indicators of cervical HPV infection. Much larger studies are required to confirm the same.

DO-14
Effect of Rapamycin on Akt Signaling in Hepatoma HepG2 and HepG2 Cells Over-expressing Constitutively Active Akt/ PKB

Shailly Varma, Jenny Oberg, Dhananjay Gupta and Ramji L Khandelwal*
Department of Biochemistry, College of Medicine, University of Saskatchewan, Saskatoon, S7N 5E5, Canada.
*ramji.khandelwal@usask.ca

Mammalian target of rapamycin (mTOR) is a serine-threonine kinase, which is known to play an important role in the regulation of cell growth. It activates ribosomal $p70^{S6k}$ and inhibits elongation factor elF4E inhibitor (4E-BP). Rapamycin inhibits mTOR and its downstream signalling. The mechanism of action of rapamycin is that it forms a complex with immunophilin FK506 binding protein 12 (FKBP12) which in turns complexes with mTOR thereby inhibiting its activity. mTOR phosphorylation levels are controlled by the activation of Akt /PKB. Therefore, the effects of rapamycin on Akt /PKB signal transduction pathway both in the absence and presence of insulin were examined in parental HepG2 and HepG2 cells constitutively over-expressing Akt /PKB (HepG2-CA Akt /PKB). Cells were treated with insulin (1-100 nM) in the presence and absence of rapamycin (20 nM). The phosphorylation status of mTOR (Ser 2448) and p70S6K (Thr 389) were investigated. In HepG2 cells, the phosphorylation of mTOR was stimulated 4- fold by insulin and it was decreased to 2-fold in the presence of rapamycin. Since, HepG2-CA Akt/PKB already have high levels of Akt /PKB activity, phosphorylation status of mTOR was high and did not change in the presence of insulin ± rapamycin. The phosphorylation of $p70^{S6K}$ was completely abolished by rapamycin in both types of cells. The reason for differential effects of rapamycin on the phosphorylation of mTOR and $p70^{S6K}$ in HepG2 and HepG2-CA Akt/PKB cells is not clear and requires further investigation. It can be concluded, however, that rapamycin can regulate $p70^{S6K}$ (and protein synthesis) both in parental hepatoma and cells overexpressing Akt /PKB.

DO-15

Altered Expression of Anti and Proapoptotic Proteins during Chemoprevention of Hamster Buccal Pouch Carcinogenesis by Tomato and Garlic Combination

V. Bhuvaneswari[a], K. S. Rao[b] and S. Nagini[a]*

[a]Department of Biochemistry, Faculty of Science, Annamalai University, Annamalainagar-608 002, Tamil Nadu, India. [b]Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad-500 007, India. *r_kumar24@rediffmail.com

Effective combinations of dietary agents are promising candidates for cancer chemoprevention because of their safety and the fact that they are not perceived as medicine. The present study was designed to investigate the apoptosis-inducing effect of combined administration of tomato and garlic during 7, 12-dimethylbenz[a]anthracene (DMBA)-induced hamster buccal pouch (HBP) carcinogenesis. Hamsters were divided into four groups. The right buccal pouches of animals in group 1 were painted with 0.5% DMBA three times a week. Animals in group 2 painted with DMBA as in group 1, received in addition, intragastric administration of a combined dose of tomato and garlic on days alternate to DMBA application. Group 3 animals were given chemopreventive agents alone. Animals in group 4 served as control. All the animals were killed after an experimental period of 14 weeks. DNA fragmentation and the apoptosis-associated proteins-Bcl-2, Bax, Bim, p53 as well as caspases 8 and 3 were used as markers of apoptosis. Topical application of DMBA for 14 weeks resulted in well-developed squamous cell carcinomas (SCCs) associated with increased expression of Bcl-2 and decreased expression of Bax, Bim, P53 and caspases 8 and 3. Combined administration of tomato and garlic significantly inhibited the development of HBP carcinomas and induced apoptosis. This was evidenced by downregulation of Bcl-2 and upregulation of Bax, Bim, p53 and caspases 8 and 3. These observations suggest that induction of apoptosis may be one of the mechanisms through which functional foods such as tomato and garlic exert their anticancer properties.

DO-16

Role of Vascular Endothelial Growth Factor-A (VEGF-A) in 2 Methoxyestradiol-Induced Tumour Cell Proliferation Inhibition

Samarendra Nath Banerjee*[1]*, *Snigdha Banerjee and Sushanta K.Banerjee

Cancer Research Unit, University of Kansas Medical Centre and Division of Hematology and Oncology, Department of Medicine, University of Kansas Medical Centre, Kansas, U.S.A. 1Present Address: Department of Zoology, Rammohan College, 102/1 Raja Rammohan Sarani, Kolkata-700009, India. *sbanerjee2@kumc.edu

Angiogenesis, the sprouting of new blood vessels, is essential for progressive solid tumour growth and thus constitutes a very promising therapeutic target. Tumor angiogenesis process is regulated by enhanced secretion of growth factors. Among several tumour angiogenic factors, vascular endothelial growth factor (VEGF) has been recognized as a prime regulator of tumour angiogenic process. It stimulates the proliferation of endothelial cells for the formation of new blood vessels as well as increasing their permeability, which may also be important for the provision of nutrient to tumours. Recent developments in understanding of the molecular mechanisms involved in tumor blood vessel formation provide a rational basis for anti-angiogenic drug development. 2-Methoxyestradiol (2-ME_2), once considered an inactive end metabolite of estradiol, has recently emerged as a very promising agent for cancer treatment. It is synthesized by sequential hydroxylation of the parent compounds followed by methylation in the liver. 2ME2 was reported to elicit both stimulation and inhibition of tumor angiogenesis and growth depending on the dosage used. However, the mechanism(s) of the biphasic action of $2ME_2$ has been elusive. The present study was undertaken to determine the dose dependent effect of $2ME_2$ on VEGF Mrna and protein levels in MCF-7 human breast cells, MIA-PaCa-2 pancreatic cancer cells and GH3 rat pituitary tumor cells, and to correlate these with cell proliferation. Mrna and protein levels were determined using Northern blot and Western blot analyses. Cell proliferation and death rates were measured using 3H-thymidine incorporation into untreated and 2ME2 treated cells. A dose-dependent biphasic effect of $2ME_2$ on VEGF-A Mrna and protein expressions and cell proliferation was found in MCF-7 and GH3 cells, while this biphasic effect was undetected in MIA-PaCA-2 cells. One mM $2ME_2$ significantly increased VEGF Mrna and protein as well as ^{3}H-thymidine incorporation in both MCF-7 and GH3 tumor cells as compared to untreated cells. In contrast, a trend of decreasing expression of VEGF-A Mrna and rate of cell proliferation was noted with higher (5-10 mM) $2ME_2$ concentrations. A low dose of 2-ME_2 also increased the VEGFA Mrna expression in ER-a-transfected normal breast epithelial cells. Moreover, the enhanced expression could be blocked by an antiestrogen ICI 182,780 and suggest that 2-ME_2-induced VEGF-A expression is mediated through estrogen receptor. Furthermore, the studies demonstrate that induced VEGF protein is functionally active because it potentiates proliferation of adjacent endothelial cells. Taken together, the results of these studies indicate that $2ME_2$ is a novel therapeutic drug for breast cancer.

DO-17

Identification of the Signal Transduction Pathways Associated with the Enhanced Survival Ability of Fibroblasts after Exposure to Repetitive Low-grade Stress: Reversal of the Anti-apoptotic Effects by Tea Polyphenols and Resveratrol

Sanghamitra Raha*

Crystallography & Molecular Biology Division, Saha Institute of Nuclear Physics, 1/AF Bidhan Nagar, Kolkata-700064, India. * sanghamitra_r@yahoo.co.uk

V79 fibroblasts were repetitively stressed through multiple exposures to a low dose (30 mM) H_2O_2 in culture for 4 weeks. p38MAPK became dually phosphorylated and ATF-2, a p38MAPK substrate also became increasingly phosphorylated over the repetitive stress period. Transcriptional activity driven by Nuclear Factor kappa B (NF-kB) was significantly (4 fold) enhanced by repetitive oxidative stress and was completely blocked by inhibition of p38MAPK activation. Catalase activity, protein levels and mRNA levels rose markedly (5-6 fold) during this time. Furthermore, Akt/Protein kinase B (PKB) became gradually phosphorylated at $Serine^{473}$ and $Threonine^{308}$ during this period of repetitive stress. The repetitively stressed cells demonstrated a significant resistance to apoptosis by subsequent acute stress in the form of ultraviolet radiation (UVR) at $5J/m^2$ or H_2O_2 (7.5 mM). Inhibition of p38MAPK activation by repetitive stress either through exposure to pharmacological inhibition or siRNA-induced silencing of p38MAPK resulted in inhibition of down-stream activation of some anti-apoptotic signaling pathways. Pharmacological blockage of Akt activation also resulted in partial inhibition of the survival effects. The opposition to apoptosis conferred by repetitive stress was drastically reduced by constant exposure to tea polyphenols or resveratrol during the stress period through their restraining effects on the signaling pathways associated with cellular survival. An overview of the multiple signaling events triggered by repetitive low-grade stress and inhibition of these pathways by natural compounds will be presented.

DO-18

Role of 5'Untranslated Regions of Human Cathepsin L mRNA Species in Determining Their Stabilities and Translational Efficiencies

Shyam S. Chauhan*

Department of Biochemistry, All India Institute of Medical Sciences, Ansari Nagar, New Delhi-110029, India.
*s_s_chauhan@hotmail.com

Cathepsin L, is over expressed in a variety of human tumors. A majority of the procathepsin L synthesized by transformed cells in culture is secreted into the medium for which it requires an intact carboxy terminus. It has been established that the switch from nonmetastatic to highly metastatic phenotype of human melanoma cells is directly related to secretion of procathepsin L. Blocking the secretion of this protease by anti cathepsin L SC-FV could abolish the tumorigenic and metastatic potential of melanoma cells in nude mice. Others and we have established that human cathepsin L is encoded by at least five mRNA species namely hCATL A, AI, AII, AIII and hCATL B. All these mRNA species contain identical open reading frames but differ in their 5'untranslated regions. These mRNA species are transcribed from two different promoters which are differentially regulated. HCATL A, AI, AII and AIII are produced by the alternate splicing of the same primary transcript transcribed from proximal promoter while hCATL B is transcribed from the alternate promoter. My laboratory identified hCATL AIII for the first time, and established that this most abundant splice variant of cathepsin L is also most efficiently translated. It also exhibit higher stability as compared to other splice variants. We further demonstrated that the 5' UTR of hCATL AIII is enough to confer translational advantage to a hetrologous mRNA. Thus the 5' untranslated regions of human cathepsin L mRNA species is sufficient for determining their translational efficiencies and stabilities.

DO-19

Investigation on the Role of Thymic Peptides in Tumor-Induced Immunosuppression

Pratima Shrivastava, Sukh Mahendra Singh*

School of Biotechnology, Banaras Hindu University, Varanasi, India. *sukh2001@sify.com

Introduction: Progressive growth of a spontaneous T cell lymphoma, designated as Dalton's lymphoma (DL), causes suppression of immune responses and thymic atrophy. It was hypothesized that the decline in the production of thymic peptides could be one of the causes of the inhibited immune responses of DL-bearing host. **Objective:** The effect of thymic peptide: thymosin 1 (thya1) on the functions of tissue macrophages, tumor-associated macrophages (TAM), TAM-derived dendritic cells (DC) and tumor-induced apoptosis of thymocytes was studied. **Methods:** Effect of *in vivo* administration of thya1 on the activation of various types of macrophages, DC, bone marrow hematopoiesis of myeloid cells and tumor-induced apoptosis of thymocytes was investigated using cell culture based techniques and bioassays. **Results:** *In vivo* administration of thya1 or thymus extract resulted in augmentation of cytotoxic and accessory functions of TAM and DC. Macrophages displayed heterogeneity in their response to thya1. Thya1 was also found to prime the macrophages to show an augmented responsiveness to activation signal of endotoxin. Thya1 administration and adoptive transfer of thya1 treated macrophages or DC to tumor-bearing mice prolonged their survival along with inhibition of tumor progression. Thya1 suppressed myeloid differentiation of bone marrow progenitor cells. Thya1 antagonized tumor-associated apoptosis of thymocytes by altering the expression of apoptosis related genes. **Conclusion:** This study shows that thya1 can be used to overcome the immunosuppressed state of macrophages and DC in a tumor-bearing host. Thus these findings may have long lasting impact on the development of tumor immunotherapy using thymic peptides.

DO-20
Exposure Estimates of Indian Chromeplaters: A Prefatory Study

Roli Budhwar, Mohan Das, Vipin Behari and Sushil Kumar*

Industrial Toxicology Research Centre, Lucknow. *sushilkumar_itrc@hotmail.com

Introduction: Chromate compounds are occupational carcinogens. Exposure to carcinogenic Cr results in a variety of adverse health effects ranging from dermatitis or skin ulcer to nasal septum perforation, inflammation & carcinoma of respiratory tract. A substantial population of chromeplating subjects in India gets exposure to carcinogen occupationally. However there is a paucity of information on their exposure estimates and health risk assessments in India. **Objectives:** A preliminary study of chromeplaters (n=24) and suitable control (n=35) with a cross-section study design was, therefore, undertaken. **Methods:** A structured interview recorded the prevalent adverse health effects. Analyses of Cr and DPC content in biological specimens (blood/urine) provided internal Cr exposure and related biological effect estimates. The metal was quantified by direct dilution method using AAS & graphite furnace. The DNA protein crosslink (effect biomarker) was investigated bio-chemically in peripheral lymphocytes. **Result**: Clinical symptoms were present in 1/3rd population of chromeplaters (n=8). These included redness in conjunctiva with prominent bulbar vessels, congestion of nasal mucosa and dermatological ailments. No clinical symptoms were present in non-chromeplating subjects. The urinary Cr levels were found to be greater in chromeplaters (n=18). Blood Cr levels (n=24) also showed an increasing trend. DPC content was greater significantly in chromeplaters (n=17). An investigation of the DPC coefficient (a quotient of DPC and total DNA) also revealed a similar result. **Conclusion:** This exploratory study validated the Cr monitoring biomarkers. It has revealed the importance and need for similar endeavors, albeit with a large sample size. More studies are needed to explore (a) the prevalent morbidity pattern, (b) its association with (both systemic as well as topical) exposure and (c) intervention strategies in Indian chromeplating subjects.

DO-21

Transdifferentiated Monocytes as Markers for Endothelial Differentiation, Tumor Angiogenesis and Growth

***Vimlarani Chopra*[1]*, *Edward V Hannigan*[1] *and Cherylyn A Savary*[2]**

The University of Texas [1]Medical Branch, Galveston, TX and [2]M.D. Anderson Cancer Center, Houston, Texas, USA. *vchopra@utmb.edu

We have detected the presence of $CD1a^+$ Langerhans/Dendritic cells (DCs) in monocultures of tumor-derived epithelial cells and their cocultures with mononuclear cells from patients with cervical cancers (>CIN II) maintained as three-dimensional (3-D) culture in bioreactors. These cells were also found in circulation of cervical cancer patients. In the 3-D culture environment, the generation of $CD1a^+$cells was accompanied by changes in expression of pSTAT3, IL-8, IL-10, IL-12, TNF-a, and NO radicals. We are using the bioreactors (mimics the in vivo host microenvironment) to gain insight into the effects of tumor environment on DC differentiation, maturation and functions. Phase contrast microscopy confirmed the formation of very large, nonadherent, and loose 3-D aggregates of developing CD1a+cells (in presence of GM-CSF+IL-4) which consisted of $Lin^-HLA\text{-}DR^+$, Lin^-B7-2+ (CD86+) and Lin- CD40+ cells. These plastic adherent cells cultured in the presence of conditioned medium from 3-D grown HeLa were CD34+ (stains endothelial precursors and mature endothelial cells), and showed evidence of tube formation and formation of network-like structures was observed by adherent cells after immunofluorescence staining. These cells were also PECAM-1^+(CD31+, constitutive endothelial marker), and VCAM-1 (CD106+) and ICAM-1 (CD54+,inducible endothelial markers).This transdifferentiation appeared to be due at least in part to the presence of VEGF, since no CD31+ cells were detected in the presence of neutralizing antibodies to VEGF. Hence under local angiogenic conditions myeloid cells, including DC progenitors might be directed towards differentiation into endothelial-like cells and can serve as early markers of tumor growth, angiogenesis and metastasis.

DO-22

Trends in Incidence of Breast Cancer-Indian Scenario

***NS Murthy*[*1], *S Saxena*[2], *K Chaudhry*[1] *and A Pandey*[3]**

[1]Indian Council of Medical Research, [2]Institute of Pathology (ICMR), [3]Institute for Research in Medical Statistics (ICMR), New Delhi. *nsmurthy44@yahoo.com

Globally, Breast cancer is the most frequent female malignancy. It is the most frequent cancer among women in Delhi, Mumbai, Ahmedabad, Calcutta and Trivandrum. In other registries, it is listed as the second leading site among women. Reports of increasing rates of breast cancer in several Indian registries prompted this analysis of trends in the incidence rate of breast cancer by age, period and cohort. Incidence data for the various Indian registries for 5-year periods from 1968-72 to 1993-97 were obtained from the different volumes of Cancer Incidence in Five Continents or from the publications made by the individual registries. Annual percentage changes (APC) in incidence rates were computed using relative difference between the two time periods for crude rate (CR), age adjusted rate (AAR) and age-specific incidence rates (ASIR). In 1993-1997, AAR of breast cancer were highest in Mumbai (28.9/100,000 woman-years) and lowest in the rural registry at Barshi (8.1 per 100,000 woman-years) The rise in ASIR of breast cancer was seen up to ages of 59 years in most of the registries followed by a slight drop and a slower increase in older age groups. The APC by age

revealed different patterns of changes in different registries. Trend analysis by period revealed that breast cancer incidence rates rose among women in all the registries with an exception of Ahmedabad registry. The highest annual percentage increase in AAR was noted in Mumbai (2.0%) and lowest for Pune (0.5%). Risk was found to be increasing in successive birth cohorts. Efforts should be made to detect breast cancer at an early stage by educating the population about the risk factors and through periodic screening either by physical self-examination or by self-breast examination. Mammography will be difficult to implement in Indian for the prevention and control of breast cancer.

DO-23

Detection of DNA Damage (Strand Breaks) in Marine Mollusks - An Early Warning Signal of Carcinogenesis

A.Sarkar**, D. Gaitonde and D. Ray

Marine Pollution Assessment and Ecotoxicology Group, National Institute of Oceanography, Dona Paula, Goa - 403004, India. *asarkar@darya.neo.org

DNA strand breaks are regarded as one of the primary causes of cancer. It can occur due to various factors. DNA strand breaks in marine organisms can occur due to interaction with various genotoxic substances such as PCBs, PAHs, PCDDs, PCDFs, TBT, methylmercury, lead etc. which are found to be prevalent in the marine environment. Cytochrome P450 enzymes generally metabolize PCBs to mono and dihydroxy-PCBs. It is evident that dihydroxy PCBs can potentially be oxidized to corresponding quinones which could probably produce reactive oxygen species leading DNA- strand breaks. In case of PAHs, Benzo (a) pyrene is generally converted to chemically reactive diol epoxide (BaPDE) at cellular level and subsequently interact with DNA to form stable adduct resulting into single strand breaks. In order to protect human health from carcinogen it is indeed of prime importance to identify the sources of geotaxis contaminants along the coastal region. In this context, detection of DNA damage (strand breaks) in terms of DNA integrity in marine mollusks (snails, oysters and clams) acts as early warning signal of carcinogen sis. DNA strand breaks in marine mollusks were determined in terms of DNA integrity (expressed as F) following the technique of partial alkaline unwinding assay in which three different parameters were measured - percentage of double strandedness, single strandedness and alkaline unwinding under a defined condition of pH and temperature. The results show significant variation in the integrity of DNA in marine mollusks at different locations. The DNA integrity in oysters (*Saccostrea cuculata*) was found to be quite low at Murmugao harbour (F, 0.177) and Chicalim (F, 0.266) while in clams (*Grafrarium divaricatum*) it was considerably higher at Dona Paula (F, 0.473) and Chicalim (F, 0.313). In the case of snails (*Planaxis sulcutas*) it was found to be in the higher side. (F 0.33 and F 0.553). The lower the integrity of DNA higher will be the strand breaks. Thus it is clearly shows presence of genotoxic substances in the marine environment, which could be transmitted to human tissues through the food chain leading to carcinogenesis.

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AWARD NOMINATIONS

C. Oral Presentations
Dr. V.B. KAMAT MEMORIAL AWARD & SHRI R.H. JAJU AWARD

KJ-1
Early Detection of Breast and Cervix Cancers among Women in India: A Cohort Trial in Mumbai

Gauravi Mishra**, K.A. Dinshaw, S.S. Shastri

Tata Memorial Hospital and Research Center, Mumbai. *mgauravi@rediffmail.com

Breast and cervix cancers account for over 50% of cancers among Indian women. Unfortunately, women have very poor health seeking behaviour, resulting in over 80% reporting for treatment at advanced stages of the disease. To investigate the efficacy of low-cost screening methods viz. clinical breast examination and visual inspection of cervix painted with 4% acetic acid (VIA), performed by trained primary health workers, in down-staging and thereby reducing the incidence of cervix cancers and mortality due to breast and cervix cancers. This is cluster randomized trial and cohort study involving 1,52,239 women between the ages of 35-64 yrs residing in the slums of Mumbai, India, randomly allocated into an Intervention Arm, to receive 4 rounds of intervention at 24 months interval, followed by 4 rounds of Active Surveillance over next 8 years and a Control Arm. The study has completed 6 years and the average compliance for screening is 68%. 1% of women are screened positive for breast and 1.5% for cervix. 70% of screened positive women have complied for further diagnostic confirmation. Cancer detection rate is 7% for breast and 3% for cervix among the complied women. Till date, there are 109 and 71 cases of breast and cervix cancers respectively along with 48 HSIL and 92 LSIL cases from intervention arm and 52 and 40 cases of breast and cervix cancers along with 1 HSIL and 9 LSIL from control arm. 90% of patients have completed their treatment with regular follow-up.

KJ-2
Establishment of Experimental Model for Oral Cancer: Use for Understanding the Molecular Mechanism of Retinoid Action

Kaur J** and Ralhan R

Department of Biochemistry, All India Institute of Medical Sciences, Ansari Nagar, New Delhi-110029, India. *jatin_kaur@hotmail.com

In view of the rapid upsurge in consumption of smokeless tobacco products, a challenging future goal is to develop an experimental model for smokeless tobacco associated oral carcinogenesis. Using this model, the network of regulatory circuitry in the cancer cells originating from smokeless tobacco consumption could be studied in depth. The insights obtained from this new level of analysis will provide powerful approaches for cancer prevention and treatment. These studies underscore the need to establish experimental models for oral squamous cell carcinoma originating from tobacco chewers for studying

the basic mechanism underlying chewing tobacco associated oral carcinogenesis.In this context, a cell line, AMOS-III, has been established from the surgically resected specimen of an untreated primary human oral squamous cell carcinoma of the floor of mouth from a chronic smokeless tobacco consumer. Characterization of this cell line included demonstration of markers of epithelial cell lineage including epithelial specific antigen, cytokeratins 5, 10, 13 and 16 and integrin 6, ultrastructure analysis, anchorage independent growth for confirmation of the transformed phenotype of epithelial cells and karyotyping for confirmation of human origin of AMOS-III cells. The status and expression of genes implicated in cell cycle regulation, apoptosis, angiogenesis, invasion and metastasis was determined. This experimental model has been used to (i) identify novel molecular targets of tobacco constituents in oral cancer and (ii) delineate the mechanism of action of retinoids. The molecular mechanism(s) underlying the action of retinoids in preventing the occurrence of second primary tumors in oral cancer patients remain to be clearly defined. Treatment of MOS-III cells with all-trans retinoic acid (ATRA, 10^{-4}mM) resulted in nuclear translocation of retinoic acid receptors, enhanced expression of p21 $^{cip/waf1}$ and apoptotic cell death. The genes that are differentialy expressed in ATRA treated cell were identified by Differential Display RT-PCR. Thus, AMOS-III cell line provides an in vitro model for elucidating the mechanism underlying smokeless tobacco induced oral cancer. Furthermore, the ATRA responsiveness of the cell line underscores its potential utility in identifying the retinoid responsive molecular targets in oral cancer cells.

KJ-3

In Vivo Magnetic Resonance Spectroscopic (MRS) evaluation and its histopathological correlation in diagnosing Soft Tissue Sarcomas

Madhabananda Kar*[*1], N.K.Shukla[1], N.R.Jagannathan[2], S.V.S.Deo[1], Uma Sharma[2], S.Datta Gupta[3], Virendra Kumar[2]

[1]Department of Surgical Oncology, IRCH, [2]Department of N.M.R and [3]Department of Pathology, All India Institute of Medical Sciences, New Delhi-29, India. *mkar279@yahoo.co.in

Introduction: The grade, size and depth of the sarcomas are the important factors to prognosticate the disease. But it is very difficult to grade tumor even with experienced pathologists. This emphasizes the need for an accurate assessment of sarcoma grading and typing. The biochemical changes in tissue lipid found to correlate with sarcoma cellularity, growth rate and differentiation. **Objective:** To Evaluate- 1. *In vivo* MRS in different sarcomas, 2. Its histopathological correlation, 3. MRS role in post surgery recurrence / residual disease. **Methods:** All patients submitted for in vivo proton MRS study after routine tissue diagnosis and imaging method. MRS performed at 1.5 Tesla. Following the scout image, T1/T2-weighted MR images obtained in 3 orthogonal planes and then depending on the tumor size, voxels of appropriate dimension have been positioned well within the tumor area for MRS study. Volume localised *in vivo* proton MRS carried out using the STEAM (Stimulated-echo acquisition-mode) & PRESS pulse sequence. Multiple scans with / without water suppression have been collected using a fixed echo time (TE) and repetition time (TR). All patients have been operated by single oncosurgeon and histopathological evaluation done by single oncopathologist. Adjuvant therapy has been decided by the tumor histopathology. **Results:** Five patients have been evaluated by the MRS study. Two patients have single voxel and three by CSI (Chemical shift Imaging) study. All MRS findings have been correlated with histopathological grade of the tumor and CSI has effectively diagnosed the tumor activity in the periphery as well as core of the tumor in all cases. **Conclusion:** MRS study can diagnose the tumor grade, margin status and tumor activity in recurrent and / or residual soft tissue sarcoma patients and thus may guide the proper management of the disease.

KJ-4

Epigenetic Modification(S) and Genomic Instability during Onset and Progression of Liver Cancer

Sanjay Gupta*, N Vishnoi, B Khade and KVK Rao

Chemical Carcinogenesis Group, ACTREC, Tata Memorial Centre, Kharghar, Navi Mumbai 410 208, India. *sgupta@actrec.res.in

Chromatin is an important and dynamic regulator of transcription, replication, and chromosome segregation. Post-translational modification of histones appears to play a central role in the regulation of neoplasia, tumor suppression, cell cycle control; hormone responsiveness and oncogenesis. Our work strives to elucidate the signalling pathways responsible for specific histone modifications and the molecular mechanisms by which these modifications affect chromatin structure and DNA-template processes in the progressive growth of an early lesion to neoplastic nodule and which sequentially progresses to malignant tumor. Western blot analysis shows increased levels of H1, H3 and H4 Ser- and Tyr/Thr phosphorylation with the progression of tumor without affecting the levels of histone proteins. The levels of acetylated histones are undetectable in control liver but H1, H3 and H4 show increase in the acetylation of lysine residue with the progression of tumors. The silver stained PAGE shows standard pattern of H1, H2A, H2B, H3 and H4 in control. After DEN-treatment, an extra band, named, as H3X is present between H2A and H4. Western blotting with H3 antibody shows two bands in spite of a single band of H3 in DEN-treated rats that confirm the fragmentation of H3 into H3X. The fragmentation of histone H3 increases with the sequential development of tumor. The specific amino acid residue in the amino terminus, Ser-10 and Lys-18 of H3 and Lys-16 of H4 show no significant post-translational modifications, whereas acetylation of Lys-9 of H3 and Lys-8 of H4 increases significantly with sequential development of liver tumor. Flow cytometric analysis of purified nuclei showed increased 4N fraction or aneuploid cells and G_0/G_1 population in neoplastic nodules and hepatoma in comparison to control. These results suggest that aberrant modification of nuclear proteins; a focal point for both positive and negative transcriptional control may be implicated in the etiology of human diseases including cancer and will enable the development of new therapeutic strategies.

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SHRI SITARAM JOGLEKAR AWARD & SMT. MANGALA BAMANE AWARD

JB-1

Neem Leaf Preparation Enhances *in vitro* Cytotoxic Efficacy of Peripheral Blood Mononuclear Cells from Head and Neck Squamous Cell Carcinoma Patients by Modulating Cytokine Signaling Pathway

***Anamika Bose*[1]*, *Diptendu Ghosh*[1], *Smarajit Pal*[2], *Soumitra Chaudhuri*[3] and *Rathindranath Baral*[1]**

[1]Department of Immunoregulation and Immunodiagnostics, [2]Unit of Clinical Biochemistry, [3]Department of Environmental Carcinogenesis and Toxicology; Chittaranjan National Cancer Institute, 37 , S. P. Mookherjee Road, Kolkata -700026, India. *bose_shasika@yahoo.co.in

Introduction: Different preparations from the plant, neem (Azadirachta indica), are known to have potent immunomodulatory properties. Here, this unique property of neem leaf preparation (NLP) is attempted to utilize for the restoration of altered immune responses in head and neck squamous cell carcinoma (HNSCC) patients. **Objectives**: To know whether NLP helps to restore suppressed cytotoxic activity of peripheral blood mononuclear cells (PBMC) and to elucidate underlying immune mechanism. **Methods**: PBMC were cultured in RPMI 1640 medium. Immunosuppression was monitored by PBMC proliferation MTT assay. Cytotoxicity towards various cancer cells (KB, MCF7 and K562) was determined either by LDH release assay. Cytokines were measured by ELISA. Status of various immune cells and intracellular secretion of cytokines were assessed by flow cytometry. Composition of NLP was assessed by Laser Capture Mass Spectroscopy (LC-MS). **Results**: HNSCC-PBMC showed lower proliferative index and lesser number of NK and T cells. Cytotoxic efficacy of HNSCC-PBMC towards KB and MCF7 cells was significantly less ($p<0.001$), in comparison to healthy individuals. This suppressed killing activity could be partially restored by in vitro treatment of PBMC with NLP, which contains a good amount of flavonoids. These activated cells were cytotoxic to NK specific target, K562 too. Analysis of NLP stimulated HNSCC-PBMC revealed the higher secretion of IL-12 and IFNg. Greater intracellular secretion of IL-12 was observed from NLP activated monocytes. Co-culture of HNSCC-PBMC with IL-12 containing culture supernatant resulted increased release of IFNg, which was inhibited upon addition of anti-IL-12. Moreover, NLP induced higher number of NK and T cells *in vitro*, may secrete greater amount of IFN . Involvement of Th1-Th2 crosstalk and JAK-STAT signaling pathway in the cytokine secretion was investigated. **Conclusion**: *In vitr*o results are suggestive that NLP may be useful therapeutics during treatment of immunosuppressed HNSCC patients.

JB-2

Role of Genetic Predisposition in the Development of Multiple Primary Neoplasias (MPN)

***Ashwin L. Kotnis*[1]*, *Rajiv Sarin*[2], *Rita Mulherkar*[1]**

[1]Genetic Engineering, Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Kharghar, [2]Department of Radiation Oncology, Tata Memorial Hospital, Parel, Navi Mumbai - 410208. * ashamit2@rediffmail.com

Head and neck cancers (HNC) are significant cause of cancer mortality in India and are widely believed to be due to carcinogens. However, carcinogen exposure entirely cannot explain why ~16% of patients with HNC subsequently develop multiple tumors. Inter-individual differences in genetic susceptibility can be an

important determinant for risk of MPN. In our study we hypothesize that MPN may be a manifestation of polygenic susceptibility to carcinogens, presenting MPN as a biological model to understand gene-environment interactions. We have genotyped MPN patients with at least one HNC, for genes implicated in tobacco related cancers. The phenotype has been analysed using carcinogen-treated lymphoblastoid cell lines established from patients and controls. Genotyping of *GSTM1, GSTT1, GSTP1, MPO, SULT1A1 p53, p21, FAS* was done using PCR-RFLP/ DHPLC of 150 ethnic patients with MPN and 100 healthy matched controls. PCR product authenticity was confirmed by sequencing 5% of the samples. Difference in genotype prevalence in cases and controls was done using Chi square analysis. Phenotypic analysis was done on BPDE (tobacco specific carcinogen) treated lymphoblastoid cell lines (10 each from patients and controls), by assessing cell death using flow cytometry and CELLQUEST software. GSTM1 and GSTT1 genotype show significant difference in MPN patients as compared to controls. The phenotypic analysis shows that MPN cell lines are more susceptible to carcinogen-induced damage as compared to controls. The analysis of cumulative effect of all genes in predisposing to MPN is underway. Cumulative effect of polymorphisms in low penetrance genes together with carcinogen exposure, predispose an individual to MPN in the Head and Neck.

JB-3

Turmeric Mediated Activation of Lymphocytes and Induction of Apoptosis of Tumor Cells

Hadida Yasmin*, *Tamal Mazumdar, Pragya Mittal and Ashim Kumar Chakravarty

Immunology & Cell Biology Lab, Centre for Life Sciences, North Bengal University, Rajarammohan Pur, Siliguri- 734430, India. *hadida_y@yahoo.com

Turmeric has been shown to possess variety of pharmacological properties such as anti-inflammatory, anti-carcinogenic and anti-oxidant by different workers. We have reported that turmeric also activates the lymphocytes and induces apoptosis to tumor cells. Thus, the turmeric has two-way efficacy in controlling tumors in murine model. Present investigation is to delineate details of blastogenic response of T cells and B cells, cytotoxic response of effector lymphocytes by 51Cr-release assay and conjugate formation between lymphocytes and tumor target cells as basic steps toward cytotoxicity in the presence of ethanolic turmeric extract (ETE). ETE was able to activate blastoid transformation of murine lymphocytes, especially the T cells, but the level of stimulation was found to be lower than that of Con A, a polyclonal stimulator. Furthermore, the ETE stimulated cells have been found to become cytotoxic to tumor cells in 51Cr-release assay. The percentage of cytotoxicity obtained with ETE treated cells was higher in comparison to the alcohol treated and untreated normal lymphocytes. Interestingly number of conjugates formed by tumor target cells and effector lymphocytes increased with ETE treatment. The conjugate formation is a prerequisite for mounting cell mediated response. ETE in all likelihood initiates well the cell mediated immune response as evident from cytotoxicity index and microscopic observations of blebbing of tumor cells which indicates setting on of apoptosis. The results of this investigation clearly suggest ETE as a potent immunotherapeutic agent to treat malignancy in murine model. These findings should find applicability for human after determining proper dose and route of ETE.

JB-4

Immunomodulatory and Antitumor actions of Medicinal Plant Tinosora cordifolia in Tumor-Bearing Host

Nisha Singh*, Sukh Mahendra Singh

School of Biotechnology, Banaras Hindu University, Varanasi, India. *nishasingh4@sify.com

Introduction: We had previously shown that progressive growth of a spontaneous T cell lymphoma, designated as Dalton's lymphoma (DL), results in immunosuppression along with derailment of T cell homeostasis resulting from tumor-associated thymic atrophy. Our quest, therefore, was to identify an appropriate biological response modifier with the potential to reverse such tumor growth associated immunosuppression. There has been a considerable interest in *Tinospora cordifolia* an Indian medical plant with powerful immunostimulant activity has been evaluated as an adjuvant in clinical conditions of some immunodisorders. We were interested to investigate if *Tinospora cordfolia* could augment antitumor defense responses in a tumor-bearing host. **Objectives:** In the present study we used alcoholic extract of *Tinospora cordfolia* (ALTC) for investigating its effect on the tumor-induced immunosuppression and tumor progression. **Methods:** Effect of in vivo administration of ALTC on the activation of tumor-associated macrophages (TAM), TAM-derived dendritic cells, myeloid differentiation of bone marrow progenitor cells and T cell homeostasis in relation to progressive tumor growth was investigated. **Results:** In vivo administration of ALTC resulted in an augmented antitumor and accessory function of TAM and TAM-derived dendritic cells. ALTC administration caused reversal of tumor associated thymic atrophy and restoration of Th1/Th2 cytokine balance along with an augmentation of myeloid differentiation of bone marrow progenitor cells. ALTC administration and adoptive transfer of ALTC-treated TAM or TAM-derived DC to tumor-bearing host resulted in slowing down of tumor progression and increased survival. **Conclusion:** This is the first study of its kind regarding the effect of Tinospora cordifolia on the reversal of tumor growth associated immunosuppression and may thus help in designing immunotherapeutic protocol of cancer.

JB-5

Establishment of Cell line Model to Study Signal Transduction Events in CML

Poonam R. Molli and Nishgandha R. Naik*

Cancer Research Institute, Advanced Centre for Treatment, Research and Education in Cancer, TMC, Kharghar, Navi Mumbai - 410208. *poonammolli@yahoo.com

Chronic Myeloid Leukemia (CML) is a stem cell disorder of haematopoeitic system. CML is characterized by presence of Philadelphia (Ph1) chromosome. Ph^1 formation results in a fused bcr-abl gene. Bcr-abl translates a chimeric protein p210 that has increased and unregulated tyrosine kinase activity. Polymorphonuclear leukocytes (PMNL) are the terminally differentiated cells of myeloid series. Chemoattractants, the key components of the cellular immune system, are involved in trafficking and activation of various functions in PMNL. Binding of a chemoattractant to its receptor on PMNL initiates a series of coordinated biochemical and functional events. CML PMNL exhibit defects in several actin dependent functions such as motility, chemotaxis, adhesion, aggregation, phagocytosis/endocytosis and microbicidal activities. A definite abnormality was observed in actin polymerization and actin per se. Major components of signal transduction pathways leading to actin polymerization in PMNL are similar to targets predicted for bcr-abl. Studies based on clinical samples show lot of variations. Therefore, a need was felt for a model system to study signal transduction events in CML. RIN/FPR/bcr-abl cell line was developed that expressed receptors for chemottractant - formyl peptide (FPR) and the bcr-abl protein simultaneously. RIN/FPR/bcr-abl is an unlimited source of material and it shows 100% tumorigenicity in scid mice. Moreover, it shows a lot of similarities with CML PMNL at the FPR-ligand interactions level and at the signal transduction level. Therefore, RIN/FPR/bcr-abl may serve as a unique model system to study pathogenesis of CML especially signal transduction events which would ultimately enable to identify new targets for drug development.

JB-6

Ethanolic Leaf Extract of Neem (Azadirachta idica) Inhibits Buccal Pouch Carcinogenesis in Hamsters

***R. Subapriya*[1], *V. Bhuvaneswari*[1], *V. Ramesh*[2] *and S. Nagini*[1]**

[1]Department of Biochemistry, Faculty of Science, Annamalai University, Annamalainagar-608 002, Tamil Nadu, India. [2]Department of Oral Pathology and Microbiology, Mahatma Gandhi Dental College and Hospital, Pondicherry-605 006, India. *r_kumar24@rediffmail.com

Chemoprevention by medicinal plants is a promising approach for controlling cancer. *Azadirachta indica* A Juss (Meliaceae), commonly known as neem is recognized to possess anti-inflammatory, hepatoprotective, antioxidant and anticarcinogenic properties. We evaluated the chemopreventive effects of ethanolic neem leaf extract (ENLE) on 7,12-dimethylbenz[a]anthracene (DMBA)-induced hamster buccal pouch (HBP) carcinogenesis. Hamsters were divided into four groups of six animals each. The animals in group 1 were painted with 0.5% DMBA three times a week. Hamsters in group 2 painted with DMBA as in group 1 received 200 mg/kg body weight ENLE by gavage three times a week on days alternate to DMBA application. Group 3 animals received ENLE alone while group 4 served as untreated control. The hamsters were killed after an experimental period of 14 weeks. Multiple markers reflecting different aspects of the carcinogenic process were used to monitor chemoprevention. These include the ability of ENLE to scavenge reactive oxygen species, modulate phase I and phase II detoxification enzymes and induce apoptosis. Administration of ENLE to DMBA-painted animals reduced the incidence of HBP tumours as well as bone marrow micronuclei, modulated the extent of cellular redox status as well as phase I and II detoxification enzymes and induced apoptosis as evidenced by downregulation of Bcl2 and upregulation of Bim and caspases 8 and 3. These results provide evidence that ENLE exerts multifunctional inhibitory effects on HBP carcinogenesis by inducing changes in the cellular redox status, carcinogen detoxification and apoptosis.

JB-7

Regulators of Telomerase and Telomere Length in Oral Cancer: Telomere Repeat Binding Proteins, Rb and c-Myc

***Rachana N. Sainger**[1], *Shaila D. Telang*[2], *Shilin N. Shukla*[1], *Prabhudas S. Patel*[1]**

[1]Biochemistry Research Division, The Gujarat Cancer & Research Institute, Ahmedabad - 380 016 Gujarat, India. [2]Biochemistry Department, Faculty of Science, M. S. University of Baroda, Vadodara - 390 001. *rachnasainger@yahoo.com

Telomere-telomerase hypothesis implicates telomere attrition and telomerase activation as critical factors in cancer. It has led current research to get insights into the mechanisms underlying specific activation of telomerase in cancers for clinical intervention and as a therapeutic target. Present work evaluated telomerase activation, telomere length alterations and regulatory proteins like pRb, c-myc, and Telomere Repeat binding factor-1 and -2 expression in oral cancers. We studied Telomerase activation by Telomeric Repeat Amplification Protocol (TRAP assay), telomere length alterations by Southern Hybridization method and protein expression by western blot method. Images were scanned and analysed using Gel Documentation unit and statistical analyses was done with SPSS software. We observed telomerase activation in 75% of the malignant and 63% adjacent normal tissues. Peak Telomere length was significantly shorter in tumor tissues ($p=0.015$) with over-expression of TRF-1 and TRF-2 ($p=0.141$ and $p=0.004$ respectively). c-myc oncoprotein

expression was associated with malignancy, however Rb expression was not, suggesting that there may be other pathways operating for telomerase activation. Kaplan Meier survival analysis revealed poor five-year disease free survival in the patients showing telomerase activation in the adjacent normal (p=0.004) and higher telomere lengths in the malignant tissues (p=0.05) as compared to their respective counterparts. Thus, our work shows significant utility of the parameters in the prognosis of oral cancer patients and demonstrates the importance of telomerase and telomere regulators in oral cancers. Also, it is the only report from India on oral cancer, analyzing telomerase and telomere regulators in patients, where oral accounts for 1/3rd of all malignancies.

JB-8

Unraveling Novel Molecular Targets in Esophageal Cancer by Gene Expression Profiling

Rinu Sharma*[1], T.K.Chattopadhayay[2] and Ranju Ralhan[1]

Department of 1Biochemistry, 2G.I.Surgery, All India Institute of Medical Sciences, Ansari Nagar, New Delhi-110029, India. *rinusharma@rediffmail.com

Introduction: Despite intensive multimodality therapy including surgery, radiotherapy and chemotherapy, prognosis of esophageal cancer remains poor with a 5 year survival rate of only 5-10%. Therefore, the development of diagnostic or preventive strategies requires an in-depth understanding of molecular mechanisms involved in the multistep process of esophageal tumorigenesis. **Objectives:** The aim of the study was to identify and characterize differentially expressed genes (cDNAs) in human esophageal cancer, to understand the molecular mechanism underlying tumorigenesis. In adittion the clinical significance of TC21R-Ras2, one of the differentially expressed genes, was determined in ESCCs. **Methods:** Differential display reverse transcription PCR was carried out to compare normal and malignant esophageal tissue. Differentially expressed bands were eluted, reamplified, cloned, sequenced and analyzed. The differential expression of these genes was confirmed by reverse northern blot analysis and RT-PCR. Immunohistochemical analysis and Confocal microscopy was carried out in ESCC tissues to analyze the localization and clinical significance of TC21. **Results:** In silico analyses revealed several clones that showed homology to metabolic enzymes, components of transcriptional/ translational machinery, cell proliferation, apoptosis, signal transduction , complement pathway , cell adhesion molecule and cell surface protein. Expression of TC21 protein (one of the differentially expressed genes) was observed in 60/83 (73%) ESCCs predominantly localized in tumor nuclei. Intriguingly, intense TC21 immunoreactivity was observed in all endoscopic biopsies with histological evidence of dysplasia as well as in dysplastic areas distant to ESCCs, while matched distant histologically normal epithelia did not show detectable TC21 expression. Immunoblotting and semi-quantitative RT-PCR confirmed TC21 expression in dysplasias and ESCCs. **Discussion:** Expression profiling of ESCC demonstrated complex alterations in expression of several novel genes in esophageal tumorigenesis. TC21/R-Ras 2, a member of the Ras superfamily of small GTP-binding proteins, found to be upregulated in ESCCs, is the first Ras related protein shown to have potent transforming activity. Its presence in preneoplastic lesions, dysplasia, and localized expression of TC21 in areas of high proliferative activity support the hypothesis that TC21 expression is an early target in esophageal tumorigenesis and may be of potential clinical relevance. This study also provides the first evidence of nuclear localization of TC21 making it the third of over 100 small GTP binding proteins (SMG) identified to be localized in the nucleus.

JB-9

Regulatory Role of NF-kB in DNA Damage Induced Apoptosis in High-Risk HPV-Positive Cancer Cells

Sandeep Singh and Manoj Kumar Bhat*

National Centre for Cell Science (NCCS), NCCS Complex, Pune-411007, India. *ssingh@nccs.res.in; san_deepsingh@yahoo.com

Introduction: HPV infection along with high levels of Bcl-2 protein is implicated in the development of invasive and highly aggressive cancer phenotype. The mechanisms involved in deregulated expression of Bcl-2 resulting in high levels of *bcl-2* mRNA and protein are not very clear. NF- B is the one of the transcription factors which is linked with several aspects of oncogenesis and survival of various tumors including cervical carcinoma. Moreover, the presence of constitutive activity of NF-kB and high levels of Bcl-2 is associated with resistance to chemotherapy or radiation. Therefore, it is of utmost importance to understand the status, role and response of these molecules to chemotherapeutic drugs to evaluate the efficacy of chemotherapeutic drugs on HPV-positive tumors. **Objectives** of Study: To investigate the involvement and response of Bcl-2 and NF-kB in Carboplatin or 5-fluorouracil induced cytotoxicity in HPV-positive cells. **Methods:** HEp-2 and KB cells were obtained from ATCC, USA. RT-PCR analysis, western blot analysis, Gel-shift assays, luciferase reporter assays and confocal microscopy was performed to investigate various genes or proteins involved. **Results:** We report that apoptosis induced in response to DNA damage is due to inactivation of *bcl-2* promoter resulting in protein downregulation without affecting pro-apoptotic Bax levels. Additionally, ectopic expression of Bcl-2 reversed the cytotoxicity of these drugs. Further, we demonstrate the presence of constitutively high levels of active NF-kB in HEp-2 cells as an essential factor for survival. Carboplatin treatment blocked the nuclear accumulation of NF-kB via stabilization of IkBa protein. Finally, we showed the direct inhibition of NF-kB binding to Bcl-2 promoter resulting in its downregulation. **Conclusion:** We propose the inactivation of NF-kB mediated Bcl-2 downregulation as one of the mechanisms underlying the induction of DNA damage induced apoptosis in HPV positive cancer cells. Additionally, overexpression of Bcl-2 reverses the cytotoxicity of these drugs thus, we for the first time proposed that the tumor cells of certain HPV positive cancers which have impairment in the pathways responsible for downregulation of Bcl-2 will likely be resistant to Carboplatin or 5-fluorouracil treatment.

JB-10

Identification of Sialoglycoconjugate Specific Antibody in Bronchoalveolar Lavage Fluid of Patients with Non-small-cell Lung Carcinoma

Sangeeta Mehta, SC Sharma, D Behera, S Majumdar, S Radhika and S Ghosh*

Postgraduate Institute of Medical Education and Research, Chandigarh-160012, India. *sangeeta9_mehta@yahoo.co.in

Introduction: The carbohydrate moiety characterizes the cohesive, adhesive and antigenic properties by its effects on cell-to-cell contacts. These properties often change after malignant transformation of cells, thus allowing the carbohydrate specific monoclonal antibodies to discriminate among various tumors and normal tissues. Many antigens recognized by these antibodies are glycoprotein and glycolipid in nature. The presence of antibodies against modified sialoglycoconjugates has long been identified in various malignant transformations. However, no progress has been made to identify the presence of the disease specific sialoglycoconjugate specific antibody in the

bronchoalveolar lavage (BAL) fluid of lung cancer patients. Thus in the present study, an attempt was made to detect the sialoglycoconjugate specific antibodies in BAL of non-small-cell lung cancer (NSCLC) patients. **Methods and Results:** BAL fluid was collected from the diseased and normal lungs of non-small-cell lung cancer patients (n=36), and also from the lungs of patients with ILD (n=6) and sarcoidosis (n=7). Used fetuin, as the broad spectrum sialoglycoconjugate to determine the levels of antibodies, we found significantly higher levels of fetuin specific IgG present in BAL as compared to that of IgM and IgA. Moreover, the fetuin specific IgG level was higher in case of the BAL fluid collected from the lung having tumour as compared to that from each set of controls (normal lung of the same patient and that from the ILD and sarcoidosis patients). Gangliosides were used for further screening of the sialoglycoconjugate specificity of IgG present in the BAL fluid and it was observed that the level of GM3 specific IgG was significantly higher in comparison to other gangliosides. IgG was purified from the pooled BAL fluid from the diseased lung of the cancer patients as well as from controls. IgG purified from the BAL fluid collected from lung having tumour was found to interact with the membrane component of the NSCLC cell lines in cytocentrifuge preparation and the membrane component was identified in Western Immunoblot as a ~43-kDa glycoprotein. No such interaction was observed with IgG purified from control BAL. **Conclusion:** Thus, the detection of the disease specific IgG in the BAL fluid of non-small-cell lung cancer patients may be helpful in the diagnosis of NSCLC.

JB-11

Antisense Therapy in CML: Effect of Derivatization of Oligonucleotides

Shubhada S. Bharne ***and Nishigandha R. Naik***

Biochemistry and Cell Biology, CRI, ACTREC, TMC, Kharghar-Navi Mumbai-410208. *shubhadabharne2003@yahoo.com

Chronic Myeloid leukemia (CML) is a fatal disease of the haematopoietic system that accounts for 15-20% of the leukemias in the world. The current therapy for CML includes chemotherapy and bone marrow transplantation (BMT). Chemotherapy is not specific and affects normal cells while availability of matched donor is a major limitation of BMT. Development of resistance has been reported against the wonder drug-gleevec. Hence there is need for an alternative therapy. Bcr/abl gene, a chimeric gene formed as a result of rearrangement of bcr and abl genes, is a consistent genetic abnormality found in CML. Bcr/abl protein is a constitutively activated tyrosine kinase that is associated with the pathogenesis of CML. Taking advantage of this consistent abnormality in the leukemic cells; antisense oligonucleotides against bcr/abl have been designed. This paper evaluates effect of various antisense oligonucleotide derivatives like phosphodiester oligonucleotides, phosphorothioates, molecular beacons, morpholinos etc. as therapeutic modalities in CML. Effect of oligonucleotides on bcr/abl positive cells lines was studied by dye exclusion method. Expression of bcr/abl mRNA and proteins was studied by RT-PCR and Western blotting, respectively. Among the various derivatives tested molecular beacons were the best candidates as antisense agents. In addition to the oligonucleotides effect of synthetic compounds as specific inhibitors of bcr/abl positive cells is being studied.

JB-12

Role of Physical Exercise, Hormones and Cytokines in Immunological Regulation of Tumor Growth

Mahendra Pal Singh*, Sukh Mahendra Singh*

School of Biotechnology, Banaras Hindu University, Varanasi, India. *psmahen@yahoo.com

Introduction: Earlier we have shown that the ascitic growth of a transplantable T cell lymphoma of spontaneous origin, designated as Dalton's lymphoma (DL), is associated with a concomitant immunosuppression involving host and tumor derived suppressive mechanisms. However, the involvement of neuroendocrine regulation of host-tumor relationship remained unknown. **Objectives:** In order to understand the neuroendocrine connections in immunological regulation of tumor growth we studied the role of physical stress, gender-specific hormones and cytokines in the progression of a T cell tumor. **Methods:** The immunological profile of DL-bearing mice along with progressive tumor growth was investigated in relation to physical exercise, gender dichotomy, effect of castration and hormone replenishment. **Result:** DL growth was observed to be faster in females compared to males. We demonstrate the involvement of gender specific gonadal hormones, tumor-associated macrophage-derived IL-1 and differential level of IL-4, IL-10 and TNF-a in the ascitic fluid of DL bearing male and female mice. Physical exercise by DL bearing mice, on a treadmill on a daily basis for various time durations for 10 days, increased the life span along with an inhibition in tumor growth. Exercise resulted in a decreased proliferative ability of tumor cells along with an augmented induction of apoptosis. Macrophages obtained from exercised tumor bearing mice showed an augmented tumoricidal activity. **Conclusion:** This study shows for the first time the role of physical stress and gender dichotomy in the immunological regulation of growth of T-cell tumors and will thus have long lasting clinical implications in the designing of immunotherapy for such tumors.

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AWARD NOMINATIONS

B. Poster Presentations - SHRI RAJNIKANT BAXI AWARD

PA-1

Immuno-Cytokine Gene Therapy for Head and Neck Cancer

Aditya Ambade ***and Rita Mulherkar***

Genetic Engineering, Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Kharghar, Navi Mumbai - 410208, India. *adiambade@indiainfo.com

Introduction: Head and neck squamous cell carcinoma (HNSCC) is the dominant cancer among males in India due to tobacco chewing habits. A large number of patients report to the clinic at a very advanced stage wherein the conventional therapies fail. Gene therapy is an alternate treatment modality, which has shown promising results in recent past. We are working on immuno-cytokine gene therapy using interleukin 2 in a xenograft nude mouse model for HNSCC. **Objectives:** We propose to bring about effective tumor cell kill using direct injection of IL-2 plasmid DNA in a xenograft nude mouse model for HNSCC. **Methods:** The plasmid DNA *pCMV IL-2 IRES Neo*, was transfected into 293 cells and clones were selected on G418. IL-2 secreted in culture supernatants was quantified by ELISA. Biological activity of secreted IL-2 was confirmed on murine splenocytes and human PBLs by ^{3}[H] thymidine assay. *In vivo* expression of the transgene was checked in immuno-competent and nude mice. Mice were injected 50 µg of DNA intramuscularly. Blood was collected from retro orbital plexus before injection and for next 7 days. The serum was checked for secreted IL-2 protein by ELISA. Presence of IL-2 transcripts in muscles was confirmed by RT PCR. **Results:** The stable transfectants secreted 3.7 to 4.3 ng/mL IL-2 protein. The bioassay showed stimulation index of 10 to 23. Expression of IL-2 *in vivo* was seen up to 7 days with IL-2 levels reaching a peak by day 3. RT-PCR confirmed the presence of IL-2 transcripts in muscle. The data suggest that this construct is producing biologically active IL-2 and can be safely used for further in vivo gene therapy studies.

PA-2

Radiation Therapy Induced Changes in Apoptosis and its Major Regulatory Proteins, Bcl-2, Bcl-XL, and Bax in Locally Advanced Invasive Squamous Cell Carcinoma of the Cervix

[1]Amit K Adhya*, [1]Radika Srinivasan, [2]Firuza D Patel*

[1]Departments of Cytology & Gynaecologic Pathology and [2]Radiotherapy, Postgraduate Institute of Medical Education and Research, Chandigarh-12. *amitadhya2003@yahoo.com

Introduction and Aims of The Study: Radiation therapy (RT) for cancer induces cell death by apoptosis. The major apoptotic regulatory molecules induce Bcl-2, Bcl-XL (anti-apoptotic) and Bax (pro-apoptotic) proteins. Invasive cancer cervix is treated mainly by radiation and hence our aim was to evaluate the changes induced by radiotherapy in the apoptotic index and correlate this to the levels of the major pro- and anti-apoptotic molecules in invasive squamous cell carcinoma of the cervix. **Methods:** Paired biopsies were obtained in 30 cases of invasive carcinoma cervix before and after 10Gy radiotherapy. The TUNEL [Tdt-mediated deoxy-Uridine Nick End Labelling] assay was performed to detect apoptotic nuclei and Bcl-2, Bcl-XL and Bax proteins detected by immunohistochemistry

(IHC) using specific monoclonal antibodies. Statistical analysis was performed using the Spearman's Rank correlation Co-efficient test. **Results:** Following RT there was a significant increase in the mean apoptotic index (AI) [2.25 (± 2.28) in the post-RT group vs. 0.90 (±0.53) in the pre-RT group]. Bax, a major pro-apoptotic protein also showed a significant increase following RT ($p<0.05$) whereas there was no significant change in the levels of the Bcl-2 protein in the two groups. Bcl-XL showed a significant decrease in expression following RT ($p=0.006$). The Bcl-2 and Bax IHC scores and the Bcl-2/Bax ratio did not correlate with the AI in the two groups. There was an inverse correlation of Bcl-XL to the AI in the pre-RT group ($p=0.003$) but not in the post-RT group. There was no significant interrelationship in the expression of these proteins. **Conclusions:** Radiation therapy for invasive squamous cell carcinoma of cervix results in increased apoptotic cell death with up-regulation of Bax and down-regulation of Bcl-XL without any significant change in the levels of Bcl-2 protein.

PA-3

Combination Chemoprevention of Experimental Gastric Carcinogenesis by S-Allylcysteine and Lycopene

B. Velmurugan* and *S. Nagini

Department of Biochemistry, Faculty of Science, Annamalai University, Annamalainagar-608 002, Tamil Nadu, India. *r_kumar24@rediffmail.com

Dietary modification has emerged as a cost-effective approach to control the incidence of cancer. S-allylcysteine (SAC), an organosulphur constituent of garlic and lycopene, an antioxidant tomato carotenoid are recognized to possess anti-inflammatory, hepatoprotective, antioxidant and anticarcinogenic properties. We evaluated the combinatorial chemopreventive effects of SAC and lycopene on MNNG and S-NaCl-induced gastric carcinogenesis. The animals were divided into eight groups of six. Rats in group 1 were given MNNG (200 mg/kg body weight) by intragastric intubation on days 0 and 14 as well as S-NaCl (1 mL/rat) every three days during weeks 0 to 3 (six times; on days 3,7,10,14,17,21). Animals in groups 2-4 given MNNG and S-NaCl as in group 1, received in addition SAC (100 mg/kg bw), lycopene (1.25 mg/kg bw) and SAC + lycopene (100 mg/kg bw + 1.25 mg/kg bw) respectively three times per week starting on the day following the first exposure to MNNG. Rats in groups 5 to 7 received the test agents alone whereas group 8 served as controls. The animals were sacrificed after an experimental period of 21 weeks. Multiple markers reflecting different aspects of the carcinogenic process were used to monitor chemoprevention. These include the ability of SAC and lycopene to scavenge reactive oxygen species, enhance detoxification enzymes, and induce apoptosis. Combined administration of tomato and garlic significantly reduced the tumour incidence, modulated the extent of lipid peroxidation, enhanced activities of glutathione redox cycle enzymes, and induced apoptosis as evidenced by downregulation of Bcl2 and upregulation of Bax, Bim and caspases. These results demonstrate that combinatorial chemoprevention by SAC and lycopene on MNNG+S-NaCl-induced gastric carcinogenesis is mediated by multiple pathways.

PA-4

The Role of Estrogen Receptor Alpha and Beta and Progesterone Receptor in Endometrial Carcinogenesis

***Dimple Chakravarty*[1], *S. Radhika*[1], *S. Gopalan*[2], *S. Majumdar*[3] and *A. Rajwanshi*[1]**

[1]Department of Cytology & Gyneocologic Pathology, [2]Obstetrics and Gynecology, [3]Experimental Medicine &

Biotechnology. Post Graduate Institute of Medical Education and Research, Chandigarh-160012, India. *dimplyc@hotmail.com

Introduction: The endometrium is regulated by estrogen and progesterone which act through their cognate receptors, Estrogen Receptor (ER) and Progesterone Receptor (PR) There are two types of ER -a and b. The focuses of this study are ERb and ERb2/Bcx. **Objectives:** To determine the expression of ERa, ERb, ERbcx and PR in normal, hyperplastic and carcinoma endometrium. **Methods:** A total of 39 cases of normal cycling endometrium, 20 cases of endometrial hyperplasia and 26 cases of carcinoma endometrium were studied. Expression of transcripts (Era, ERb, ERbcx and PR) determined by RT-PCR and densitometry performed ER and PR protein expression determined immunohistochemically. Statistical tests: Mann-Whitney Test, Students t Test, Chi Square Test. **Results:** There was no significant difference in the levels of ERa and ERbcx among the groups. However, both ERb and PR transcripts are downregulated in carcinoma endometrium compared to normal and hyperplastic endometrium ($p<0.05$). In carcinoma endometrium, there was a significant downregulation of ERbcx in the tumors with increasing myometrial invasion or higher stage ($p=0.02$). There was good correlation of the transcript and protein levels of ERa($P=0.01$) and PR ($p=0.004$). PR also correlated well with the ERa transcript and protein levels. A definite relationship of the combination expression of the ER isoforms to PR expression was not discernable. **Conclusion:** In the endometrial carcinomas, there is a significant down regulation of ERb and PR expression as compared to normal controls. The ER cx isoform is down regulated with increasing depth of myometrial invasion in endometrial cancers.

PA-5

Detection of Mitochondrial DNA Mutations in Cervical Cancer Tissues

Himani Sharma*, Archna Singh, Sunesh Kumar Jain[1] and Neeta Singh

Department of Biochemistry and Obstetrics & Gynecology[1], All India Institute of Medical Science, New Delhi 110029, India. *himanisharma@rediffmail.com

Introduction: There are approximately 1,24,000 new cases of cervical cancer being detected annually in India. Mitochondrial DNA is more susceptible to DNA damage than nuclear DNA due to continuous exposure to ROS. Tumor formation is associated with mitochondrial DNA mutations and alterations in mitochondrial genomic function. **Objective:** In an attempt to understand the relationship of mitochondrial DNA alterations and cervical carcinogenesis, we studied the mitochondrial DNA for mutations, if any, in cervical cancer and normal cervical samples. **Methods:** We have evaluated the entire mitochondrial DNA of primary cervical cancer samples for mutations, using eight different pairs of primers by multiplex polymerase chain reaction followed by cloning and sequencing. **Results:** A significantly higher prevalence of mitochondrial mutations, mostly deletions were seen in the cervical cancer samples (15/28; 53.5%) vs the controls (2/12; 16.6%). 25/28 (89.2%) tumors and 1/12 controls were HPV positive. The highest frequency of mutations was seen in the "D loop" region. The other mutations observed were in OXPHOS complex I (ND3, ND4 and ND5) complex III (12 s rRNA and 16 s rRNA) complex IV (CO_1, CO_2, CO_3), complex VI (ATPase 6 & 8) and in Cyt b. Since these mutations occur in the coding region they are likely to have biological consequences and may play a role in the initiation and promotion of carcinogenesis. **Conclusions:** Since mitochondria is involved in apoptosis, these somatic mitochondrial mutations have potential therapeutic implications and could be useful in augmenting the definitive biological diagnosis.

PA-6

Modulation of Xenobiotic-Metabolizing Enzymes and Redox Status During Chemoprevention of Hamster Buccal Carcinogenesis by Bovine Lactoferrin

***K.V.P. Chandra Mohan*[1], *P. Vidjaya Letchoumy*[1] *and S. Nagini*[1]**

[1]Department of Biochemistry, Faculty of Science, Annamalai University, Annamalainagar-608 002, Tamil Nadu, India. *r_kumar24@rediffmail.com

The present study was designed to evaluate the chemopreventive efficacy of bovine lactoferrin (bLF), an antioxidant found in milk on 7,12-dimethylbenz[a]anthracene (DMBA)-induced hamster buccal pouch (HBP) carcinogenesis. Hamsters were divided into four groups. The right buccal pouches of animals in group 1 were painted with 0.5 per cent DMBA three times a week for 14 weeks. Animals in group 2 painted with DMBA as in group 1, received in addition, basal diet containing 0.2 per cent bLF. Group 3 animals were given basal diet containing 0.2 per cent bLF alone. Group 4 animals served as untreated control. The status of phase I (cytochrome P450) and phase II (glutathione S-transferase, DT-diaphorase) carcinogen-metabolising enzymes, the extent of lipid peroxidation and glutathione-dependent antioxidants in the buccal pouch as well as the frequency of bone marrow micronuclei were used as biomarkers of chemoprevention. All the hamsters painted with DMBA alone for 14 weeks, developed HBP carcinomas that showed diminished lipid peroxidation and increased activities of carcinogen-metabolizing enzymes and antioxidants with enhanced bone marrow micronuclei. Dietary administration of bLF significantly decreased the incidence of DMBA-induced bone marrow micronuclei and HBP carcinomas. This was associated with a significant decrease in phase I enzymes, modulation of lipid peroxidation and enhanced antioxidant and phase II detoxification enzyme activities. The results of the study suggest that the chemopreventive effects of bLF is mediated by reducing DMBA-induced genotoxicity and modulating carcinogen-metabolising enzymes and the cellular redox status.

PA-7

Correlation of Tissue Lipid Peroxidation and Antioxidants with Clinical Stage and Menopausal Status in Patients with Adenocarcinoma of the Breast

***R. Kumaraguruparan*[1], *J. Kabalimoorthy*[2] *and S. Nagini*[1]**

[1]Department of Biochemistry, Faculty of Science [2]Department of Surgery, Faculty of Medicine, Annamalai University, Annamalainagar-608 002, Tamil Nadu, India. * r_kumar24@rediffmail.com

Breast cancer is the most common cancer in women worldwide and its incidence is increasing in most countries. The etiology of breast cancer is multifactorial. Hormonal, genetic and environmental factors appear to interplay in the pathogenesis of breast cancer. The risk factors associated with breast cancer may exert their effects via generation of reactive oxygen species (ROS). The sensitivity of the mammary epithelial cells to ROS is attenuated by an array of enzymic and nonenzymic antioxidants. The present study was designed to evaluate the extent of lipid peroxidation and the antioxidant status in breast cancer patients in relation to different clinical stages and menopausal status. Fifty newly diagnosed patients with adenocarcinoma of the breast were chosen for the study. The patients were divided into different groups based on the clinical staging and menopausal status. The extent of lipid peroxidation as evidenced by the formation of thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides (LOOH) and conjugated dienes (CD) as well as the status of the antioxidants superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and glutathione

peroxidase (GPx) in tumour tissues and adjacent normal tissues were estimated in these patients. Enhanced lipid peroxidation accompanied by significant elevation in enzymatic and nonenzymatic antioxidants was observed in breast tumour tissues compared to the corresponding uninvolved adjacent tissues irrespective of clinical stage and menopausal status of the patients. The magnitude of the changes in tissue oxidant-antioxidant status was however more pronounced in stage III and in premenopasual patients compared to stage I and II and postmenopausal patients respectively. The results of the present study reveal a correlation between tissue redox status and tumour progression and suggest that upregulation of antioxidants enables tumour cells to counter oxidative stress thereby conferring a selective growth advantage over corresponding normal cells.

PA-8

Molecular Insights into Apoptosis Signaling in Human Oral Tumors and Cell lines

Sanchita Mallick**, Sagar N Pawar, #Alok Pathak and Tanuja R. Teni

Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Tata Memorial Centre, Kharghar, Navi Mumbai - 410 208, India. #Tata Memorial Hospital, Tata Memorial Centre, E. Borges Marg, Parel, Mumbai-400 012. *sanchita_mallick@rediffmail.com

An altered apoptotic response represents a pivotal feature of cancer, contributing to carcinogenesis and resistance to therapy. Our earlier studies demonstrate an inhibition of cell death and enhancement of proliferation in the transition from oral lesions to oral cancer. Frequent overexpression of p53, bcl-2 and bax, members of the p53-dependent apoptotic pathway was observed in oral cancers and a subset of oral lesions. The present study proposes to identify the apoptotic molecules and pathway(s) altered in the oral cancer cell lines Dwivedi & Gurav and Immortalized fetal buccal mucosa cell line FBM as compared to that of oral tumor tissues. The ribonuclease protection assay was used to analyse the mRNA expression of apoptotic genes of Bcl-2, Fas, IAP and Caspase family members in the above samples. A high expression of anti-apoptotic Mcl-1, Bcl-xL, bclw and proapototic bad and bax genes was observed in both the cell lines and majority of tumors. The Fas pathway members were elevated in the cell lines while the TRAIL pathway molecules were high in the oral tumors. High caspase 4 and survivin expression was observed in cell lines and tumors. However the cell lines expressed high caspase 8 while the tumors expressed high caspase 1. RT-PCR assay confirmed the high mRNA expression of Mcl-1 and survivin while immunohistochemical analysis revealed expression of p53, bclxl and Mcl-1 proteins in oral cell lines. Thus altered expression of the bcl-2 related pathway, altered caspase cascade and abnomal p53 and survivin expression observed in our studies may contribute to evasion of apoptosis and the pathogenesis of oral cancers.

PA-9

Syzygium aromaticum L. (CLOVE), a Promising Chemopreventive Agent for Lung Cancer

***Sarmistha Banerjee** and Sukta Das**

Dept. of Cancer Chemoprevention, Chittaranjan National Cancer Institute, Kolkata 700026, India. *rmisti@rediffmail.com

Introduction: In recent time, one of the most promising strategies for cancer prevention is chemoprevention by phytochemicals through intake of vegetables, fruits, herbs and spices in daily diet. Among spices, Syzygium

aromaticum L. (clove) family Myrtaceae, has drawn much attention due to its health promoting properties including radical scavenging effect, antimutagenic effect. Lung cancer is one of the most a common cause of cancer death of which about 85% is associated with tobacco use. Benzo (a) Pyrene, a polycyclic aromatic hydrocarbon present in tobacco smoke is a major risk factor for lung cancer. **Objectives:** The present investigation was an endeavor to assess the anticarcinogenic potential of an aqueous infusion of clove during BP induced lung carcinogenesis in mice. **Methods:** To assess the anticarcinogenic potentiality of clove, histopathological evaluation was carried out both in carcinogen control and treated lung of mouse. To confirm histopathological study, immunohistochemistry were undertaken to detect proliferating cells and apoptotic cells and expression of COX-2, caspase-3 were analyzed by western blotting technique. **Results:** Hyperplasia, dysplasia and carcinoma in situ evident in lung of carcinogen control group after 8th week, 17th week and 26th week respectively, were effectively reduced after treatment with clove infusion. Significant reduction in number of proliferating cells and increased number of apoptotic cells was found on 8th week, 17th week and 26th week of treatment with clove. A reduced expression of COX-2 and increased expression of caspase-3 protein were also observed on 8th week, 17th week and 26th week of BP exposure in treatment group in respect to carcinogen control group. **Conclusion:** Our observation suggests a promising role of clove in prevention of lung cancer.

PA-10
BAG-1 Expression in Premalignant and Malignant Tongue Lesions: A Preliminary Study

***Shalvi V. Mehta*[1], *Hemangini H. Vora*[1], *Kruti N. Shah*[1], *Nilima S. Desai*[1], *Birva V. Brahmbhatt*[1], *Shilin N. Shukla*[2], *Pankaj M. Shah*[3]**

[1]Immunohistochemistry Division, [2]Hon. Deputy Director Research, [3]The Gujarat Cancer & Research Institute and The Gujarat Cancer Society, Asarwa, Ahmedabad, India. *ihcgcri@hotmail.com

BAG-1 (Bcl-2 associated athanogene 1) is a multifunctional anti-apoptotic gene localised on chromosome 9p12 and it represents a link between growth factor receptors and anti-apoptotic mechanisms. The present study evaluated clinical significance of BAG-1 in tongue carcinogenesis. BAG-1 expression was studied in 12 patients with leukoplakia and in 61 patients with tongue cancer by immunohistochemistry. All leukoplakia patients exhibited BAG-1 expression. However, in tongue cancer, BAG-1 expression was noted in 70% patients whereas no expression was seen in 30% of the patients. BAG-1 expression when correlated with clinicopathological parameters, it was found that the expression was significantly lower in tobacco users (63%) as compared to tobacco non-users (93%; P=0.025). Further, patients with stage IV disease and patients with histological grade III tumors had low expression of BAG-1 as compared to their respective counterparts. BAG-1 expression when correlated with other biomarkers, showed a significant inverse correlation with p53 and cerbB2. Further, with overall survival significant association was not observed. Hence, in a preliminary study, down regulation BAG-1 is seen in tongue carcinoma.

PA-11
Immunostimulatory Activity of Herbal Preparations - NCV I and AC II and Their Usefulness in HIV

[1]Sheeja. T. Tharakan*, [1]Girija Kuttan, [1]Ramadasan Kuttan, [2]Kesavan. M, [2]Sr. Austin and [2]Rajagopalan. K

[1]Amala Cancer Research Centre, Amala Nagar, Thrissur, 680555, Kerala, India. [2]Amala Ayurvedic Hospital, Thrissur, Kerala, India.*amalaresearch@rediffmail.com

Introduction: Immunosuppression is a major problem in cancer and AIDS. Hence worldwide, there is an increase in demand for compounds that can stimulate immunity. **Objective:** NCV I and AC II, which were formulated at Amala Ayurvedic Research Centre contain plant materials with known immunostimulatory activity. Objectives of the study is to find out the immunopotentiating activity of these preparations in immunosuppressed animals as well as to determine the effect of these preparations on total viral load, CD4/CD8+ lymphocytes in HIV infected persons. **Methods:** NCV I and AC II were administered 1g/kg.b.wt and 250mg/kg.b.wt orally to mice for one month. Simultaneously animals were immunosuppressed by treatment with radiation (600 rads) or with cyclophosphamide (50mg/kg.b.wt). Parameters assessed were hematological parameters, bone marrow cellularity and a-esterase positive cells. The effect of these drugs on humoral and cell-mediated responses was also analyzed in mice models. For clinical evaluation of these drugs against HIV/AIDS, 30 HIV positive patients were asked to take the medication everyday for one year. CD4/CD8+ lymphocytes and total viral load were determined before the treatment of NCV ACII preparations and I and after one-year treatment. **Results:** NCV I and AC II stimulated total WBC count, bone marrow cellularity and a-esterase positive cells in normal, radiation as well as cyclophosphamide treated animals. Administration of these drugs also enhanced total antibody production, number of antibody forming cells and cell mediated responses such as NK-cell, ADCC and ACC. Administration of NCV I and AC II significantly improved the CD4 status in HIV infected persons. Out of 21 patients 19 patients had increased the CD4 count and 17 patients had decreased total viral load. Viral load in 6 patients were reduced to undetectable range i.e. <20. **Conclusion:** NCV I and AC II possess significant immunostimulatory activity. These non- toxic, inexpensive preparation may be found to be useful in persons with HIV- infection.

PA-12

In Vitro Studies of Human MCF-7 and OAW-42 Cell Lines in Presence of Mammalian Glutaminase Enzyme and the Glutamine-Analogue Acivicin

Subhadra Roy*, Sonali Ghosh, Palash Mallick and Putul Maity

Department of Metabolic Regulation, Chittaranjan National Cancer Institute, 37, S. P. Mukherjee Road, Kolkata-26, India. subhadra_roy@yahoo.co.in

Chemotherapy still remains the most effective modalities of managing metastatic cancer cells. Glutaminase enzyme and glutamine analogues have been used in human blood cancer patients and the glutamine- analogue acivicin is under Phase II clinical trials. Although contribution of the drug's sensitivity to tumor growth inhibition has been evaluated, its influence on tumor-induced angiogenesis has not been accounted so far. Both the enzyme and drug have previously demonstrated tumor growth inhibition in vivo. This study analyzed the effects of these agents on tumor angiogenic markers, for their possible prognostic value in human metastatic cancer cells. ELISA was performed to determine the expression patterns and levels of angiogenic cytokine such as VEGF. Cell invasion through the matrigel under drug therapy was also evaluated. The collagenase enzyme was evaluated after drug therapy with MMP-9 ELISA kit. The study showed that cell culture supernatant VEGF and MMP-9 levels correlated well with cell growth inhibition through drug treatment. These findings suggest that glutaminase enzyme and glutamine analogue influence tumor-induced angiogenic markers.

PA-13

Effect of Thuja Occidentalis on Tumor- Specific Angiogenesis in C57BL/6 Mice

Sunila E.S* and Girija Kuttan

Dept. Immunology, Amala Cancer Research Centre, Amala Nagar, Thrissur, 680555, Kerala, India. *amalaresearch@rediffmail.com

Introduction: Angiogenesis, a process by which new blood vessels sprout from existing one, is a prerequisite for outgrowth and metastasis of tumour. Growth of solid tumours depends on the induction of angiogenesis to provide adequate oxygen and nutrients to proliferating cells. **Objectives:** The antiangiogenic activity of *Thuja occidentalis* was studied using in vivo as well as in vitro models. **Methods:** *In vivo* antiangiogenic activity was studied using B16F-10 melanoma cell-induced capillary formation in animals. Methanolic extract of *Thuja occidentalis* (5 mg/dose/animal) was administered intraperitoneally for 5 consecutive days. Serum was separated and used for the estimation of various cytokines such as IL-1 , IL-2, IL-6, TNF-a and GM-CSF and inhibitors of metalloproteinases (TIMP-1) using ELISA kits according to the manufacturers instructions. Level of VEGF expression was analyzed by the ELISA as well as Quantikine m-RNA. The rat aortic ring assay was used as the *in vitro* angiogenesis study model. **Results:** Methanolic extract of *Thuja occidentalis* significantly inhibited the number of tumour directed capillaries induced by injecting B16F-10 melanoma cells on the ventral side of C57BL/6 mice. The cytokine profile in serum of angiogenesis induced animals showed a increased level of pro-inflammatory cytokines such as IL-1 , IL-6, TNF-a, granulocyte monocyte-colony stimulating factor (GM-CSF) and vascular endothelial cell growth factor (VEGF). The m-RNA expression of VEGF was also enhanced by the treatment with non-toxic concentration of *Thuja occidentalis* extract. Levels of IL-2 and tissue inhibitor of metalloprotease-1 (TIMP-1), which were enhanced in the animals during angiogenesis, were found to be reduced by treatment with the extract. Extract at non-toxic concentrations inhibited the production of micro vessel outgrowth from the rat aortic ring in vitro. **Conclusion:** Administration of *Thuja occidentalis* was found to regulate the cytokine levels during angiogenesis and this could be related to observed antitumour activity.

PA-14
Association of Antioxidant Enzymes, GST-m and Tobacco Habits with Oral Cancer

Tina K. Dave**, Beena P. Patel, Shilin N. Shukla, Prabhudas S. Patel

Biochemistry Research Division, The Gujarat Cancer & Research Institute, Ahmedabad-380 016. *richa_7cz@yahoo.com

Tobacco is a major etiological factor resulting into "New epidemic" of oral cancer in India. The present study analyzed antioxidant enzymes, thiol and GST-M1 genotype from blood samples from oral cancer patients (OCP, n=140), healthy controls with habit of tobacco (WHT, n=25) and no habit of tobacco (NHT, n=25) to rule out tobacco associated free radical induced changes in antioxidant enzymes. Tobacco use in any form as well as duration, frequency and lifetime tobacco exposure were higher in OCP as compared to WHT. WHT with elevated levels of RBC GR, SOD, CAT, lower level of plasma thiol and higher lifetime tobacco exposure showed higher risk of oral cancer development. 63% of the OCP had GSTM1 null genotype. Data revealed that higher antioxidant enzymes, lifetime tobacco exposure and lower oxidative stress markers in WHT showed increased risk of cancer development. Individuals with GST-M1 null genotype may be at higher risk of oral cancer development.

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POSTER PRESENTATIONS

Cell Cycle Regulation & Apoptosis, Molecular Drug Designing, Cancer Immunology & Vaccine (Wednesday)

W-1

On The Prevalence Pattern of Cancer in Eastern Uttar Pradesh

A. Srivastava* and S.K. Awasthi

Institute of Life Sciences, C.S.J.M. University, Kanpur- 208024, India. *akhilsrivastava@msn.com

As the death toll from infectious diseases has declined through out the world, cancer is becoming the second most potent cause of death, led by the heart ailments. Current estimates suggest that approximately 10 million new cases of cancer is diagnosed every year and out of this 50 percent are from developing countries. The cancer patterns vary not only throughout the world but also between different regions of the same country. Variation in incidence of different types of cancer have been reported among populations residing in Delhi, Mumbai, Chennai, Bangalore and some other selected districts in India (ICMR, 2001). However there is very little information available from India's most populous state, the Uttar Pradesh. Therefore studies were undertaken to find out the prevalence pattern of cancer in different anatomical sites in the eastern Uttar Pradesh. Data for the years 1990 to 2001 were collected from Hanuman Prasad Poddar Cancer Hospital, Gorakhpur by adopting Hospital Based Cancer Registry (HBCR) methodology. This hospital is the only recognized cancer hospital catering to the rural and urban populations of Gorakhpur, Deoria, Kushinagar, Maharajgunj, Basti, Siddharthnagar, Gonda, Balrampur, Azamgarh, Mau and Ballia districts which fall in the eastern belt of Uttar Pradesh. In all, cancer of 43 anatomical sites was recorded in these districts with maximum of 39 from Gorakhpur followed by 37 from Deoria. Frequency of Cancer of Cervix, Breast, Gall bladder, Liver, Tongue, Lymph nodes and Lungs were higher in that order. Cancer of Cervix and Breast in females were the major types accounting for nearly 47% of the total registered cases. Cancer of Gall bladder was next in order followed by that of Liver. In males, cancer of Larynx, Lungs, Tongue, Alveolus, Lymph nodes, Oesophagous, Cheek, Blood and Stomach were more prevalent in the above order. Higher incidence of tobacco associated cancers like that of Larynx, Lungs, Oesophagous and Alveolus seems to be due to huge consumption of tobacco by male population in different forms.The population between 35 and 65 years of age were identified more risked. Both Cervix and Breast cancer was more prounced in the age group of 35-39 to 55-59 years. Cancer of blood and lymph nodes were found more prevalent in the younger population of below 25 years. These findings have highlighted the need to undertake studies on the etiology and management of most predominant cancer of Cervix, Breast, Gall Bladder, Liver and the tobacco associated cancer of oral parts and respiratory organs in the eastern Uttar Pradesh.

W-2

Development and Evaluation of an Indigenous Reverse Line Blot Assay for Genotyping of Human Papillomaviruses

Abhilash KV[1]*, Vijayabhaskar L[2], Geetashree Mukherjee[3], BKM Reddy[2], Jayshree RS[1]

Departments of Microbiology[1], Radiotherapy[2], and Pathology[3], Kidwai Memorial Institute of Oncology, Bangalore-29. *abhilashkv@yahoo.com

Cervical cytology screening for reducing the incidence of and mortality due to carcinoma cervix (CaCx) has limitations. Vaccines against Human Papilloma Viruses (HPV) are a promising alternative. Recent vaccination trial using HPV 16 L1 VLP vaccine has been shown to be 100% efficacious. However, there is no cross protection between genotypes. Multivalent vaccines would therefore be a plausible solution to this problem. Hence, information on HPV genotypes prevailing in CaCx, in various regions of our country becomes absolutely essential for formulating vaccination strategies in the future. We developed an indigenous Reverse Line Blot Assay (RLBA) for the genotyping HPV. Further, the prevalence of various HPV genotypes was also evaluated in 100 histopathologically confirmed cases of CaCx. Multiplex PCR was performed on DNA extracted from cervical washings using biotinylated PGMY09/11 primers. HPVs in the post PCR amplicons were genotyped by RLBA using polydT probes of the following genotypes 16,18,26,31,33,35,39,45,51,52,55,56,58,59,68,73,82,83. Beta globin gene amplification was done in parallel for house keeping. Infection with a single genotype was found in 62% (62/100) of the cases. Mixed infections with two and three genotypes were found in 36% (26/100) and 2% (2/100) of the cases respectively. HPV 16 was the most common genotype (36/100 i.e. 36%); followed by 18 (33/100 i.e. 33%); 45 (13/100 i.e. 13%); 52 (12/100 i.e. 12%); 26 (11/100 i.e. 11%); 31 (9/100 i.e.9%); 58(4/100 i.e.4%); 51(2/100 i.e.3%). Genotypes 56 and 73 comprised 1 % each of the total; genotypes 33, 35, 39, 55, 59, 68, 82 and 83 however were not detected This method is cost effective (six times cheaper than the commercially available detection and genotyping methods) and can be used by routine laboratories, thus making it an economically viable option for large scale screening even in low resource countries.

W-3

Clinical Significance of Expression of Nuclear Factor Kappa B (NF-kB) in Fulminant Hepatitis

***Ajay K Singh*[1]*, Bhupesh K Prusty[2], Bhudev C Das[2], P Kar[1]**

[1]Department of Medicine, Lok Nayak Hospital, Maulana Azad Medical College, New Delhi. Division of Molecular Oncology, Institute of Cytology & Preventive Oncology (ICMR), I-7, Sec-39, Noida, India. *doctorajay76@rediffmail.com

Background: Fulminant hepatitis is a clinical syndrome resulting from large number of inciting agents including viruses and toxins. The common endpoint is massive hepatic necrosis. It has a very high mortality rate of 50-80%. Not much is known about this mortal condition. Recent evidence has suggested that inflammatory cells and their products (viz: TNF-a and IL-1) can contribute to the hepatic necrosis. Nuclear factor kappa B (NF-kB) proteins are the key regulator of genes involved in response to infection, inflammation and stress (including hepatitis). **Methods:** The study group included 60 subjects, case group included 25 patients of FHF, control group included 20 healthy voluntary (replacement and altruistic) blood donors, 10 healthy pregnant females and 5 surgical cases of non-liver disease from which control liver tissue was obtained after an informed consent. FHF was diagnosed when after a typical acute onset, patient became deeply jaundiced and went into hepatic encephalopathy within 8 weeks of onset of disease with out any past history of chronic liver disease. NF-kB expression was studied in nuclear extracts obtained from blood and liver tissues using western blotting in both cases and controls. It was also studied in patients who recoverd from fulminant hepatitis. Heparinised blood samples collected were immediately processed for nuclear extract preparation. NF-kB expression was studied in nuclear extracts by western blot analysis using different antibodies of NF-kB family. **Results:** Among pregnant FHF patient HEV was most common etiologic virus seen 10/12 (83.3%) followed by HBV 2/12 (16.7%). Mortality in pregnant FHF patient was very high 9/12 (75%). Overall Mortality in FHF group was 76% (19/25). We could not found any significant correlation ($p>0.05$) between the expression profile of p50 and p65 protein in blood with the duration of jaundice grade of encephalopathy, total serum bilirubin and prothrombin time in FHF patients. Because the principal factor responsible for the poor clinical outcome

and high mortality of FHF patients are severe liver damage, lack of liver regeneration and impaired immunity, our observation of selective suppression of p65 and an increased homodimerization of p50 subunits leading to disruption of normal NF-kB complex and its function seem to play a crucial role during viral hepatitis and fulminant hepatic failure. This hypothesis gains further support from observation of partial upregulated expression of p65 in 6 recovered FHF cases who recovered following treatment. **Conclusion:** Thus it indicates that the lack of p65 is responsible for severe liver damage and high mortality in normal as well as pregnant FHF patients

W-4

Role of Activator Protein-1 (AP-1) during Microangiogenesis in Breast Cancer

Ajay Yadav*[1], Bhupesh K. Prusty[2], Neeraj Jain[2], Ravi Kant[1], PN Agarwal[1], Vinay Kamal and Bhudev C Das[1]

[1]Department of Surgery, Lok Nayak Hospital, New Delhi 110002, India. [2]Division of Molecular Oncology, Institute of Cytology & Preventive Oncology (Indian Council of Medical Research), I-7, Sector - 39, NOIDA 201301, India. *ajayyadav_dr@hotmail.com

Background: Breast cancer is the leading cancer of the females in western countries. In India, it is the second most common malignancy of females after the carcinoma of cervix. **Aims and Objective:** The study was undertaken with the aim of evaluating transcription factor Activator Protein-1 and microangiogenesis in breast cancer and to correlate the activity of Activator Protein -1 and microangiogenesis with the clinical stage of breast cancer. **Material and Methods:** Twenty-five cases of breast cancer cases were taken in the study and five cases of normal breast tissues were taken as control. Nuclear extraction, Western Blotting and Electrophoretic Mobility Shift Assay for Analysis of DNA-Protein Interactions (EMSA) were done for all samples. The microvessel density was determined by light microscopy under H&E staining in areas of invasive tumor containing most intense neovascularisation. Individual microvessel counts were then made on 200X field within the invasive tumor neovascular hot spot. **Results:** The expression of c-Jun and c-Fos was shown to be strong to very strong in all cases of breast cancer. The presentation of c-Fos and JunB as the components of active AP-1 transcription factor was in contrast to other studies done on breast cancer cell lines. The presence of c-jun and c-fos in the cancer patients was associated with a higher microvessel count indicating neo-microangiogenesis. The higher microvessel count also correlated with a patient presenting with higher stage of breast cancer. **Conclusion:** The present study gives an insight into the signaling pathways in the normal and breast cancer cells and defines the role of Activator Protein -1 and its components in the binding activity to DNA, the formation of active AP-1 transcription factor and its role in angiogenesis.

W-5

A Correlation Study of Organochlorine Levels in Serum, Breast Adipose and Gluteal Adipose Tissue among Breast Cancer Cases in Kerala, India

Aleyamma Mathew,[1] Jennifer A. Rusiecki,[2] Susan Sturgeon,[3] Rashmi Sinha,[4] Edo Pellizzari,[5] Tongzhang Zheng,[6] Dalsu Baris[2]

[1]Regional Cancer Centre, Trivandrum, Kerala, India; [2] National Cancer Institute, US; [3] School of Public Health Sciences, University of Massachusetts, Amherst, MA, US; [4] National Cancer Institute, US [5] Research Triangle Institute International, NC, US; [6] Yale University, New Haven, CT, US. *amathew@rcctvm.org

Data from a breast cancer pilot study carried out in Kerala, in 1997, for which organochlorine (OC) levels were measured in three biological media - blood serum, breast, and gluteal adipose tissues - of 37 fasting breast cancer cases (pre-treatment) were utilized. Our objective was to investigate the relationships between OC concentrations in different biological media. Gas-liquid chromatography determined serum, breast adipose and gluteal adipose tissue levels of Dichlorodiphenyltricholorethane (DDT), Dichlorodiphenyldichloroethane (DDE), b-Benzene Hexachloride (b-HCH) and poly-chlorinated biphenyl (PCB) congeners, PCB-153 and PCB-180. Correlation plots were made and Spearman correlation coefficients (r) calculated for breast adipose versus serum, gluteal adipose versus serum and breast adipose versus gluteal adipose. There were strong correlations between serum and breast and gluteal adipose tissues concentrations for most OCs analyzed, one exception being gluteal versus serum for PCB-153. The correlations for all other comparisons ranged from r=0.65 to 0.94. Serum (ng/g) versus adipose ratios approached 1:1 for most of the OC pesticide comparisons and did not vary by summary statistic. These data indicate that blood serum reflects the present body burden of a range of OCs to the same extent as those in adipose tissue, and they support the view that serum may be collected in lieu of adipose tissue to obtain similar information. However, such measurements are a combination of both recent exposures and past exposures, which have metabolized slowly and may still persist. Therefore, investigators should use caution when assigning a level as lifetime body burden.

W-6

Hypoxia Causes Global Demethylation with Patchy Hypermethylation of CpG Islands of Repeat DNA Elements in U87MG Human Glioblastoma Multiforme Cell Line

Arnab Pal*, Abdul M Mondal, Mohit Mehndiratta, Subrata Sinha & Parthaprasad Chattopadhyay

Department of Biochemistry, All India Institute of Medical Sciences, New Delhi, India. *drarnabpal@gmail.com

Introduction: DNA methylation at CpG islands is one of the major ways to suppress long-term gene expression. The retrotransposable SINE (Short Interspersed Nucleotide Elements) contains CpG islands, which are normally fully methylated. In hypoxic conditions, which are invariably found inside all rapidly growing solid tumors, there are chances of CpG sites being demethylated as has been reported in other loci. This increases the chances of SINE being retrotransposed in the genome and cause genomic instability leading to higher grading of the tumor. This phenomenon may partially explain increased genomic instability in higher-grade tumors. **Objective:** We checked methylation status of CpG sites of a selected SINE locus in U87MG human glioblastoma multiforme cells. **Methods:** After culturing the U87MG cells in hypoxic conditions (1.33% of O2) for 6 weeks, COBRA (Combined Bisulfite Restriction Analysis) and Sequencing was done from Bisulfite-PCR amplified DNA to check the methylation status. Real Time RT-PCR of mRNA of the cells was also done to check for any increased SINE expression. **Results:** In our experimental hypoxia majority of the CpG sites at a selected SINE locus underwent statistically significant permanent hypomethylation while there was patchy hypermethylation in a few sites. There was no change in expression in SINE. **Conclusion:** Hypoxia causes site-specific demethylation of CpG island. However, very little or no hypomethylation of the CpGs in the A-box and the B-box PolIII promoter region may be the cause of no increase in SINE expression as seen by Real Time RT-PCR.

W-7

Neem Leaf Mediated Prophylactic Growth Inhibition of Murine Tumors: Possible Immune Mechanism Involving Macrophages and NK Cells

Enamul Haque and Rathindranath Baral*

Department of Immunoregulation and Immunodiagnostics, Chittaranjan National Cancer Institute, 37, S. P. Mookherjee Road, Kolkata-700026. *iaminaam@rediffmail.com

Introduction: Neem leaf preparation (NLP) could induce immunoprophylaxis for significant growth restriction of murine tumors (Int. Immunopharmacol., 4, 2004, 355-366). Underlying mechanism for tumor growth restriction is unknown. **Objective:** To elucidate the possible immune mechanism involving macrophages and NK cells for the tumor growth restriction. **Methods:** Mice were immunized with NLP and splenocytes from immunized mice were adoptively transferred. Cytotoxicity was tested in vitro by LDH release assay. Proportion of macrophages and NK cells was assessed by flow cytometry. Release of nitric oxide from macrophages was estimated using Griess reagent and secretion of IFNg was monitored by ELISA. **Results:** Adoptive subcutaneous transfer of splenocytes from NLP treated mice mixed with Ehrlich's carcinoma (EC) caused inhibition of tumor growth. Systemic transfer of immune cells from NLP induced growth regressed mice inhibited the progression of subcutaneously inoculated EC. Splenocytes from NLP treated mice were more cytotoxic to EC cells in vitro (mean specific lysis; NLP: 36% vs PBS: 16%). Spleen cells were fractionated into three fractions, i.e., macrophage rich fraction, B-T-NK cell rich fraction and T-NK cell rich fraction. Co-incubation of these fractions, from spleen cells of NLP treated mice, along with EC resulted 37%, 32% and 50% cytotoxicity respectively, in comparison to 17%, 11% and 13% killing in control. Moreover, immune cells from NLP treated mice can kill NK specific target K562 more efficiently (18% vs 2%, in control). CD14+ and NK1.1+ cells were increased after NLP treatment. In vitro and in vivo stimulation with NLP enhances LPS mediated release of nitric oxide from peritoneal adherent cells. Similar treatment stimulates the secretion of IFNg from adherent and non-adherent cells. **Conclusion:** Accumulated evidences are in favor of the involvement of NLP activated innate immune functions for the tumor growth restriction. Additionally, lack of direct participation of MHC restricted T cells in this tumor antigen free system, confirms this view.

W-8

Evaluation of Non Enzymatic Antioxidants in Patients with Breast Cancer

Franky P. Jha, Rakesh M. Rawal, Pankaj M. Shah, Prabhudas S. Patel*

Biochemistry Research Division, The Gujarat Cancer & Research Institute, Ahmedabad - 380 016 Gujarat, India. *frankyjha@yahoo.com

Several types of reactive species are generated in the body as a result of metabolic reactions. They attack macromolecules including protein, DNA and lipids etc causing cellular or tissue damage. To counteract their effects, the body is endowed with antioxidants, which are produced either endogenously (which include enzymes like superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase) or received from exogenous sources (which includes antioxidant vitamins like b-carotene, vitamin A, vitamin E and vitamin C). In a normal cell, prooxidant and antioxidants maintain a ratio and a shift in this ratio towards prooxidants gives rise to oxidative stress, which remains the cause of several disease including cancer. In the present investigation, we estimated plasma levels of nonenzymatic antioxidants (b-carotene, vitamin A, vitamin E, vitamin C) from 125

breast cancer patients and 70 female controls. Plasma samples were analysed for b-carotene, retinol (vitamin A), a-tocopherol (vitamin E), and vitamin C spectrophotometrically. It was observed that mean plasma levels of b-carotene, vitamin E, and vitamin C were significantly lower (p=0.000, 0.04, 0.000 respectively), while vitamin A levels were significantly higher in breast cancer patients as compared to the controls (p=0.000). ROC curve for vitamin levels could discriminate between control and breast cancer patients. Odds ratio also revealed that lower plasma levels of b-carotene, vitamin E, and vitamin C and higher plasma levels of vitamin A were significant predictors of breast cancer risk (p=0.000, 0.003, 0.000, 0.000, respectively). The data provided interesting clues of potential role of plasma vitamin levels in etiology of breast cancer.

W-9

Immunoexpression of Her2, Cxcr4, Erk1 and Ki 67 With Respect to Overall Survival in Breast Cancer Patients

***Geetaram Sahu*[1]*, *Bipulendu Jena*[1], *Jabed Akhtar*[1], *Rajesh BT*[1], *Gopinath Acharya*[1], *Monika Sarkar*[1] *and Sujay Singh*[1,2]**

[1]Imgenex Biotech Pvt Ltd, N1/A14, Nayapalli, Bhubaneswar, India. [2]Imgenex Corporation, Flintkote Avenue, Suite-E, San Diego, USA. *imgenexindia@imgenex.com

Introduction: The breast cancer management is currently relies on surgery, chemotherapy and radiotherapy. Despite recent progress in clinical management of breast cancer, the probability of complete cure has not been greatly enhanced. The women, who have no detectable lymph node metastases at the time of diagnosis, develop metastases in the later stage. In patients with metastatic disease that does not respond to radiotherapy or chemotherapy, immunotherapy may offer an additional form of cancer control. The chemokine receptor CXCR4 and its cognate ligand CXCL12 recently have been proposed to regulate the directional trafficking and invasion of breast cancer cells to sites of metastases. Recently it was also reported that HER2 gene in breast cancer is associated with an increased incidence of metastatic disease and with a poor prognosis in breast cancer patients. **Objective:** To evaluate the prognostic significance of the molecular markers such as HER2, CXCR4, ERK1 and Ki67 in sixty breast cancer patients of eastern Indian population. **Methods:** Immunohistochemical and western blotting techniques were used to check expression of HER2, CXCR4, ERK1 and Ki67 proteins in breast cancer tissue arrays. The overall survival analysis of these patients would be done using Kaplan-Meyer overall survival curve and Log-rank test. **Results and Conclusion:** The correlation of HER2, CXCR4, ERK1 and Ki67 proteins expression data with respect to clinical outcome might be potential predictive factor for adjuvant systemic therapy in breast cancer patients.

W-10

Involvement of Mitochondria in DEN Induced Hepatic Lesions After Orange Oil Treatment in Rat

***H. B. Bodake**, M. G. Kamble, K. V. K. Rao*

Chemical Carcinogenesis, Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Kharghar, Navi Mumbai-410 208. *hbodke@actrec.res.in

Earlier we have shown that orange oil prevents DEN induced hepatic tumors. In another study, we observed different hepatic phenotypes induced by DEN depending on dose. An attempt has been made to test the effect

of orange oil on such DEN induced hepatic phenotypes by both histology and electron microscopy in addition to "Comet Assay". Accordingly, wistar rats were administered DEN at a dose of 50 ppm (DEN 50) and 100 ppm (DEN 100) through drinking water. Two weeks after discontinuation of DEN, the rats were administered orange oil by gavage till the end of experiments. After sacrifice the animals, the livers were removed and fixed in NBF for histology and in glutaraldehyde for electron microscopy. Part of liver pieces were stored at -80°C for Comet Assay. Histologically, all the livers appeared normal except DEN 100. DEN 50 group cellular lesions showed swelling of mitochondria and loss of cristae with abnormal inter and intramitochondrial bridges as a focal lesions. DEN 100 induced hepatic lesions after orange oil treatment resulted in restoration to normal cytoarchitecture. Moreover, these lesions resulted in dispersion of rER and mitochondria that were wrapped in DEN 100 phenotypes. As a pilot study, DEN 100 induced hepatic lesions after orange oil treatment revealed cellular apoptosis. The results of the study suggest that mitochondria are the best targets for prevention of hepatocellular lesions and apoptosis of such cells may show the involvement of mitochondrial pathway.

W-11

Pre-Clinical Molecular Pharmacology of Benzamide Riboside as an Anti-Leukemic Agent

***Indhu Hariharan*[1]*, Jayaram N Hiremagalur[2], BR Das[3] and M Radhakrishna Pillai[1]**

[1]Department of Molecular Medicine, Drug Development and Chemoinformatics, Regional Cancer Centre, Thiruvananthapuram, India. [2]Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, USA. & [3]Molecular Diagnostics, SRL Ranbaxy, Mumbai, India. *indhuhariharan@gmail.com

Introduction: The identification of new and effective anti cancer drugs has always been a focal point in leukemia research. Benzamide riboside (BR) is a synthetic pro-drug that is phosphorylated to its 5' monophosphate and then converted to its active metabolite benzamide adenine dinucleotide (BAD), an analog of NAD by the action of the enzyme nicotinamide mononucleotide adenyl transferase (NMNAT). BAD exerts its cytotoxic effect by inhibiting inosine 5' monophosphate dehydrogenase (IMPDH); IMPDH is the rate-limiting enzyme of the branched purine nucleotide synthetic pathway that provides guanylates including GTP and dGTP. There are two isoforms of IMPDH; Type I that is constitutively present in all cells and type II that is inducible and present in highly proliferating cells such as cancer cells. Ongoing studies suggest that BR is more selective in inhibiting IMPDH Type II. **Objectives:** An ideal anticancer drug exerts its cytotoxicty by apoptosis causing minimal damage to its neighboring cells. The objectives of this study were to assess the molecular pharmacology of benzamide riboside as an anti-leukemic agent. **Methods:** For this study we used the Bcr-Abl positive leukemic cell line K562 and Bcr-Abl negative cell line HL60. We first investigated the extent of cytotoxic effects exerted by BR on these cell lines using the MTT assay and then determined whether the drug had any direct effect on the expression on Bcr-Abl. Further to look for any probable apoptotic mechanisms by which the drug may have induced cytotoxicity, we examined morphological changes using ethidium bromide, DNA fragmentation by TUNEL, expression of apoptotic regulatory genes p53, bax and bcl2 using immunocytochemistry, western blot & RT-PCR and the possibility of involvement of mitochondria in triggering apoptosis. NF-kB, a transcription factor, which has been identified as an inhibitor of apoptosis and a potential regulator of cellular transformation, its down stream target Cox-2 and the probable mechanism of NF-kB activation by Bcr-Abl was also studied by EMSA and RT-PCR. In addition, we also looked for any connecting links between the NF-kB transcription factor and TGF- , which are important regulators of apoptosis in some cell types for which Smad proteins are the down-stream effectors. **Results and Conclusions:** Benzamide riboside was found to induce cytotoxicity in K562 and HL-60 cell lines by apoptosis. Our preliminary results suggest that the anti-leukemic properties of

benzamide riboside involve NF-kB and smad pathways. This study may help in understanding the molecular pharmacology of benzamide riboside, setting a stage for determining the drug's effectiveness in clinical studies.

W-12
Mutation of p53 and PTEN Genes in Kangri Cancer (of Skin) in Kashmiri Population

Ishraq Hussain*[*1], Safiya Abdullah[1], M. Muzaffar Mir[1], Shabir Iqbal, M. Inam Zaroo[2], Mushtaq A. Siddiq[3]

[1]Departmen of Clinical Biochemistry, [2]Department of Plastic Surgery, [3]Department of Immunology & Molecular Medicine, Sher-i-Kashmir Institute of Medical Sciences, Soura, Srinagar, J & K, India-190011. *sishraq@yahoo.com

Kangri cancer is a unique heat-induced squamous cell carcinoma (SCC) of the skin found especially in Kashmir valley, which is believed to develop due to persistent use of Kangri - brazier used by Kashmiris to combat the chilling cold during winter months. Unlike classical SC, the sites of Kangri cancer are legs and abdomen with common features of erythematous patches, recurrence and metastasis. In absence of any molecular etiology of Kangri cancer, we studied p53 and PTEN genes that are often mutated in SCC of skin and other human cancers. Mutational analysis using PCR-SSCP and nucleotide sequencing of the conserved regions of p53 and PTEN genes revealed presence of only two mutations in exon 5 of PTEN gene in a case of primary tumor but no mutation in the p53 gene could be detected out of 34 cases studied. One of the mutations resulted in premature truncation of the PTEN protein and the second mutation was a missence alteration. The absence of p53 mutations and a low frequency of PTEN mutations indicate that these may not be involved in the development of Kangri cancer.

W-13
Clonal IgV_H dominance of B-CLL cells in Response to Chemokine SDF1

Jyoti A. Kode**, Reena Nair[#] and Shubhada V. Chiplunkar

Immunology, Advanced Centre for Treatment, Research & Education in Cancer, Tata Memorial Centre, Kharghar, Navi Mumbai-410208; # Tata Memorial Hospital, Tata Memorial Centre, Parel, Mumbai 400012; *jkode@actrec.res.in

B-CLL (Chronic Lymphocytic Leukemia) / SLL (Small Lymphocytic Lymphoma) is characterized by the clonal expansion and accumulation of anergic, self-reactive and mature CD5+CD19+CD23+ B-cells in the blood, secondary lymphoid tissues, and the bone marrow. The extent of infiltration correlates with clinical stage and prognosis and could be due to different regulatory mechanisms in stromal microenvironment of marrow. The present project is aimed at a) understanding the role of chemokine SDF1-CXCR4 axis in accumulation of mature B-lymphocytes in bone marrow of CLL patients and b) study of Immunoglobulin Variable Heavy Chain (IgV_H) gene rearrangement in B-CLL/SLL cells transmigrating in response to chemokine SDF1. Increased expression of mRNA and protein of CXCR4 was observed on CD19+ B-lymphocytes from peripheral blood of CLL/SLL patients using RT-PCR and dual-color flow cytometry, respectively. In transmigration assays, CD19+/ CXCR-4+ B-CLL cells demonstrated good migration in response to chemokine SDF-1. In a PCR-coupled

heteroduplex assay, clonal IgV_H gene rearrangements were analysed in these patients using primers specific for CDR3 junctional region of IgVH 1 to 7. Clonal IgV_H 3 gene was predominantly observed in 54% of the patients while 30-38% patients demonstrated clonal IgV_H 1, 2, 4 and 6 gene rearrangements. $CD19^+/CXCR\text{-}4^+$ B-CLL/SLL cells transmigrating in response to SDF1 gradient exhibited dominant IgVH3 gene rearrangement. Studies are in progress to understand the prognostic significance of somatic mutations in IgV_H CDR3 sequences of B-CLL/SLL cells and their response to chemokine SDF1.

W-14

Expression Pattern of Tumor Associated Genes in Small Cell Carcinoma and Non-small Cell carcinoma of Lung

K Raveendran Pillai**, TP Brinda, Jayaprakash Madhavan, Elizabeth K Abraham

Division of Cytopathology and Radiotherpy, Regional Cancer Centre, Thiruvananthapuram, Kerala, India. *ravi@rcctvm.org

Introdution: Lung cancer is one of the most prevalent cancer among males in Kerala. Tobacco smoking is considered to be an important aetiological factor, though lung cancer occurs in non-smokers also. Histological diagnosis is essential for selecting the mode of treatment in patient with lung cancer, hence it is broadly categorized into small cell carcinoma (SCC) and non- small cell carcinoma (NSCC). SCC is characterized by more rapid growth rate and early metastatic dissemination. NSCC is a morphologically diverse group which includes squamous cell carcinoma, adenocarcinoma and large cell carcinoma and is strongly associated with smoking. **Objectives:** The present study evaluates the difference in the expression of select tumour suppresser genes p53 and Rb, oncogenes Bcl2 and Her2 and proliferation markers PCNA and AgNORs in SCC and NSCC. **Methods:** The standard Avidin Biotin Complex immunohistochemical staining method was used to study the expression of p53, Rb, Bcl2, Her2 and PCNA in paraffin sections from SCC (17) and NSCC (82) of patients who underwent brochoscopic biopsy or surgical resection. AgNOR was performed by using the one step staining technique. NSCC includes squamous cell carcinoma (29), adenocrcinoma (32) and poorly differenciated carcinoma (21). The expression indexes of these proteins were statistically compared between various histopathological groups of lung cancers. **Results:** Her2 (p=0.018), Rb (p=0.013) and Bcl2 (0.015) showed significant difference in expression between NSCC and SCC groups. Rb and Her2 expression were more in NSCC lesions than in SCC cases. However, bcl-2 expression was high in the SCCs. Significant difference in expression pattern of Bcl2 (p=0.003) and Her2 (p=0.017) were also observed between various histopathological groups of lung cancers by one way ANOVA. Expression of p53and PCNA and AgNOR count were not significant. **Conclusion:** Thus Bcl2 and Her2 are good immunohistochemical markers to differentiate NSCC and SCC. Also its expression pattern showed significant difference between various NSCC groups.

W-15

H-Ras 81T -> C Polymorphism Associated with Oral Cancer Risk

KM Sathyan**, KR Nalinakumari and S Kannan

Division of Cancer Research, Regional Cancer Centre, Trivandrum - 695 011, Kerala.
*sathyankm@yahoo.co.in

Introduction: Oral cancer is a major health problem in many parts of the world especially in India. Eventhough the tobacco use and alcohol consumption are the major risk factors for oral cancer, only a small fraction of tobacco and alcohol users develop oral cancer. It points that the interaction between genetic and environmental factors plays an important role in the aetiology of oral cancer. Ras gene is found to be mutated in 30% of the Indian oral cancer, while low frequency has been reported from the Western countries. Recently a polymorphism at 81st nucleotide of H-Ras (81T->C) was found to be associated with bladder carcinoma. **Aim**: The present study evaluated whether H-Ras 81T->C polymorphism has any influence on oral cancer susceptibility. **Study subjects and Methods:** A total of 175 patients with oral cancer and 142 hospital based age and sex matched healthy controls were included in the present study. Genotyping was carried out by PCR-single strand confirmation polymorphism (SSCP) analysis. Genotypes were confirmed by sequencing. **Results:** The observed distribution of H-Ras 81T->C genotypes in cases and controls were not statistically different from the frequency expected from the Hardy-Weinberg equilibrium equation. The frequencies of the H-Ras 81 TT, TC and CC genotypes were 94, 69 and 12 respectively, in cancer cases and 92, 43 and 7 respectively, in controls. Compared with wild type (TT), CC genotype was not associated with an increased risk, but when compared TT with TC+CC, a significantly increased risk was observed (p = 0.047, OR = 1.57, 95% CI = 1.006 - 2.499) for the latter group. The risk remains more or less unchanged (p = 0.046, OR = 1.59, 95% CI = 1.008 - 2.513) after adjusted for age and sex. **Conclusion:** We found that H-Ras 81T->C polymorphism appears to be epidemiologically relevant for assessing oral cancer risk.

W-16

Cellular Manifestations of Inflammatory Mediators COX-2 and NF- B in Oral Submucous Fibrosis

Lekshmy Srinivas*, Santhi W.S, and M Radhakrishna Pillai

Department of Molecular Medicine, Regional Cancer Centre, Trivandrum, Kerala, India. *leppy@rediffmail.com

Introduction: The use of smokeless tobacco usually involves chewing of a betel quid (areca nut, betel leaf, tobacco and slaked lime mix or as "pan masala") and has led to the development, in a large proportion of users, of a unique generalized fibrosis of the oral soft tissues, called oral submucous fibrosis (OSMF). Oral submucous fibrosis is a pre-malignant fibrotic condition of the mouth that exhibits excessive collagen production. It is an inflammatory condition and also involves disturbances in the homeostatic equilibrium between synthesis and degradation of extracellular matrix molecules (ECM). Cyclooxygenase (COX)-2 is an inducible enzyme responsible for prostaglandin synthesis in most inflammatory diseases. A major pathway by which COX-2 associated with malignancy is through the NF-kB pathway. Matrix metalloproteinases (MMPs) can play an important role in the development and progression of OSMF. The tissue inhibitors of metalloproteinases (TIMPs) are natural inhibitors of the matrix metalloproteinases that form tight complexes with the activated forms of MMPs, thereby inhibiting the catalytic activity of these enzymes. **Objectives:** To understand the cellular manifestations of inflammatory mediators COX-2 and NF-kB in oral submucous fibrosis. **Methods:** Immunocytochemistry was done using specific monoclonal antibodies against COX-2, p65, p50, IkB, MMP-2, MMP-9, TIMP-1, and TIMP-2. **Results:** There was significant difference in expression of COX-2 and NF- B components (p65, p50 and IkB) in OSMF compared to normal oral mucosa. A marginal increase in MMP expression was detected in OSMF samples compared to normal oral mucosa. TIMP expression in OSMF samples was found to be significantly high compared with control tissue. **Conclusion:** This study is to our knowledge, the first report in OSMF demonstrating a relationship between MMPs, TIMPs, COX-2 and NF-kB, thus elucidating inflammatory pathways in the disease.

W-17

DEN Induced Carcinogenesis in F344 Rat: A Model to Study Early Cellular Lesions

M. G. Kamble*, H. B. Bodake, K. V. K. Rao

Chemical Carcinogenesis, Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Kharghar, Navi Mumbai-410 208. *mkamble@actrec.res.in

It is well known that experimental induction of animal tumors depends on strain, dose, and route of administration of carcinogen. Earlier we found putative precursors during development of NNK induced lung tumors in F344 rat as early as 8 wk which also exist by 30 wk. An attempt has been made to observe such alterations by using oral administration of another carcinogen such as DEN to the same strain. F344 rats were administered DEN orally at a dose 20 mg biweekly and animals were sacrificed at 4, 8, 12 & 16 weeks after carcinogen administration. Lungs were removed and fixed in NBF for histology and in glutaraldehyde for TEM study. Part of lung tissues were collected and stored at -80 ℃ for protein analysis. Histologically, the cellular lesions in the lung tissue were not clearly differentiated. Electron microscopy revealed early cellular changes by 4 wks followed by intermediate cellular lesions between 8-12 weeks and undifferentiated cellular lesions were observed by 16 weeks. Abnormal keratinization, indented nuclear changes were characteristics features of late stage. The collagen fibrils were loosely disorganized in such lesions. However, early cellular alterations such as association between epithelial (basal) and clara cells were more frequently observed. Abnormal accumulation and clustering of mitochondria with vacuolated bodies and ribosomal rosettes were suggestive of transitional stage. Western blotting showed gradual increase in p53 expression in response to induction period which reached at maximum by 16 weeks. In this study the induction period was short and involvement of Clara and basal cells seems to be responsible for lung carcinogenesis induced by DEN.

W-18

Identification of Differentially Expressed Genes and Their Transcriptional Regulation in a Radio-Resistant Chinese Hamster Cell Strain Derived From V79

Paromita Roy Choudhury,* Ramanuj Banerjee, Susanta Roychoudhury, Nitai P. Bhattacharyya, Keya Chaudhuri

Human genetics and genomics group, Indian Institute of Chemical Biology, 4, Raja S.C. Mullick Road, Jadavpur, Kolkata-700032, India.*paro8@rediffmail.com

Introduction: Many studies have identified genes induced in response to DNA damaging agents such as ultraviolet and ionizing radiation. A number of genes induced in response to ionizing radiation have been identified in mammalian cells that code for transcription factors, proteins inhibiting cell cycle progression, family of heat shock proteins. **Objectives:** The objectives of the study is to identify the genes that are differentially expressed in a radio resistant and methotrexate resistant cell strain M5, compared to its parental Chinese hamster lung fibroblast cell line V79. Transcriptional regulation of those differentially expressed genes were also examined. **Methods:** Total RNA was extracted from V79 and M5 cell lines and subjected to RNA finger printing by arbitrary primed polymerase chain reaction (RAP-PCR). Differentially expressed RAP-PCR products were cloned and sequenced. Differential expression was confirmed by Northern blot or semiquantitative RT-PCR. Electrophoretic mobility shift assay was performed to understand transcriptional regulation. **Results:** The expression of mitochondrial genes ND1, ND4 and COXI under the H strand promoter were upregulated in M5

compared to V79 while the mitochondrial copy no. remained unchanged. Interestingly, the expression of mitochondrial gene ND6 under the L strand promoter was unaltered. EMSA studies showed that binding affinity of cytosolic and mitochondrial protein for H strand promoter was much higher in M5 than V79. **Conclusion:** Ionizing radiation causes increased expression of mitochondrial genes under H strand promoter. Expression of mitochondrial genes under the L strand promoter remains same in V79 and M5 cell lines. Cytosolic and mitochondrial protein binding to H strand promoter regulate the transcription of mitochondrial genes under this promoter.

W-19

Association of DNA Repair Genes, Tobacco Habit and Multiple Primary Neoplasias (MPN) in the Upper Aero-Digestive Tract (UADT)

Rita Mulherkar*, Rekha Rai, Naresh Mahida, Sadhana Kannan and Rajiv Sarin

Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Kharghar, Navi Mumbai, 410208, Tata Memorial Hospital, Parel, Mumbai, 400012, India. *rmulherkar@actrec.res.in

Patients with sporadic cancers in the upper aero-digestive tract (UADT) are at a much higher risk of developing a second cancer in the same region due to repeated carcinogenic insult. In the Multiple Primary Neoplasia (MPN) Registry at Tata Memorial Hospital, the commonest site of cancer is the UADT. About 80% of these patients have tobacco habit, indicating a strong predisposition to tobacco-related cancers. In the study of sporadic cancers, the focus of research has traditionally been on the exogenous and endogenous carcinogens and how they cause genetic alterations that result in cancer. However, it is being increasingly realized that genetic predisposition due to polymorphisms and mutations in the low penetrance genes play an important role in determining the outcome of carcinogen exposure. MPN patients may be more susceptible to carcinogenesis in the mucosa of the UADT thereby accumulating genetic alterations, resulting in the induction of multiple, independent malignant lesions. Second cancer occurs in these individuals after exposures to endogenous and exogenous agents and could be because of an inherently less efficient carcinogen detoxification or DNA repair system. Variation in the DNA repair capacity and cancer susceptibility is known to be associated with the polymorphism in DNA repair genes. We have studied polymorphisms in DNA repair genes - XRCC1, XRCC3, Ape1, XPD and hOGG1, representing two different repair pathways, in a case-control study. Data pertaining to more than 60 MPN patients with at least one cancer in the UADT has been compared to equal number of matched, healthy controls.

W-20

Counseling of Cancer Patients: A Study from a Tertiary Care Center

S Jaju*[1], SK Pal[2], SK Mishra[1], AK Verma[1], NR Dutta[3], A Agarwal[1],G Agarwal[1], A Mishra[1], Farah[1]

Department of Endocrine Surgery 1, Gastroenterology 2, Radiotherapy 3 Sanjay Gandhi Postgraduate Institute of Medical Sciences, Raebareli Road, Lucknow, UP 226 014, India. *caid@sancharnet.in

Introduction: A diagnosis of cancer sends one's life into a new direction, one that is not asked for. It is like being pushed out of a helicopter into a jungle war with no training, no map, and no idea how to survive. Research studies have shown that effective counseling and information can be an important tool in helping

people to cope with cancer. **Objectives:** To study the effect of counseling in cancer patients coming to our institute for treatment. **Methods:** In the last 8 years a total of 902 cancer patients (male - 203) were counseled. Which included 519 (57.6%) breast, 134 (14.8%) GI tract, 120 (13.3%) bladder, 82 (9.1%) thyroid, 36 (4%) hematological and 11 (1.2%) prostrate cancer patients. **Results:** Majority of patients viz. 837 (93%) was from the urban areas of Uttar Pradesh. The three main areas where most of the patients needed counseling were: 1) treatment options, 2) financial constraints, 3) concern about their family. Few cancer patients received financial support viz. 96 (10.4%) from Cancer Aid Society, 42 (4.6%) chief Minister's Fund, 9 (1%) Prime Minister's Fund and 3 (0.3%) from Kamdhenu SGPGIMS Fund. **Conclusion:** Counseling had a beneficial effect on patients in helping them understanding the stage and ature of disease. It helped them overcome the fear of treatment and improve their understanding about the basis of treatment. It also motivated them psychologically o fight the disease. The family members also learnt how to detect cancer in early stages with self-examination, especially braest cancer.

W-21

Significance of Intraoral Site-wise Analysis of Clinico-Biological Factors in Oral Cancer

R Jayasurya, R Sailasree, Elizabeth K Abraham, and S Kannan*

Division of Cancer Research, Regional Cancer Centre, Trivandrum - 695 011, Kerala, India. *kannan@rcctvm.org

Introduction: Earlier clinico-epidemiological studies showed that the behaviour of the tongue cancer is different from the cancer originating at other sites of the oral cavity. However, studies to identify the reason for such difference is lacking in the literature. **Objectives:** In the present study we attempted to see whether any difference existed in the cell cycle regulatory mechanism of these tumours by comparing the expression of major cell cycle regulatory proteins in carcinoma of the buccal mucosa and carcinoma of the anterior two third of tongue prospectively. **Methods:** The study population constituted of 241 consecutive cases of previously untreated oral carcinomas. Out of the 241, 147 cases were buccal carcinoma and 94 were tongue carcinoma. Patient's demographic and clinical details were collected. Expressions of major cell cycle regulatory proteins were assessed by immunohistochemical methods. Appropriate statistical methods were used to analyze the data. **Results:** Epidemiological data showed that tongue cancers occurred in comparatively young age patients and they do not have a strong association with tobacco chewing habit as that of buccal cancer and are clinically more aggressive than buccal cancer. When comparing the expression pattern of cell cycle regulatory proteins, significantly high expression of cyclin D1 and low expression of Rb were observed in tongue cancers than in buccal cancers. When relating with disease prognosis, tongue cancers exhibited poor prognosis than that of buccal cancer. The interesting finding is that none of the clinico-biological factors studied have exhibited any relation with treatment response in tongue cancers. However, in buccal cancers the biological factors like p53 and Cyclin D1 and a clinical factor, stage of disease, has independently influenced the disease prognosis. **Conclusion:** Thus the present data indicates that tongue cancers are aetiologically, clinically and genetically distinct from that of buccal cancers. Further molecular level studies are required to pinpoint the exact reason behind these differences.

W-22

Cytohormonal and Cytomorphological Alterations in Cervico-Vaginal Smears of Postmenopausal Women on Hormone Replacement Therapy - A Preliminary Study

***Sanjay Gupta*[1]*, *Neeta Kumar*[2], *Niti Singhal*[2], *Usha Manektala*[3], *Shyama Jain*[2], *Pushpa Sodhani*[1]**

[1]Divisions of Cytopathology and Obstetrics & Gynecology, Institute of Cytology and Preventive Oncology, I-7, Sector 39, NOIDA, [2]Maulana Azad Medical College and [3]Lok Nayak Hospital, New Delhi, India. *sanjaydr17@hotmail.com

To study the cyto-hormonal and morphological alterations in cervico-vaginal smears, associated with the use of hormone replacement therapy (HRT). Ninety postmenopausal women; 30 on Estrogen - progesterone combination (HRT) for 1 to 24 months (User1); 30 on estrogen therapy (ERT) for 1 to 44 months (User 2) and 30 not on any hormones (Non Users) were included in the cross-sectional study. Their lateral vaginal wall smears and cervical smears were examined for hormonal and morphological assessments respectively. The smear pattern showed predominance of parabasal cells in 46.6 % of Non Users while none of the Users had >70% parabasal cells. A high percentage (>70%) of intermediate cells was found in 46.6 % of Users and only in 16.6 % of Non Users. A high Maturation value (MV) was found in 75% of Users as compared to only 16.6% of Non Users. The women with high MV (>50) were significantly less symptomatic than Non Users. Atrophic changes were present in cervical smears of 14/20 (46.6%) Non Users as compared to 1/60 (1.66%) users. Atypical squamous cells of undetermined significance (ASCUS) was diagnosed in 7 Users and 3 Non Users. It persisted on follow up in 4 Users and 1 Non User. Histology revealed one mild dysplasia among users. The cyto- hormonal pattern on vaginal smears correlates well with the response to hormonal therapy and clinical symptoms. Cervico-vaginal smears provide an opportunity to the cytopathologists to evaluate the hormonal and morphological alterations associated with the usage of replacement hormones.

W-23

Identification of Tumor Antigens Eliciting Humoral Immune Response in Oral Cancer Using Proteomics

***Sanjeev Shukla**, S.M. Zingde**

Advance Centre for Treatment, Education and Research in Cancer, Tata Memorial Centre, Kharghar, Navi Mumbai, India. *sanjeevtmc@yahoo.com

Conventional methods, which are used for cancer therapy, fail to cure the disease totally. Therefore molecular markers are now being used for management of cancer. Several molecular markers have been identified in oral cancer, a major cancer in males in India. These have provided a sequence of events for transformation of the normal cell to the tumor cell. These studies show that alterations in multiple genes/ proteins are necessary for transformation. Different combinations of alterations occur in each individual, thereby necessitating a holistic/global analysis such as proteomics to obtain a true picture and to identify more markers for optimising cancer treatment. Present study aims to identify tumor antigens in buccal mucosa oral cancer. Proteomics-based approaches have been used in various cancers to identify tumor markers based on their occurrence as tumor antigens that elicit a humoral immune response. Auto and heterologous sera from patients with cancer of the buccal mucosa were used to immunoprecipitate antigens from tumor tissue lysate. The proteins were resolved by 1D/2D SDS-PAGE and the antigens eliciting an immune response identified by immunoblotting with auto/heterologous sera. Proteins obtained with serum from normal individuals and from those with breast and liver cancer were used as controls. Preliminary results indicate that the sera from patients with buccal mucosa cancer detect specific antigens. The identity of these antigens is being determined. These antigens could be further validated for their use in cancer management by immune intervention.

W-24

Expression of NF-kB and COX-2, During Oral Tumorigenesis and in the Assessment of Minimal Residual Disease in Surgical Margins

***Santhi W.S.*[1]*, Paul Sebastian[2] and M. Radhakrishna Pillai[1]**

Departments of Molecular Medicine[1] and Surgical Oncology[2], Regional Cancer Centre, Thiruvananthapuram, India. *santhiws@gmail.com

Oral cancer incidence varies strikingly around the world; in parts of India and Southeast Asia it is one of the most common cancers. Despite multimodality treatment methods including surgery and chemoradiotherapy, the overall survival rate for oral cancer patients have not significantly improved in the last three decades. The outcome of any cancer treatment depends on the eradication of minimal residual disease or MRD. The tumor specific genetic alterations (tissue-specific markers) can be exploited in the evaluation of MRD. The tissue specific markers selected for this study are nuclear factor-kappa B (NF-kB) and cyclooxygenase-2 (COX-2). NF-kB is a ubiquitous transcription factor that controls a large number of genes including those involved in tumorigenesis. COX-2 is an enzyme, whose byproducts play major role in tumor progression. This study included two set of sample tissues; one set included tissues taken from normal oral mucosa, oral premalignant lesions and oral cancer while the other set of tissues included tumor, surgical margin and extra margin, taken from the same patient undergoing surgery for oral cancer. Expression of NF-kB was evaluated using immunohistochemistry, EMSA and ELISA. RT-PCR was done to evaluate mRNA levels of COX-2. We found a differential NF-kB expression (both p50 and p65 as well as the cytoplasmic inhibitor IkB) between the normal oral mucosa, oral premalignant lesion and primary oral tumor. The same pattern of expression was observed in primary tumor, surgical margin and a further extra margin tissue. The expression of NF-kB paralleled the increased detection of COX-2 mRNA in these two set of tissues. These findings may help us to understand the process of oral tumorigenesis and also raise the possibility of such results being used as prognostic markers in understanding the completeness of surgical resection and minimal residual disease.

W-25

Purification of Disease Specific IgG and its Interaction With Sialoglycoconjugate(s) on ALL Cells

***Sarika Kapoor*, Ram Marwaha, Siddhartha Majumdar and Sujata Ghosh[#]**

Department of Pediatrics, [#]Department of Experimental Medicine & Biotechnology, PGIMER, Chandigarh 160012, India. *kapoorsarika@yahoo.com

Introduction: Malignant transformation of cells is associated with alteration of the cell surface carbohydrates. Various leukemic cell lines express specific glycoconjugates with altered pattern of sialylation on their surfaces. In this study an attempt was made to identify the disease specific sialoglycoconjugate (DSSG) specific antibody in the sera of children with acute lymphoblastic leukemia (ALL). **Methods and Results:** Fetuin was taken as the representative sialoglycoconjugate and the level of fetuin specific IgG was found to be maximum as compared to that of IgM and IgA. It has been observed that the level of fetuin specific IgG decreased in the sera of patients during follow-up i.e. at the end of induction phase and at 3 monthly intervals of maintenance therapy for 1 year. The disease specific IgG was purified and characterized. The membrane component(s) of lymphoblast of ALL patients was found to interact with the purified DSSG specific IgG in cytocentrifuged preparation. Further, the DSSG(s) was identified in the membrane fraction of patients' cells in

Western Immunoblot. In all the experiments, appropriate controls were run in parallel. **Conclusion:** Thus, the increased level of fetuin specific IgG can serve as an index to monitor the disease status of ALL patients during therapy. Moreover the characterization of disease specific sialoglycoconjugate(s) may have some therapeutic implication to control the disease progress.

W-26
pS2 Protein in Prognostication of Human Breast Cancer

Trivedi Sunil[1], Dave Heena[1], Trivedi Trupti[1], Shah Rohini[1], Shah Manoj[2]

[1]Receptor & Growth Factor Laboratory, [2]Pathology Department, The Gujarat Cancer Research Institute, Asarwa, Ahmedabad 380 016, India. *drsuniltrivedi@hotmail.com

Stamping of hormone dependence would qualify the administration of antihormonal agents for effective treatment of breast cancers. Estrogen receptor (ER) was the first to be used. Subsequently, concept of intact ER pathway implicated pS2 as marker of estrogen responsiveness. Higher pS2 expression increases chances of tamoxifen responsiveness, a longer relapse free- and over all- survival. This study performed on 250 breast cancer patients estimated ER from breast tumor cytosols by Radio-receptor assay. pS2 protein was estimated using IRMA. The expression levels of both these molecules were compared to established histological prognosticators, relapse-free and over-all survival. Our findings suggest a minimal role of pS2 protein in advanced breast tumors.

W-27
Potentiation of Cytotoxicity of Liposomal Ricin by monensin in Human Nasopharyngeal Carcinoma Cells

Shailendra Singh Rathore and Prahalad C. Ghosh

Department of Biochemistry, University of Delhi, South Campus, New Delhi-21, India. *suwagmani@yahoo.com

Ricin is a heterodimeric (consists A and B chain) toxic protein, inhibits protein biosynthesis in mammalian cells and can be used as an anticancer agent. The potential application of ricin as an anticancer agent is limited due to the non-selectivity of the B-chain. To reduce the passive targeting, ricin was encapsulated in various liposomal formulations and its effect on the cytotoxity in KB (Human Nasopharyngeal Carcinoma) cells was compared with native ricin in presence of monensin. Liposomes were prepared by hand shaken method followed by sonication and extrusion through polycarbonate membrane. The cytotocicity of ricin was reduced markedly following encapsulation in conventional charged liposomes depending on the charge of the liposomes. Monensin (50nM) potentiates the cytotoxicity of free ricin (21.78) fold. The fold potentiation of the cytotoxicity of liposomal ricin by monensin was found to be highly dependent on the charge as well as density of DSPE-Mpeg on the surface of liposomes. Maximum potentiation of cytotoxicity of ricin by monensin was observed when ricin was delivered through negatively charge liposomes (69.80 fold) followed by positively charge liposomes (48.46fold) and neutral liposomes (40.43fold). The incorporation of DSPE-mPEG in different charge liposomes significantly modulates the cytotoxicity of liposomal ricin depending on the charge of liposomes. The maximum potentiation of ricin cytotoxicity was found 85.78 fold in 5 mol% PEGylated negatively charge liposomes, 72.56 fold in 2.5 mol% PEGylated positively charge liposomes and 38.71 fold in 7.5 mol% PEGylated neutral liposomes. These

results clearly showed that liposomal ricin with appropriate lipid composition, surface charge and incorporation of optimum density of DSPE-Mpeg might have potential application for selective elimination of malignant cells in combination with monensin.

W-28

Inactivation of *hMLH1* and *hMSH2* genes in HNSCC tumors by promoter methylation and its relation with MSI phenotype

***Shiladitya Sengupta*[1]*, *Susmita Chakrabarti*[2], *Chinmay K. Panda*[3] and *Susanta Roychoudhury*[1]**

[1]Human Genetics and Genomics Division, Indian Institute of Chemical Biology, Kolkata, India. [2]Metro Hospital, Cleveland, USA.[3]Department of Oncogene Regulation, Chittaranjan National Cancer Institute, Kolkata, India. *shiladitya_sg@yahoo.co.in

Introduction: Inactivation of mismatch repair genes *hMLH1* and *hMSH2* by promoter methylation (pMe) in different sporadic cancers has been reported and found to be associated with MSI phenotype. Although MSI has been observed in head and neck squamous cell carcinoma (HNSCC), no association with MMR gene defect has been clearly demonstrated. **Objective:** The objective of the study is to determine the promoter methylation status of *hMLH1* and *hMSH2* genes in HNSCC and to correlate it with MSI status of the tumor. **Methods:** Genomic DNA isolated from microdissected tumour tissues and corresponding normal counterparts were subjected to MSI analysis. Methylation status of the *hMLH1* and *hMSH2* promoters was carried out by HpaII/MspI restriction digestion PCR method. Methylation status was confirmed by immunohistochemistry of the respective proteins in corresponding tissues. **Results:** 63% of primary HNSCC tumors were MSI+. Although 29% (19/66) of MSI^+ tumours were pMe^+, about 33% (22/66) MSI^+ were pMe^-. Interestingly, 23% (15/66) of MSI- tumors were also pMe^+. In 74% (14/19) of MSI^+-pMe^+ tumours these genes were hypermethylated both in tumor tissues and their normal counterparts. Immunohistochemistry of tissues revealed pMe+ tumours to have reduced or no expression of proteins as compared to pMe- tumors. **Conclusion:** Two independent pathways may be involved for MSI phenotype in HNSCC tumors, one involving inactivation of *hMLH1* and/or *hMSH2* by promoter hypermethylation and another due to some other factors. A statistically significant ($p < 0.05$) association was observed between MSI phenotype of HNSCC tumors and hypermethylation of the *hMLH1* and *hMSH2* genes in the paired normal tumor tissues.

W-29

Study on the effect of Jacalin on Non-Small Cell Lung Cancer Cell Line NCI-H520

***Shilpa Bhatia**, Sangeeta Mehta, S Majumdar and S Ghosh*

Postgraduate Institute of Medical Education & Research, Chandigarh, 160012, India.
*shilpabhatia26@yahoo.co.in

At present, lung carcinoma accounts for a major load of cancer related deaths worldwide. One of the key factors that is thought to contribute to the development of this killer disease is the derangement of apoptotic pathway. Despite major advances in cancer treatment over the past few decades, the prognosis of patients with lung carcinoma has improved only to a small extent. Thus there is an urgent need to develop new and

effective strategies for the prevention and treatment of cancer. Plant lectins are thought to play a very significant role in this context. Thus the main focus of this study is to explore the potential of a plant lectin-Jacalin (derived from Artocarpus integrifolia) in view of apoptosis. This particular lectin has already been known to exert anti-proliferative effects in human colon cancer cell line. Our study revealed that Jacalin could induce apoptosis in non-small cell lung cancer cell line NCI-H520 in a dose dependent manner. Further, the expression analysis of pro- and anti-apoptotic markers was also carried out by means of Western blotting. To conclude, this study may provide new insights in designing the therapeutic tools against this dreaded disease.

W-30

"Paper Smear" A Simple Method for Dry Collection, Transport and Storage of Cervical Specimens for Detection of HPV Infection: A National Multicentric Study

Shirish Shukla*[1], Suresh Hedau[1], Sudhir Krishna[2], M R Pillai[3], S V Chiplunkar[4], H Tongaonkar[4], S. Batra and BC Das[1]

[1]Division of Molecular Oncology, Institute of Cytology and Preventive Oncology (ICMR), I-7, Sector -39, Noida - 201 301. [2]National Centre of Biological Sciences, Bangalore.[3]Regional Cancer Centre, Trivandrum.[4]Tata Memorial Centre, Mumbai. *shirishshukla31@rediffmail.com

Background: Human Papillomaviruses (HPVs) are major pathogens associated with the development of cancer of the uterine cervix, the most common malignant tumours of women worldwide. Reliable diagnosis of HPV infection, particularly the "high-risk" types (16/18), may facilitate early identification of "high risk" populations for developing cervical cancer, and may augment the sensitivity and specificity of primary cervical cancer screening programmes by complementing the conventional Pap test. **Objective:** To develop a simple, cheaper and easy to handle, method for detection of HPV infection that cause cervical cancer. **Methods:** 20 paper smears from cervical cancer patients were collected from each of the four different participating centers in India. These paper smears were employed for a simple single tube boiling method of DNA extraction and a simple PCR amplification procedure using HPV consensus and HPV type-specific 16 and 18 primers. **Results:** The quantity and quality of DNA extracted from the dried paper smears by boiling were comparable to those obtained by standard collection and Phenol-chloroform extractions, as estimated by ethidium bromide stained agarose gel electrophoresis. Out of 80 samples analyzed, 42 (57.5%) were found to be positive for HPV infection by L1 consensus primer. Of these 30 cases (71.5%) were found to be HPV 16 positive and none of the samples were found to be HPV 18 positive. **Conclusion:** The collection of cytological samples on small filter paper and their dry shipment and storage at room temperature, coupled with a single tube boiling method of non-organic DNA extraction, solves several disadvantages and reduces biohazards associated with the conventional handing of biological specimens and DNA extraction for large-scale population/ epidemiological screening. This is a user-friendly, cost-effective and reliable method for screening HPV and other genes interest.

W-31

Expression of DNA-Damage Repair Proteins in Relation to Viral Infection in Primary Nasopharyngeal Carcinoma.

Smriti. M. Krishna*[1]*, Jayashree Kattoor[3], Prabha Balaram[1]

[1]Div. Of Cancer Research, Regional Cancer Centre, Trivandrum 695 011, [2]Div. Of Cytopathology, Regional Cancer Centre, Trivandrum 695 011, India. *smritisunil@yahoo.co.in

Introduction: Viral interactions with tumor suppressor genes abrogate cell cycle arrest and disturb DNA repair of therapy-induced DNA lesions. Nasopharyngeal carcinoma (NPC) is a unique cancer due to its viral etiology and radiotherapy (RT) is the usual treatment modality. The variation in RT sensitivity shown among the tumors of same stage, suggest a strong need for additional predictive factors to improve therapy outcome of these patients. **Objectives:** In the present study the effect of EBV and HPV on DNA-repair proteins, ATM and DNA-PKcs was assessed in benign and malignant nasopharyngeal epithelium. The rational of study was that evaluation of role of viruses and expression of DNA-repair proteins in NPC may predict response to RT. **Methods:** Expression of DNA-PKcs and ATM were assessed by immunohistochemistry and presence of EBV and HPV by PCR using specific primers. **Results:** 63% of NPC were EBV+ and 30% were HPV+. Expression of ATM and DNA-PKcs were significantly increased in NPC when compared to benign samples ($p=0.000$). NPC showing EBV infection showed reduction in expression of ATM and DNA-PKcs ($p=0.009$ & $p=0.011$) where as, in NPC's with HPV infection, the mean expression values of the proteins was increased ($p=0.001$ & $p=0.003$). NPC patients with HPV infection also showed significant bad response to radiation ($p=0.002$). **Conclusion:** The study indicates that EBV infection down regulates the expression of DNA-repair proteins and renders NPC sensitive to RT, where as HPV infection upregulates expression of DNA repair proteins making the tumors resistant. The assessment of expression of DNA-PKcs and ATM in biopsy specimens can be used as a criterion to identify radio-resistant NPCs.

W-32

Identification of a Novel Interacting Protein for Tumor Suppressor Proteins Hamartin and Tuberin

Sonal Khare*, S. Chakraborty and Arun Kumar

Department of Molecular Reproduction, Development, and Genetics, Indian Institute of Science, Bangalore, India. *khare_sonal@yahoo.com

Tuberous sclerosis complex (TSC) is a human genetic disorder. It is characterized by hamartomas in multiple organs, which is caused by mutations in either TSC1 or TSC2 tumor suppressor genes. TSC1 encodes for hamartin, which contains a putative transmembrane domain and two putative coiled-coil domains (CCDs). A stretch of 133 amino acids close to the potential transmembrane domain is conserved across species, suggesting an unknown functional role. TSC2 encodes tuberin, which contains two CCDs, two transactivation domains, and a GAP domain. Tuberin and hamartin interact with each other. This interaction is mediated by N-terminal CCD (N-CCD) of tuberin and C-terminal CCD (C-CCD) of hamartin. In order to understand the function of 133 amino acid long stretch of hamartin and C-CCD of tuberin, we have used yeast-two-hybrid approach to identify proteins, which interact with them. The above mentioned domains were cloned in Gal4 DNA binding domain vector pGBT9. Approximately $1x10^6$ clones from a fetal mouse brain cDNA library cloned in Gal4 activation domain vector, pACT2, were screened separately with pGBT9-Ham133 containing 133 amino acid long stretch of hamartin and pGBT9-TubCCD2 containing C-CCD of tuberin. The transformants thus obtained were assayed for interaction using nutritional selection, and alpha- and beta-galactosidase assays. A novel interacting protein was identified which interacts with both the proteins. The co-immunopreciptation experiments have confirmed the interaction of this novel protein with hamartin as well as tuberin. The results and its implication will be discussed. (Financial support from CSIR and UGC are acknowledged).

W-33
Apoptotic and Proliferative Related Gene Expression in Non - Hodgkin's Lymphoma

Sumita Saluja*, Rekha Ghai, Sujala Kapoor, M. Sharma, Sumita Bhasin, D. K. Gupta, Sunita, S.P. Kataria*

VMMC and Safadarjung Hospital in collaboration with Institute of Pathology, New Delhi - 110029, India. * ssaluja@vsnl.com

Introduction: The growth of both indolent and aggressive NHL depends upon the proliferation and death rate of cancer cells. Cell proliferation is determined by using antigens like PCNA while Bcl-2 and BAX are known to regulate the apoptotic pathway. Recent studies favor that most indolent lymphoma are characterized by inhibition of apoptosis. In contrast aggressive lymphoma seems to result from increase in cell proliferation rather than inhibition of apoptosis. **Aim and Objective:** To correlate the expression of apoptotic proteins Bcl-2, and Bax and proliferative antigens like PCNA with the histological grade and immunophenotype of non-Hodgkin's lymphoma. Material and **Methods:** The study was conducted on 35 lymphnode biopsies of non Hodgkin lymphoma. **Results:** The expression of Bcl-2 and BAX and their ratio BBPR was correlated with the immunophenotype and histological grade of tumors. Bcl-2 expression was high in SLL while bax expression was low this giving a high BBPR. Conversely aggressive lymphomas have low Bcl-2 and high Box and hence lower BBPR. The expression of PCNA showed significantly lower labeling index in indolent lymphomas as compared to higher values in aggressive lymphomas. **Conclusion:** There is a significant correlation between the expression of apoptotic and proliferative antigens and the predicted biological behavior of NHL.

W-34
Cancer of Gall Bladder in Dehradun region

Sunil Saini*

Dept. of Surgery, Himalayan Institute of Medical Sciences, Dehradun Uttaranchal State 248140. *drsaini@sancharnet.in

An analysis of cancer patients seen over last 6 years in cancer clinic was done. 2913 patients were diagnosed having cancer. 618 (21.2%) patients were treated for gastrointestinal cancers. 134 (4.6%) were diagnosed having CAGB and 11 Biliary tract cancer . CAGB was second most common GI cancer after cancer of Oesophagus 210 (7.2%), other common GI cancers were stomach 80 (2.5 %) and colorectal cancer 78 (2.5%). In females (8.48%) it was four times common than males (1.96%), and was the fourth most common cancer after breast (21.5%),Head and Neck 14.7%, uterine cervix (9.91 %) in females. Average age was 51 yr females and 55 yr males. All patients had ultrasound of abdomen for upper abdominal symptoms. 45% patients had obstructive jaundice at initial presentation. 95% had associated cholelithisis. Majority had CT / MRI scan done by their referring doctor for evaluation. Histological /cytological diagnosis was confirmed in 93 (70%) patients, by USG guided FNAC or post operative. Rest had radiological diagnosis only, could not get a cytological confirmation for various reasons. 90 patients had adenocarcinoma and 2 had squamous cell carcinoma one small cell CA. Staging details were available for 68(50%) patients, 53 had stage four disease, 10 stage three and only 5 had stage one or 2 disease. Procedures done were segment three Biliary-enteric by pass 22, including gastrojejunostomy in 6, with significant morbidity and two mortality. Extended cholecystectomy in 9 patients. 18 patients had Biliary stent elsewhere. CAGB common cancer in female, diagnosed late, needs palliative care; biliary bypass has significant morbidity, only few suitable for radical surgery.

W-35
Cancer of Laryngopharynx: Results of Treatment

Sunil Saini*

Dept. of Surgery, Himalayan Institute of Medical Sciences, Dehradun Uttaranchal State 248140. *drsaini@sancharnet.in

Background: Over a period of 6 years (1998-2003) approximately 2820 patients were treated cancer clinic, Head and Neck cancer was seen in 794 (28.35%) patients, of which 619 (38.00%) were males (average age 54.97 yrs) and 175 (15.0 %) were females (average age 48.6 yrs), Head & Neck cancer was the commonest malignancy in males and third commonest in females seen in this region. Oral Cavity: 140 (4.96%) Oropharynx: 218 (7.73%) Laryngopharynx: 252 (8.93%) Larynx 111 (4.00%) Supra glottis 35, Glottis 25, Subglottis 4. Hypopharynx 141 (5.00%) PFF 97, Post Cricoid 11. Others 284 (6.52%). All patients had squamous cell carcinoma, except one having chondrosarcoma of larynx. **Interventions:** Diagnostic endoscopy was performed in all patients, Total 104 surgical procedures were performed in 86 patients. 52 patients had post operative radiotherapy. Salvage surgery was performed in 18 patients (including 10 for post radiation recurrence). **Surgical procedures:** Total Laryngectomy 28, Total Laryngectomy with partial pharyngectomy 34, Total laryngopharyngectomy 9, Conservative Laryngectomy 12, partial glossectomy 16, B/L Modified neck node dissection in 58, radical neck dissection in 18, Emergency tracheostomy in 16, Feeding gastrostomy 2. Primary closure performed in 51 patients, patch pharyngoplasty with PMMC flap in 18, gastric pull up in 9, Oesophageal speech rehabilitation by oesophageal speech mainly, few using electrolarynx. **Results:** Operative mortality nil, Morbidity 20%, temporary pharyngocutaneous fistula 4, lymphorea 10, Wound infection 7, pharyngo-esophageal stenosis 10, Tracheostoma stenosis 10, phrenic nerve palsy 3, Good speech quality 38. Longest followup 6 years, 34 patients disease free survival more than three years, common site of recurrence neck nodes. **Conclusion:** Common cancer, most patients diagnosed late, need combination treatment, only few suitable for conservative surgery.

W-36
Epitopes of Estrogen Receptor : Do They Have A Place in Breast Cancer Prognostication?

***Trivedi Sunil*[1]*, Dave Heena*[1]*, Trivedi Trupti*[1]*, Shah Rohini*[1]*, Shah Manoj*[2]*, Shukla Shilin*[3]

[1]Receptor & Growth Factor Laboratory, [2]Pathology Department, [3]Department of Medical Oncology, The Gujarat Cancer & Research Institute, Asarwa, Ahmedabad 380 016, India. * drsuniltrivedi@hotmail.com

Estrogen receptor (ER) is an accepted 'gold standard' in identifying hormone dependent breast cancers. Six functional domains of the protein have differing/multiple functions. ER 'in vivo' is susceptible to several proteolytic enzymes likely to cleave the molecule affecting the functionality. Availability of a plethora of antibodies permit identification of the molecule in different domains and allowed us to evaluate the presence of 'ligand binding' (H222) and 'hinge region' (D547) in the prognostication of breast tumors. We employed the technique of covalent labeling of cytosolic ER with ^{125}I-tamoxifen aziridine and immunoprecipitation followed by SDS-PAGE and autoradiography. Presence of a 43 KDa ER band was connected to a poor prognosis in advanced breast tumors.

W-37

BRCA1 Promoter Hypermethylation and Reduced BRCA1 Protein Expression in Sporadic Breast Carcinomas

***Suresh Hedau*[1]*, Bhupesh K Prusty[1], Neeraj Jain[1], Prachi Saxena[1], Rakesh Kumar[1], Usha R Singh[2] and B C Das[1]**

[1]Division of Molecular Oncology. Institute of Cytology and Preventive Oncology (ICMR), I-7, Sector-39, Noida - 201301 (UP) and [2]Department of Pathology, University College of Medical Science, Shahdara, Delhi - 110 093. * suresh_hedau@hotmail.com

Background: Breast carcinoma is one of the most common malignancies among women worldwide. In India, breast cancer is the second most common cancer in women after the cancer of uterine cervix except in Mumbai and Delhi where it is the leading cancer. Breast cancer susceptibility gene BRCA1 has been found to be mutated in majority of familial breast cancer but not in sporadic breast cancer. However, reduced expression of BRCA1 protein has been reported in sporadic form indicating its involvement in sporadic breast cancer also. **Objective:** The aim of this study was to analyze the expression of BRCA1 protein and the role of BRCA1 promoter methylation. **Methods:** We analyzed one hundred sporadic breast cancer patients for methylation-specific PCR (MSP) and Immunohistochemistry. BRCA1 promoter methylation was analyzed by using methylation-specific PCR (MSP) on DNA from breast tumor and normal controls. Expression of BRCA1 protein was detected in paraffin sections of breast cancer tissues using immunohistochemistry. **Results:** Immunohistochemical analysis of breast cancer biopsy showed reduced BRCA1 protein expression in 40/100 (40%) of the sporadic breast cancer cases. DNA from these tissues were further analyzed by methylation-specific PCR that showed that 20/40 (50%) were having hypermethylation of BRCA1 promoter. Decreased expression of BRCA1 protein correlated with promoter hypermethylation and increased histologic grade and the age group. **Conclusions:** The study showed that an aberrant cytosine methylation of the BRCA1 promoter may be one of the major etiological factors leading to decreased level of BRCA1 expression in sporadic breast cancer.

W-38

Genetic Predisposition to DNA Repair and its Effects on Tumor Response to Radiotherapy in Oral Cancer

***Surya Ramachandran*[1], K Ramadas[2], Ramkumar Hariharan[1], R Rejnish Kumar[2] and M Radhakrishna Pillai[1]**

Departments of Molecular Medicine[1] and Head and Neck Oncology[2], Regional Cancer Centre, Thiruvananthapuram, India. *srnair142@gmail.com

Oral squamous cell carcinoma is a significant public health problem in many parts of the world particularly in South East Asia. It accounts for 40% of all cancers in the Indian subcontinent. Polymorphisms in DNA repair genes may alter protein function and an individual's capacity to repair damaged DNA. Deficiency in repair capacity may lead to genetic instability and carcinogenesis. Tobacco users with diminished ability to repair somatic mutations may be more susceptible to tobacco attributable cancers. Tumor response to radiation therapy depends on the extent of DNA damage induced and its

repair. This study has looked into two major roles of DNA repair genes viz its association with extent of DNA damage during tumorigenesis in the oral cavity as well as its role in response of individual tumors to radiation treatment. We examined polymorphisms of two DNA repair genes in 110 oral and oropharynx cancer, 84 leukoplakia and 110 controls belonging to the Travancore population [XRCC1 (*Arg194Trp, Arg280His Arg399Gln*), and XPD (*Lys751His*)]. Genotyping was done by PCR-RFLP. There was a positive association between the polymorphisms in XRCC1 and XPD and cancer risk. The polymorphic variant of XRCC1 codon 194 and 399 had a 2.3 and 2.7 fold-increased risk (95%CI=1.44-3.89 and 1.69-4.29 respectively) of cancers of the oral cavity compared to the wild genotype. Similar results were observed with the XPD gene, where the polymorphic variant had about 2- fold increased risk (OR=2.3, 95% CI = 143-3.69) of oral cancer. Comparative modeling was done for assessing the functional significance of these polymorphisms. The *Arg399Gln* position was mapped to the surface of BRCT1 domain, which might interfere with the protein function and stability. The actual extent of DNA damage was established by *in vitro* studies using the Cytokinesis Block Micronucleus Assay (CBMN). The standard mean calculated was higher in oral cancer cases than in controls. The results of molecular mapping together with the epidemiological data and *in vitro* DNA damage assay confirms our hypothesis that polymorphisms in functionally important and conserved areas of repair genes may alter the protein structure thus interfering in its function. This may be the reason for increased oral cancer risk.

W-39

Modulation of Enhancing Potency of Liposomal Monensin on Ricin Cytotoxicity in Transformed Mammalian Cells

Suwagmani Das and Prahlad C. Ghosh*

Department of Biochemistry, University of Delhi South Campus, New Delhi-110021. India. * suwagmani@yahoo.com

Monensin, a carboxylic ionophore is known to enhance the cytotoxicity of tumor cell specific immunotoxins and also has potential antimalarial activity. But the biological activities of monensin are significantly inactivated in the presence of serum. With a view to prevent its inactivation by serum, we have intercalated monensin in various liposomal formulations and studied its biological efficacy in these formulations using its ability to enhance cytotoxicity of ricin in CHO Pro cells as an assay system. Ricin cytotoxicity enhancing potency of monensin in liposomal formulations in the presence of serum was found to be highly dependent on both the charge and the density of DSPE-mPEG on the surface of liposomes. Intercalation of monensin in various conventional liposomal formulations has marginal effect on it ability to enhance the cytotoxicity of ricin in the presence of serum. On the other hand, incorporation of 2.5 mol% of DSPE-mPEG in these liposomal formulations significantly stimulated the ricin cytotoxicity enhancing potency of monensin in the presence of serum. Maximum fold enhancement (26.45 fold) of ricin cytotoxicity by monensin in the presence of serum was observed when it was delivered through negatively charged liposomes having 2.5 mol% DSPE-mPEG on the surface followed by positive charged liposomes (21. 22 fold) and neutral liposomes (15.04 fold). These values are 2-3 fold higher as compared to free monensin. These results clearly showed that monensin incorporated in the negatively charged liposomes having 2.5 mol% DSPE-mPEG significantly improve its biological efficacy and can effectively be employed to enhance the cytotoxicity of tumor cell specific immunotoxins for their selective elimination and also for the chemotherapy of malaria under *in vivo* condition.

W-40

Expression and Significance of Telomerase and Its Components in Lung Cancer and Their Relationship to c-myc Expression

[1]Teena Pasrija*, [2]S. Radhika, [1]D Behera, and [3]S Majumdar

Departments of [1]Pulmonary Medicine, [2]Cytopathology and [3]Experimental Medicine & Biotechnology, PostGraduate Institute of Medical Education and Research, Chandigarh, INDIA-160012. *pasrijateena@rediffmail.com

Introduction and Objectives: Telomerase is a ribonucleoprotein complex consisting of 3 main components, hTERC (telomerase RNA component), hTERT (telomerase reverse transcriptase) and telomerase associated proteins. It is a biomarker of malignancy and is regulated by several oncogenes, principally the c-myc oncogene. Lung cancer is one of the common cancers with a high mortality and early detection may improve survival. We aimed to study the expression of telomerase and its components and c-myc in lung cancer patients. We also aimed to determine telomerase activity in sputum samples. **Material and Methods:** A total of 40 patients were studied so far. These included 28 histologically proven lung cancer patients and 12 cases with respiratory diseases clinically proven to be non-malignant. In 18 of these cases, paired biopsy and pre-bronchoscopy sputum samples were available. In all cases fiberoptic bronchoscopy was carried out and tissue pieces obtained for routine histopathology and for molecular analysis. Telomerase Repeat Amplification Protocol (TRAP) assay for telomerase activity and RT-PCR for hTERT and hTERC and c-myc was carried out. **Results:** Out of 28 biopsies tested, hTERC, hTERT and c-myc expression was present in 27 (96.4%), 26 (92.8%) and 24 (85.7%) cases. In 18 out of the above cases, telomerase activity was seen in 94.4% biopsies and 83.3% matched sputum samples. Cytology was positive in only 3/18 sputum samples. Out of 12 negative control sputum samples tested for telomerase activity, 3 were positive and all these were cases of sarcoidosis. **Conclusion:** The preliminary results of our study indicate that, in lung cancer, telomerase is a promising non-invasive biomarker of malignancy that improves the sensitivity of its early detection in sputum, however false-positivity is of concern. c-myc expression is in concordance with the expression of h-TERT and h-TERC.

W-41

Effect of Allyl Isothiocyanate (AITC) and Phenyl Isothiocyanate (PITC) on Tumor Specific Angiogenesis

Thejass P* and Girija Kuttan

Dept. Immunology, Amala Cancer Research Centre, Amala Nagar, Thrissur, 680555, Kerala. *amalaresearch@rediffmail.com

Introduction: Angiogenesis is critical to the transition of premalignant lesions in a hyper proliferative state to the malignant phenotype, which leads to tumour growth and metastasis. **Objectives**: To evaluate the antiangiogenic activity (*in vivo* as well as *in vitro* models) of two naturally occurring isothiocyanates; AITC and PITC. **Methods:** *In vivo* antiangiogenic activity was studied by intradermal injection of B16F10 (1x106) melanoma cells to the shaven ventral side of C57BL/6 mice. AITC & PITC were administered daily at a concentration of 25µg/dose/animal for 5 days. Serum from angiogenesis induced animals treated with these ITCs were used for the estimation of TNF-a and nitric oxide levels. In vitro studies were done by employing

rat aortic ring assay. **Results:** AITC, which produced 40.43% inhibition of tumour, directed capillary formation was found to be more potent than PITC (30%). The serum nitric oxide level was significantly reduced by the administration of AITC (40.86%) and PITC (37.64%) as compared to untreated control animals. Treatment with these ITCs down regulated the activity of TNF-a. *In vitro* studies by rat aortic ring assay showed that the compounds at non-toxic concentrations inhibited the micro vessel out growth from the aortic ring, there by indicating the inhibitory effect of AITC and PITC on the secretion of proangiogenic factors from the B16F10 melanoma cells. **Conclusion:** Both AITC and PITC were shown to inhibit tumour directed capillary formation. The observed antiangiogenic activity of these ITCs is related to, at least in part, the down regulation of nitric oxide, TNF- and proangiogenic growth factors.

W-42
Circulating IGFBPs in Prognostication Of Human Breast Cancers

Trivedi Sunil[1], Dave Heena[1], Trivedi Trupti[1], Shah Rohini[1], Shah Manoj[2], Patel Yatin[3]

[1]Receptor & Growth Factor Laboratory, [2]Pathology Department, [3]Department of Surgical Oncology, The Gujarat Cancer & Research Institute, Asarwa, Ahmedabad 380 016, India. * drsuniltrivedi@hotmail.com

Insulin like growth factor axis is a multi-component network of molecules composed of 2 growth factors, two receptors, six binding proteins and IGFBP proteases. Expression of all these molecules affects the mitogenic stimulation to breast epithelium. Increased expression of IGFBPs lower the bioavailability of growth factors in the target tissues. High IGFBP-3 levels indicate unfavorable prognosis. We estimated circulating IGF binding proteins (IGFBPs) -2 and -3 from 166 breast cancer patients by RIA/IRMA. The levels were correlated to known histologic prognosticators, relapse free- and over all- survival. Circulating IGFBP-2 and -3 were about 2 fold higher in the patients with malignant disease as compared to the patients with benign disease or controls.

W-43
Curcumin Down-regulates the Proliferative Effect Induced by Nicotine in Lung Cancer Cells

Vinesh kumar T.P.*, Smitha Bava, and Ruby John Anto

Rajiv Gandhi Centre for Biotechnology, Jagathy, Thiruvananthapuram, Kerala, India. *kumartpvinesh@yahoo.co.uk

Introduction: Cigarette smoking remains the major cause of lung cancer, with more than 90% of cases in men attributable to tobacco use. Nicotine which provides the subjective pleasure of smoking contributes a large extent to cigarette smoke-induced lung cancer. In this scenario, chemoprevention by harmless natural products is very much promising. Here we have explored the possibility of using curcumin, the active component of *Curcuma longa*, a commonly used food additive of Indian population, as a chemopreventive agent against nicotine-induced lung cancer. **Objectives:** The aim of this study was to find out the molecular mechanism behind the carcinogenic effect of nicotine and also to see whether the nontoxic food additive, curcumin can regulate the same. **Methods:** In this study we have used the lung cancer cell line, H1299. The cell viability was assessed by MTT and tritiated thymidine incorporation assays. Activation of NF-kB was detected by Electrophoretic Mobility Shift Assay and the expression of IkB and IKK was detected by Western blot.

Results: Lower concentrations of nicotine (10^{-9} - 10^{-7}M) induced a slight proliferative effect on H1299 cells. But when pre-treated with curcumin (10mM), the same concentrations of nicotine were found to be cytotoxic to the cells. We observed an activation of NF-kB, a strong antiapoptotic signal, by these concentrations of nicotine, which was down-regulated by curcumin. This may be one of the reasons for the increased cytotoxic effect. **Conclusion:** Curcumin may be used as a potential chemopreventive against nicotine-induced lung cancer.

W-44

TCR Associated Signaling Defects in Oral Cancer Patients as a Possible Reason for Immune Impairment

Vino T. Cheriyanu, Suresh Chandra Dutt and Prabha Balaram

Division of Cancer Research and Division of Radiotherapy, Regional Cancer Centre, Trivandrum, India. *smritisunil@yahoo.co.in

Introduction: T-lymphocytes have been shown to be the principal effecter cells in immunological responses and a hierarchy of immunosuppression exists in cancer patients. The mechanism of immunosuppression has not been clearly defined. Recent reports suggest modification of signal transducing molecules to be responsible for impaired immune responses in various tumours. The status in cancer of the oral cavity is not clear. **Objectives:** To analyse the signal transduction mechanisms in T lymphocytes at the molecular level and assess its role in the immune impairment in patients with cancers of the oral cavity. **Methods:** Percentage population of CD3 +, CD4+ and CD8+ cells were enumerated by surface phenotyping using FACS Calibur. MTS non radioactive cell proliferation assay and Thymidine incorporated cell proliferation assay was used to check the proliferation status. T-cell functions were detected by IL-2 production assay, after stimulation with PHA/anti-CD3, in oral cancer patients. We examined the signaling molecules of peripheral blood T lymphocytes of the oral cancer patients by western blotting. **Results:** Oral cancer patients' lymphocytes were unable to proliferate to the same extent as in controls in the presence of the PHA, anti-CD3 in presence of r-IL2. MTS non radioactive cell proliferation assay and Thymidine incorporated cell proliferation assay clearly demonstrated the defects in cell proliferation after incubation with T cell specific mitogens. Defects in T-cell functions were detected by IL-2 production assay. We also detected abnormal low levels of the signaling molecules (TCR-z, CD3-e, zap-70, p^{56}lck, PKC, rel-B and c-rel) and an impairement in the transduction of rel-B to the nucleus. **Conclusion:** Impairment in the translocation of rel-B to the nucleus and the reduced levels of signal transducing proteins might be the reason for the decreased production of interleukin-2 and immune impairment in oral cancer patients.

W-45

Modulation of Phage-Displayed scFv Antibody Binding by Substrates and Inhibitors: A Case of Placental Alkaline Phosphatase (PLAP)

Vishal Jain[1]*, Deepti Saini[2] and Subrata Sinha[1]

[1]Department of Biochemistry, All India Institute of Medical Sciences, New Delhi, India. [2] University of Arkansas, USA. *aiimsvishal@hotmail.com

Introduction: Human antibodies against specific targets of the tumor cells are the most desirable molecules

for possible cancer immunotherapy. Using the combinatorial antibody library displayed on a phage one could develop such antibodies. Placental alkaline phosphatase (PLAP), the heat stable isozyme of alkaline phosphatase is a potential target for immunotherapy as it is expressed on the membrane of several malignancies eg. choriocarcinomas, seminomas and tumors of ovary, uterine cervix, breast and lung. Objectives: The intention, here, is to develop an antibody specific against the placental isozyme (PLAP), with little or no cross-reactivity to other isozymes and plasma proteins. **Methods:** A synthetic human antibody library (Griffin Library) was used for antibody isolation. A novel selection strategy was adopted employing known uncompetitive inhibitor of PLAP, like L-Phe-gly-gly. Soluble expression of the phage-displayed antibodies (scFv) was carried out and scFv obtained was purified with metal affinity columns. Binding assays were done by ELISA using PLAP-conjugated magnetic beads, Hela cells expressing PLAP on their surface and by antigen capture. Modulation of enzyme activity was judged by plotting Lineweaver-Burk plot. **Results:** The clone VE5 showed binding in both Magnetic Bead Phage-ELISA, antigen capture ELISA and HeLa cell ELISA. Binding to the antigen (PLAP) was inhibited by L-Phe, L-Phe-gly-gly and L-leu. One of the significant findings was the enhanced binding of the antibody in the presence of the substrate, para-Nitro Phenyl Phosphate and inhibition of binding in the presence of another substrate Disodium Phenyl Phosphate. Also, the purified scFv inhibited PLAP activity in a competitive manner. **Conclusion:** The differential binding of the antibody to the putative enzyme-substrate complex is interesting and these binding characteristics of the clone can be exploited to study the structural differences in the critical epitopes on PLAP that are concerned with antigen antibody binding.

Human Papillomavirus and Cervical Cancer (Thursday)

TH-1

Human Papillomavirus DNA in Urine Samples of Women with or Without Cervical Cancer and Their Male Partners Compared With Simultaneously Collected Cervical/ Penile Smear or Biopsy Specimens

Amita Gupta*[*1], Raksha Arora[1], Sanjay Gupta[3], Uma Kailash[2], Bhupesh K. Prusty[2], Swaraj Batra[1] and Bhudev C. Das[2]*

Department of Gynecology and Obstetrics, Maulana Azad Medical College, Lok Nayak Hospital, New Delhi,[1] Division of Molecular Oncology,[2] Division of Cytopathology, [3] Institute of Cytology and Preventive Oncology {ICMR}, I-7, Sector-39, Noida, UP, India. *amitagupta98@rediffmail.com

Background: Infection of specific types of high-risk human papillomaviruses (HPVs) cause cervical cancer in women. Conventional test for genital HPV infection requires collection of scraped cervical cells or biopsy specimens, which involves invasive procedures. Utility of non-invasive urine sampling for detection of HPV in women and her male sexual partners is controversial. There is a need for validation of this urine-based HPV DNA test, which will be of immense value not only in screening large population and children but also for HPV vaccine monitoring in adolescents. **Methods:** We have examined the prevalence of high risk HPV types 16 and 18 in the simultaneously collected urine samples and cervical scrapes or biopsy specimens from women with cervical cancer and their single lifetime male sexual partners in order to validate the utility of urine sampling as a reliable noninvasive method for detection of HPV infection. Thiry women with invasive cervical cancer and their husbands along with 30 age-matched normal healthy women including their husbands were recruited for the study. Cervical biopsies/scrapes from women subjects and penile scrapes from their husbands, and urine samples from all of them were collected before taking biopsy or scrapes. HPV-L1 consensus primer

as well as high-risk HPV (HPV 16 and 18) type-specific oligo-primers were used for PCR detection of HPV DNA sequences. **Results:** The total prevalence of HPV in women with cervical cancer was found to be 83% (25/30) while it is only 66% (20/30) in their male partners but there was virtually no difference in results between urine and scrape or tissue biopsy specimens either in women or their male partners. Interestingly, although healthy control women and their husbands showed similar frequency of HPV infection both in urine and scrape samples, there is a significant difference (P=0.05) in the prevalence of high risk HPV type 16 in women with cervical cancer (70%) and their male partners (30%). Similar was the trend between control women and their male partners. No HPV type 18 could be detected either in cancer or in controls. The results also show a very high prevalence of HPV type 16 among Indian women with cervical cancer while its frequency is significantly low in their single lifetime male partners. **Conclusion:** The case by case matching results of HPV positivity between urine and cervical / penile scrapes or biopsy specimens obtained from women and their male partner demonstrate that the non-invasive urine sampling can be reliably used for screening genital HPV infection in both men and women.

TH-2

Polymorphisms at *GSTM1, GSTT1* and *GSTP1* Loci and Susceptibility to Cervical Cancer and Chronic Myeloid Leukemia in Indian Population

Anita Sharma**, BC Das and JK Sharma

Institute of Cytology and Preventive Oncology (ICMR), I-7, Sector 39, Noida, India. *dranitasharma@yahoo.co.in

Introduction: The GSTs represent a super family of phase II enzymes that play an important role in cellular protection against the toxic effects of pollutants, drugs, environmental carcinogens and xenobiotic through their conjugation with glutathione. Since many GST genes are polymorphic, there has been considerable interest in determining whether particular allelic variants are associated with susceptibility to various cancers. **Material and Methods:** In the present study, we report here the polymorphism of *GSTM1, GSTT1* and *GSTP1* in normal controls, chronic myeloid leukemia (CML) and cervical cancer cases. *GSTM1* and *GSTT1* polymorphisms were analyzed by multiplex PCR method and *GSTP1* polymorphism by Southern blot hybridization, using Bam HI restriction enzyme. **Results:** The result observed revealed that a particular genotype is associated with an increased risk of developing a particular cancer but not the other. **Conclusion:** Presumably different cancers have different causative substrates and this is reflected in the genes associated with susceptibility. Results will be presented.

TH-3

Evaluation of See-and-Treat Approach for Urban Slum Dwellers in a Camp as a Preventive Measure to Control the Incidence of Cancer Cervix in the State of Chhattisgarh

***Asha Jain** and Pratima Chaudhary**

Cancer Prevention & Relief Society, Sri Srimaa Sharda Street Cancer Suraksha & Anusandhan Kendra, 58, Jalvihar Colony, Raipur,(C.G.) India, 492001. *aasha221@rediffmail.com, drashajain@yahoo.co.in.

Introduction: In Raipur, capital of Chhattisgarh, nearly 2,000 women present with late stage invasive cancer every year though Cancer Cervix is Preventable if CIN is diagnosed and treated. **Objectives:** We establish an objective of identifying and treating CIN by Colposcopic 'See& Treat' approach by conservative

modalities in the high-risk group of Urban Slum Dwellers, who do not return later for treatment. **Method:** Detailed history, general & gynecologic examination & Pap smear was done on all women followed by Video Colposcopy with image capture. HPVHC2 specimens were collected only from acetowhite lesions and from symptomatic cervical erosions due to paucity of funds. Treatments of CIN were done as per the See& Treat protocol by Cryo/Leep/Cold Cautery. It was predefined to treat symptomatic cervical erosions. In Invasive disease only biopsy was taken. Over treatment was understood as a distinct but acceptable possibility. **Results:** Adequacy of treatment was correlated with reports of Pap smears& HPVHC2 with Histopathology as the Gold Standard. All three were done in Delhi. Out of 259 women, 16 had biopsy / LEEP and 16 had Cryo. 4 biopsies were positive for Squamous carcinoma and 10 Leeps done were positive for CIN1&/2. Out of 30 HPVHC2 samples 10 were positive. Sensitivity and Specificity of Colposcopy was 94% and 99% respectively. **Conclusion:** As unnecessary biopsies were not taken and HPV positives & Pap smear positives had already received treatment, Colposcopic 'See& Treat' approach was found an effective method to deal with women for controlling the incidence of Cancer Cervix.

TH-4
Molecular Markers for the Detection and Progression of Cervical Cancer

***Bharathi Ramdass*[1]*, *Pradip Nair*[1], *Geetashree Mukherjee*[2] *and Sudhir Krishna*[1]**
[1]National Centre for Biological Sciences, GKVK Campus, Bangalore-560 065. [2]Kidwai Institute of Oncology, Bangalore. *bharathi@mailsvr.ncbs.res.in

Introduction: The infection by high-risk human pappillomavirus (HPV) and the persistent expression of viral oncogenes E6 and E7 are associated with the progression of human cervical cancer. In the present investigation we are trying to identify novel genes that could potentially act as predictive markers for human cervical cancer. **Objectives:** The first phase includes six molecules p53, Bcl2, c-Fos, NF-kB p50, NF-kB p65 and IkBa. The second phase of study includes Notch, Jagged1, Hes1, cyclin D1, and cdk9 and pAkt molecules. To study whether these proteins have a role in determining cervical cancer progression, a study involving the distribution of these molecules across various cervical cancer grades has been initiated. **Methods:** Immunohistochemical staining was done on 80 tissue samples covering normal (normal -25 patient samples), preinvasive (CINI, CINII-III-19 samples) and invasive (36 samples). **Results:** p53 and NF-kB (p65 and p50) were significantly over-expressed in invasive samples as compared to normal and preinvasive samples. These results would suggest that p53 ($p< 0.001$), NF-kB proteins [p65 ($p< 0.001$) and p50 ($p<0.001$)], actually shows over expression in cervical carcinomas as compared to pre-invasive and normal samples. c-Fos ($p<0.019$) and IkBa ($p<0.007$) is also significant in cervical cancer, while Bcl2($p<0.122$) is not significant in tumor progression. **Conclusion:** Consequently it may be summarized that p53, NF-kB and c-Fos are the three proteins that are overexpressed in cervical cancer. Notch, Jagged, Hes1, cyclin D1, cdk9 and pAkt molecules are being studied to determine their role in cervical cancer progression. Future studies will involve the ability of these proteins to predict patient response to therapy. [Note: This project is a part of DBT sponsored multi-centric programme.]

TH-5
Colposcopy on Cervical Condylomas - Case Reports

***Geethanjali Amin**, S.S.Shastri and R.Sankarnarayanan*

Mumbai CEDS Project, IARC, Department of Preventive Oncology, Tata Memorial Centre, Parel, Mumbai

40012, India. *geethanjaliamin@yahoo.co.in

Human Papilloma Viruses (HPV) has been implicated as an important etiological factor in cervical carcinogenesis. The diagnosis of HPV lesions could be difficult because it presents differently in the various tests i.e. cytology, colposcopy, histopathology and HPV DNA testing and thus can pose contradictory in the management of the disease. To present varied colposcopic findings pertaining to HPV in few case reports. We report few cases of colposcopically diagnosed HPV lesions presented differently at varied menopausal age. Case 1 - Peri menopausal asymptomatic lady with large polypoidal exophytic lesion on the cervix. Case 2 - Postmenopausal widowed lady with history of abnormal discharge per vagina with a colposcopic picture of florid large condyloma involving the whole of cervix and the left fornix. Case 3 - Pre menopausal lady with colposcopic picture of flat geographical lesion. Case 4 - Pre menopausal lady with leukoplakic lesions on the cervix extending on to the vagina which recurred even after leep and simple hysterectomy. Colposcopic, cytological and the histopathological findings of all the cases will be discussed in the presentation. 1. Women with HPV lesions considered at higher relative risk for malignant transformation since the underlying histopathology may vary from immature squamous Metaplasia to HGSIL and even to carcinoma. 2. Patient with atypical condyloma form a special group at definite high risk and should be immediately managed and followed up at frequent intervals.

TH-6
p53 Mutation Profiling and HPV Typing in Oral Carcinoma

Geo Francis*, Thomas Abraham, and S. Kannan

Division of Cancer Research, Regional Cancer Centre, Trivandrum - 695 011, Kerala, India. *geoflip@yahoo.com

Introduction: Oral cancer remains one of the key health issues in India. Tobacco chewing is considered to be the major causative factor of Indian oral carcinoma. Besides tobacco chewing, viral etiology is also suspected in oral cancer. Dysfunction of p53, a multi functional tumor suppressor protein with pivotal role in many cell fates determining process, is implicated in oral tumorigenesis. Gene mutations constitute the major form of p53 inactivation. Although p53 gene has 11 exons, exons 5 through 9 harbors 90% of observed mutations. In addition to mutation, high risk HPV, types 16 and 18, also reported to inactivate p53 proteins. Aim: The present study analyzed the mutation spectrum of p53 gene in oral carcinoma and also looked for HPV infection rate in these samples. Materials and **Methods:** Total 103 oral carcinoma cases were screened for p53 mutations in exons 5, 6, 7, 8, 9. Primary screening of mutations was carried out by PCR-SSCP/Heteroduplex method and further mutations were confirmed by direct sequencing. PCR analysis using type-specific primers were performed to detect the presence of high risk HPV 16 and HPV 18 DNA in these cases. Beta-globin gene was used as PCR internal control. **Results:** Out of 103 cases, p53 mutation was found only in 9 cases (8.7%). None of the cases has HPV 16 infection and HPV 18 genome was detected in 2 samples. **Conclusion:** The present study suggests low frequency (9.7 %) of p53 mutations and little role of HPV in OSCC.

TH-7
Prevalence of HPV in Laryngeal Papillomatosis, Benign and Premalignant Lesions of Head and Neck and its Prognostic Significance

Gitanjali Bugnait*[1], Alok Mishra[2], BC Das[2], TS Sidhu[1]

[1]Deptt. of Otolaryngology, Dr.RML Hospital, New Delhi. [2]Division of Molecular Oncology, Institute of Cytology

and Preventive Oncology, I-7 Sector-39, NOIDA, UP. *bcdas48@hotmail.com

The aim of the study was to determine the prevalence of HPV infection in benign, premalignant and malignant lesions of Head and Neck to ascertain the role of HPV infection in the tumor progression and prognosis of disease following treatment. Patients of the study comprised a heterogenous population of disease types, sites and stages.A total of 19 patients have been assessed.These included patients of carcinoma of larynx, oropharynx, oral cavity and oral laryngeal papillomatosis, vocal poly and leukoplakia. PCR was performed for the detection of HPV DNA sequence using consensus as well as type -specific HPV primers. HPV was detected in four out of 19 specimens assessed.Of these three patients were of laryngeal Papilloma and one of carcinoma tonsil (T2, N1, M0).Two of the laryngeal Papilloma patients had HPV-11 infection and one had both HPV 16 and 11.The lone case of carcinoma tonsil tested positive for HPV-16. All three patients of laryngeal papilloma who tested positive for HPV are symptomatic after year follow up.The positive case of carcinoma tonsil was referred for radiotherapy and is alive 6 months post therapy. The preliminary study indicates that HPV infection particularly high risk types may play an important role in progression of Head and Neck cancer.

TH-8

Polymorphism of Cytokine Gene, Tumor Necrosis Factor in Cervical Cancer

***Indu Kohaar*[1]*, Nisha Thakur[1], Sudha Salhan[2], Pushpa Sodhani[3], Mahendra P[1], Alok Mishra[4], B.C. Das[4], Debi P Sarkar[5]and M Bharadwaj[1]**

Divisions of [1]Mol. Genetics, [3]Cytopathology and [4]Mol. Oncology, Institute of Cytology & Preventive Oncology (ICMR), I-7, Sector 39, Noida. [2] Safdarjung Hospital, New Delhi. [5]Department of Biochemistry, University of Delhi (South Campus), New Delhi. *kohaarindu@rediffmail.com

Introduction: Infection of high risk human papillomavirus types 16 and 18 is the major cause of cervical cancer. However, cervical infection with HPV usually results in a transient infection, with 70 to 90% of individuals showing virus clearance, as revealed by repeated HPV DNA testing, within 12 to 24 months of detection. The importance of persistent HPV infection in the remaining 10 to 30%, especially with oncogenic HPV types, lead to the development of high-grade cervical intraepithelial neoplasia (CIN) that progress to invasive carcinoma. Factors that contribute to viral persistence have not been elucidated but the host immune responses may be one the most important factors in the natural history of HPV infection. Inherited polymorphisms in immunomodulatory genes may contribute to variations in immune function and genetic susceptibility for complex disease like cancer. Tumor necrosis factor alpha (TNF μ), a cytokine to have a potent immunomodulation activity and is induced by bacterial lipopolysaccharide, mitogens and viruses. **Objectives:** The aim of the present study was to find polymorphisms in TNFμ promoter region in pre-cancerous and cancerous lesions of uterine cervix, which could be interesting not only as genetic marker but also for their involvement in clearance of HPV infection. **Method:** PCR-RFLP methodology was employed for identifying the polymorphisms at -308 and -238 loci of TNFμ . A total of 55 samples of pre-cancerous and cancerous lesions were screened for these loci. **Results:** Analysis of these loci of TNF μ reveals that -308 locus is highly polymorphic as compared to the other. **Conclusions:** Further analysis with larger sample size is needed.

TH-9

Assessing the Involvement of HPV in Oral Cancer Based on E-6 M-RNA Expression

Jatin K.Nagpal*and Bibhu. R. Das
Research & Development Division, SRL- Ranbaxy, 113, MIDC, 15th Street, Andheri (E), Mumbai - 400 093, India. *jatin852@hotmail.com

Several studies over the past decade show HPV 16/18 infection in epithelial cells of oral cavity and their role in development of cancer. It has been proved that two early genes E-6 and E-7 of high risk HPV's (16/18) are transforming genes which are responsible for maintenance of tumorigenic phenotype. The E-6 oncoprotein specifically inactivates wild type p53, and the E-7 oncoprotein inactivates Rb. A total of 110 patients of oral squamous cell carcinoma from the Eastern region of India addicted with tobacco chewing habit for more than 10 years were selected for study. We observed that 33.6% (37/110) patients showed presence of HPV DNA among which the presence of HPV-16, 18 and 16/18 co-infection was 12.7%, 4.5% and 10% respectively. We also studied the genotypic distribution of p53 codon72 polymorphism in these patients. It has already been reported that HPV-16 E6 oncoprotein more readily targets Arginine form of p53 for degradation. Our studies strikingly showed that high HPV infection rate was observed in patients with Arg/Arg genotype as compared to Pro/Pro genotype. Further 20 HPV-16 positive tumors were selected and studied for the E-6 and p53 m-RNA as well as protein expression. Here HPV-16 positive SiHa cervical cancer cell line was used as positive control. We observed that 7/20 (35%) of HPV 16 positive tumors showed E-6 m-RNA expression, indicative of viral activity. Whereas only 3 out of 7 E-6 m-RNA positive tumors infact showed the presence of E-6 protein. 14/20 (70%) of HPV-16 positive oral tumors expressed p53 m-RNA whereas only 10/20 (50%) showed accumulation of p53 protein. Our results suggest that role of HPV in oral cancer can be overestimated when using DNA assays only. Hence analysis of E-6 m-RNA expression by RT-PCR or semi-quantitative analysis of the viral load, seem more reliable assays to assess HPV involvement in oral cancer that the very sensitive DNA PCR analysis.

TH-10

Association of Telomere-repeat fragment 1 (TRF I) expression with telomerase activity and its catalytic components and the role of human papillomavirus infection during cervical carcinogenesis

Uma Kailash*[*1], CC Soundarrajan[2], R Arora[3], S Vivekanandan[2], R Lakshmy[4] and BC Das[1]

[1]Division of Molecular Oncology, Institute of Cytology and Preventive Oncology (ICMR), Sector 39, NOIDA, [2]Department of Neuro-Biochemistry, All India Institute of Medical Sciences, Ansari Nagar, New Delhi. [3]Department of Obstetrics and Gynaecology, Lok Nayak Hospital, New Delhi. [4]Department of Cardiac Biochemistry, All India Institute of Medical Sciences, Ansari Nagar, New Delhi. *umakailash@yahoo.com

Introduction: Telomerase is activated in more than 95% of malignant tumors, with the exception of some self-renewing tissues with high regenerative potential. Telomerase activity is usually repressed in normal somatic tissues. A number of telomerase relevant genes have been cloned, including those, which encode three major components of human telomerase: human telomerase RNA component (hTR), human telomerase reverse transcriptase (hTERT), and telomerase-associated protein-1 (TEP1). Recently, two important genes encoding human telomeric-repeat binding factor protein (TRF) 1 and 2 have also been cloned. To clarify mechanisms regulating telomerase activity, in the development of cervical carcinogenesis, we studied telomerase activity, expression profile of telomerase components hTERT, hTR, hTEP1 and the telomeric-repeat binding factor protein TRF1 in normal, dysplastic and invasive cervical cancer tissues and the presence/absence of oncogenic humanpapillomavirus (HPV) infection. **Methods:** DNA, RNA and protein extracted from the cervical tissues using the methods routinely followed in the lab were used, for studying HPV, telomerase catalytic components and TRF1 expression respectively. Protein for TRAP assay was extracted using CHAPS lysis buffer. Presence of

oncogenic HPV types 16 and 18 DNA was done by polymerase chain reaction. Telomerase activity was analyzed by TRAP assay. Expression analysis of telomerase components hTERT, hTR and hTEP1 was done by RT-PCR whereas telomere-repeat fragment 1 (TRF1) expression was studied by western-blot technique. **Results:** We observed that both telomerase activity and hTERT were up regulated as the disease progressed. The up-regulation of hTERT mRNA was associated with reciprocal changes in the expression of TRF1. Up- regulation of telomerase activity and hTERT expression in malignant tissues may be due to transcriptionlly active form of hTERT. We also correlated HPV infection on telomerase activity, its catalytic components and the expression of TRF1. **Conclusions:** Our results suggest that not only hTERT but also TRF1 as an important regulator of telomerase activity.

TH-11

Targeted Screening with Visual Inspection: A Method for Increasing the Yield of Cervical Smear Positivity in Urban Women

K Kumar**, V K Iyer, N Bhatla[1], A Kriplani[1] and K Verma

Cytopathology laboratory, Department of Pathology and Department of Obstetrics & Gynaecology[1], All India Institute of Medical Sciences, New Delhi, India. *drkamlu04@yahoo.co.in

Objectives: To establish the utility of cervical smear screening by targeted approach in an urban population. **Methods:** Three hundred sixty-one women in reproductive age group were selected on the basis of a per speculum examination (visual inspection) by a gynaecologist showing a suspicious lesion. Cervical smears using Ayers spatula and endocervical brush were made and examined by a consultant cytopathologist. Colposcopy and biopsy were done in 221 cases, including all cases with abnormal smears. Routine smears from a rural setting served as a control population. **Results:** 12/361(3.32%) smears showed ASCUS, 16/361(4.43%) showed LSIL, 23/361(6.37%) HSIL and 3/361(0.83%) showed carcinoma on cytology. On biopsy 28/221(12.67%) showed LSIL, 21/221(9.50%) showed HSIL and 7/221(3.17%) showed carcinoma. On correlation of cytology with histology, the sensitivity of cytology screening was 46% while specificity was 91.41% for LSIL and above. Sensitivity for HSIL & above on cytology was 76.9% with specificity of 94.47%. Yield of 12.67% for LSIL and 12.67% for HSIL & carcinoma together on histology is higher than that reported for routine screening (0.5% to 2.6%). **Conclusion:** Targeted screening of women in an urban setting on the basis of visual inspection has a higher yield of significant abnormalities on cervical smear examination and biopsy.

TH-12

Endogeneous Expression of Gal-1, Gal-3 and a Galactose Specific Plant Lectin Binding in Cervical Intra Epithelial Neoplasia: Its Significance to Assess the Malignant Potential

K Sujathan[1], Tharasomanathan[1], HJGabius[2], S Kannan [3], P Remani [3]

Divisions of Cytopathology[1], Cancer Research[3], Regional Cancer Centre, Trivandrum, Kerala, India. & Institutfür Physiologie,Physiologische Chemie and Tierernährung Veterinärstr, Germany[2]. *ksujathan@yahoo.co.in

Cancer of the uterine cervix is characterized by a series of progressive pre-malignant changes ranging from CIN I to CIN III. HPV infection has been reported to involve in the etiology of at least 90% of the CIN lesions. The rate of progression of these lesions to cancer has been reported to vary from 4.4% to 65% depending upon the severity of the lesion If biological markers can accurately predict the CIN lesions which have more chance to progress to higher up lesions, treatment and further cytological follow up can be limited to them alone and that can be used as a pre-selection criteria for further management. Studies on oral pre-

malignant lesions exhibiting epithelial dysplasia have shown changes in the glycosylation pattern, whereas, such changes were only occasionally seen in lesions without dysplasia. Lectins are reported to be useful in identifying these changes in the glycosylation pattern. The present study analyzed endogenous lectins, gal-1, gal-3 and a galactose specific plant lectin binding pattern in 66 archival cervical biopsies, which includes normal, different grades of CIN and malignancy to see whether SGL binding pattern or the galectin expression show any significant difference between different grades of dysplasia. Uniform cytoplasmic expression of galectins in the differentiated areas were observed in all the samples of invasive squamous cell carcinoma but in the CIN lesions, the expression was similar to that of normal samples except for CIN III. Similar tissue binding patterns for gal-3 as well as for gal-1 and for the plant lectin with identical sugar specificity were observed in the squamous epithelium of the uterine cervix. In normal endocervical cells and in adenocarcinomas the expression of both of the galectins were confined to the apical membrane and the lumen whereas uniform binding of the plant lectin with moderate intensity was observed all over the cytoplasm. The present study found no application for snake gourd lectin or for galectins in predicting the malignant potential of CIN and suggest these lectins as markers of differentiation, which may have some application in pathology and cytology to distinguish differentiated tumors from undifferentiated ones.

TH-13
Assessment of Agnor Counts as Tumor Marker in Cervical Carcinogenesis

J.S. Misra, Vinita Das and Madhulika Singh*

Department of Obstetrics and Gynaecology, King George's Medical University, Lucknow, India. *hrrc_lko@sancharnet.co.in

The present investigation is aimed at finding out diagnostic value of AgNOR counts and its potentiality as tumor marker in the process of cervical carcinogenesis. Silver nitrate staining was performed in 50 cervical smears each cytologically diagnosed as normal, inflammatory, low-grade squamous intraepithelial lesions (LSIL- mild dysplasia), HSIL (moderate and severe dysplasia) and squamous cell carcinoma. A progressive increase in AgNOR counts was noticed when the severity of the pathological lesion increased. The statistical analysis revealed significant variation in AgNOR dots between normal and inflammatory smears ($p<0.05$) and highly significant difference between inflammatory and LSIL cases, between LSIL and HSIL and between severe dysplasia and frank malignancy of cervix ($p<0.01$). Follow up smears were available in 16 mild and 5 moderate dysplasia cases. While all 8 mild dysplasia cases with initial low AgNOR counts revealed regression of the lesion on follow-up, 75% of the cases with initial high counts showed progression or persistence of dysplastic lesion. Similar trend was also seen in 5 cases of moderate dysplasia followed. The study thus points out the diagnostic importance of AgNOR counts specially in discriminating SIL cases. First it is hoped that follow-up of large number of dysplasia cases will help in ascertaining relationship of high AgNOR counts with progression of dysplastic lesion thus singling out high risk SIL cases and to reach a definite conclusion regarding tumor marker potential of AgNOR counts in cervical carcinogenesis.

TH-14
Role of different epidemiological factors, colposcopy and cytology in the screening of cervical cancer in symptomatic patients

Rajyashri Sharma[1], Veena Maheshwari[1], Mehreen Aftab[1] and B.C. Das[2]

[1]Jawaharlal Nehru Medical College & Hospital, Aligarh Muslim University, Aligarh. [2]Institute of Cytology & Preventive Oncology (ICMR), Noida, 201301. * mehreen_aftab2004@yahoo.com

To Asses the epidemiological factors including human papilloma virus and pre-cancerous lesion for the early detection of cervical cancer by clinical assessment colposcopy & cytology. The present study by conducted for a period of one year from July 2003 - June 2004. A detailed clinical history and epidemology factors were studied colposcopy and cytology were done and these were correlated with each other. The highest incidence of HPV was detected in the age group of 30-40 years. Patient with parity 3 (30.18%) were associated with pre- cancerous lesion of cervix, whereas those with p5(45.45%) had a higher incidence of cancer cervix those Pre- cancerous (92.45%) and cancerous(96.96%)lesion had a higher incidence in the lower socio-economics status. The age at marriage ranged from <18-25 years show Pre- cancerous (49.05%) and cancerous (81.13%) and cancerous (90.90%) were seen in Hindu religion. Associated cases of tobacco chewing in Pre cancerous is 3% and in cancerous is 6% and in smoking Pre cancerous is 3.77% nil in cancerous. Complains of vaginal discharge 31.32% cases, contact bleeding 18.2% in cancerous patients. Association of HPV with koilocytic changes in cytology find in Pre- cancerous 50% and 11.11% in cancerous cases. Presence of aceto white area in colposcopy expected for HPV its confirmed by biopsy. 153 Cases were screen for HPV out of 184 cases. 53(28.8%) were diagonased as pre- cancerous lesion and 33(10.7%) as cancer cervix. HPV 16+ve in pre- cancerous is 20.6% and in cancerous 78.2% HPV with Koilocytic changes in cytology find in Pre- cancerous 50% and 11.11% in cancerous cases.

TH-15

Role of Human Papillomavirus DNA Testing in Screening for Cervical Cancer

Neha Gami*, Gauri Gandhi, Swaraj Batra, B.C.Das, Suresh Bhambani, Vijay Zutshi

Department of Obstetrics and Gynaecology, Maulana Azad Medical College and associated Lok Nayak Hospital, New Delhi, India. *nehagami@hotmail.com

Introduction: Human papillomavirus is an established risk factor for cervical cancer. **Objectives:** To evaluate the role of testing for high risk human papillomavirus DNA as a screening test for cervical dysplasia and cancer. **Method:** 400 patients from the gynecology outpatient department were screened using Pap smear and VIA. HPV DNA testing was done for 62 VIA positive and 100 VIA negative women. Colposcopy was done for all women. Those found positive on any or all of the screening tests were subjected to cervical biopsy. **Results:** VIA had the highest sensitivity (91%) to detect any grade of dysplasia. The sensitivity of the combination test (VIA + HPV) was 80.6% which was lower than that of VIA (91%) and also lower than that of HR HPV DNA detection (86%). The specificity of the combination test (VIA + HPV) was 68.3% which was significantly higher than that of VIA alone (56%). Pap smear had the highest specificity. **Conclusion:** Thus a large number of cases missed by Pap smear would be picked up by the combination test. However due to its lower specificity, the combination test would result in a large number of women being unnecessarily referred for colposcopy and further treatment regarding cost analysis, the combination test (HR HPV done only for VIA positive cases) would be more economical than universal screening by Pap smear.

TH-16

Involvement of Molecular Marker in Cervical Cancer

NishaThakur[1]*, Indu Kohaar[1], S Batra[2], V Singh[3], S Gupta[4], B C Das[5] and M Bharadwaj[1]

Divisions of [1]Mol. Genetics, [3]Clinical Research, [4]Cytopathology and [5]Mol. Oncology, Institute of Cytology & Preventive Oncology (ICMR), I-7, Sector 39, Noida. [2]Division of Gynecology, LNJP Hospital. *thakurnisha@hotmail.com

Introduction: Cervical carcinoma is one of the most common malignancies affecting women worldwide as well as in India. Since the prognosis of invasive cervical cancer is generally poor, the dissection of the molecular events that mediates the pathological changes from non-invasive to invasive tumors is of considerable importance. Clinico-epidemiological studies suggested role of multiple risk factors including infections of HPV. It has been found to be an infection with certain high-risk HPV subtypes substantially increases the relative risk for cervical cancer but involvement of host genetic events appear to play a role in the process of cervical carcinogenesis. In any kind of cancers, one or many candidate genes involved in cell cycle control, signal transduction, DNA repair, cell to cell communication, tumor suppressor pathways are found to be mutated, deleted, methylated or polymorphic or found as combination of any of these two. Emerging concept is variation in nucleotide sequence either in coding or non-coding sequences could have correlation with the cancer incidence. So it is important to evaluate the nucleotide variations in those candidate genes involved in the development of the disease. **Objectives:** Since in cervical cancer precancerous stage is well defined but conversion of precancerous stage to neoplasia is variable, the aim of this study was to evaluate whether single nucleotide polymorphisms (SNPs) in Cyclin D1 (CCND1) in different stages of precancerous stages may shed new sight to understand the disease manifestation and the biological significance of the molecular markers involved in these cancer. **Method:** PCR-RFLP methodology was employed for screening of CCND1-A870G polymorphism in different grades of cervical cancer and urinary bladder cancer tissues and sequencing for confirmation. A total of 22 cervical cancer and 15 urinary bladder cancer tissues were screened. **Results:** Analysis of preliminary results the genotype frequencies of CCND1-A870G polymorphism in tumor tissues were 11.6% and 9% for GG in urinary bladder cancer and cervical cancer tissues respectively. **Conclusion:** Further analysis with larger sample size as well as with control will give the actual genotyping frequencies in our population.

TH-17

Human Papillomavirus Type 16 E6 / E7 Transcripts in Patients with Cervical Neoplasia

Sathish N, Abraham P, Peedicayil A*, Sridharan G, John S, Chandy G

Depts of Gynaecology, Radiation Oncology & Clinical Virology, Christian Medical College Hospital, Vellore, India. *og3@cmcvellore.ac.in

Background: The viral transforming genes E6 and E7 of HPV cause the degradation of tumor suppressor proteins. **Objectives:** To study oncogenic transcripts and E2 DNA of HPV 16 in patients with cervical neoplasia. **Methods:** Thirty-six women with cervical cancer and six women with cervical intraepithelial neoplasia (CIN) who were positive for HPV 16 DNA by PCR / RFLP were included in the study. Reverse transcription PCR for HPV-16 E6 / E7 mRNA transcripts and a PCR to detect HPV-16 E2 DNA were performed. **Results:** HPV-16 E6 / E7 mRNA transcripts were detected in 93 % (43/46) biopsy specimens. The full length E6 transcript alone was seen in two patients while the others had combinations of the full length, E6*I and E6*II transcripts. Episomal E2 DNA was seen in 52 % (22/46) of patients. **Conclusions:** The majority of cervical cancers express oncogenic transcripts. The occurrence of intact HPV-16 episomal E2

DNA suggests that disruption of the E2 open reading frame is not mandatory for oncogenic expression.

TH-18

Role of Colposcopy in the Primary Screening of Cervical Cancer in India

Asha Jain* and Pratima Chaudhary

Cancer Prevention & Relief Society, Sri Srimaa Sharda Street Cancer Suraksha & Anusandhan Kendra, 58, Jalvihar Colony, Raipur,(C.G.) India, 492001. *aasha221@rediffmail.com, drashajain@yahoo.co.in.

Introduction: Our center is conducting Colposcopic examination for the last 5 years as a measure of spreading awareness regarding the preventability of cervical cancer in women. We conduct VIAM/ Colposcopy screening camps regularly without help from any funding agency. **Objective:** The present study was undertaken to evaluate "Colposcopy & See& Treat Approach" as an optimally useful screening tool for Cancer Cervix in India. **Methods:** Starting with an awareness camp in year 2000, subsequently we have done VIAM+VILI, Colposcopy, Colposcopy& Pap, then with Cryotherapy and in 2004- Colposcopic See& Treat with Pap smear + HPVHC2 in selected cases. This camp was for urban slum dwellers. Anganwadi workers were used to fetch needy patients. Colposcopy was utilized as a primary tool to screen the cervix with counseling and written consent. Similar approach was utilized in private patients. Total 295 patients were examined. Detailed history, complete gynecological examination with Pap smear collection & Video Colposcopy with image capture was performed. Specimens for HPV DNA were collected on women with acetowhite lesions or cervical erosions, which bled on touch. Treatment was by LEEP, Cryo or Electric Cautery with biopsy of clinical cancer. **Result:** Histopathology reports were the Gold Standard. Colposcopy and the histopathology correlated in 90% cases whereas HPV DNA arm showed above 75% correlation. Sensitivity and Specificity of Pap smear and Colposcopy was 58% & 90% and 90% & 97% respectively. **Conclusion:** As is evident from results, quality assured Colposcopy could be used as a primary screening tool in low resource setting like India very effectively.

TH-19

Public Health Implications of Dietary and Genetic Folate Deficiency in HPV Mediated Cervical Cancer

Preethi. N. R[1]*, Asok Antony[2] and M. Radhakrishna Pillai[1]

Department of Molecular Medicine[1], Regional Cancer Centre Thiruvananthapuram and Hematology-Oncology[2], Department of Medicine, Indiana University, Indianapolis, USA. *preethinr@gmail.com

Introduction: India has one sixth of the world's total population and one third of the world's cervical cancer burden. Estimates of ~ 371 000 new cases every year represents 24% of world's annual incidence. More than 79% of these cases are reported from developing or underdeveloped countries alone, which emphasizes the importance of socioeconomic factors in the progression of the disease. Human papillomavirus is causally related to cervical cancer by being present in more than 90% of invasive cervical cancer cases. However the role of underlying micronutrient deficiencies in disease progression is unclear. The detrimental effects of hyperhomocysteinemia in various disease conditions have been well identified. However, studies on the role of increased levels of plasma homocysteine in cancer progression are still in its infancy. **Objective:** Micronutrient deficiencies such as that of folate, vitamin B6 and B12 have been found to be associated with malignant transformation of cells. Folate deficiency co-existing with HPV increased the risk of developing Cervical Intraepithelial Neoplasia (CIN). Folate metabolism is also influenced by Single Nucleotide Polymorphisms (SNPs) of methylene tetrahydrofolate reductase (MTHFR). Folate deficiency (either due to a genetic reason or dietary deficiency) also resulted in accumulation of homocysteine. This study

therefore aims at understanding the effects of hyperhomocysteinemia secondary to folate/B12 deficiency in HPV initiated cervical carcinogenesis. **Methods:** A total number of 93 cases and 68 controls were selected for the study. Chemiluminescence assay was used to identify serum folate and B12 levels and Enzyme Immuno Assay for serum Homocysteine levels. HPV genotyping was done with DNA extracted from tissue/exfoliated using PCR. MTHFR polymorphisms were studied by PCR/RFLP. Consumption patterns of different foods were identified using a Food use Frequency Questionnaire. Statistical analysis was done using SPSS software 10.0. **Results:** We observed that SNPs of MTHFR at nucleotides 677 (C T) and 1298 (A C) in the gene sequence impaired folate metabolism. Consumption of folate rich foods were observed to be far less than the recommended levels and this was reflected in serum levels of folate. Reduction in folate levels and homocysteine accumulation was associated with the presence of HPV DNA in all grades of cervical lesions. **Conclusions:** Serum homocysteine was strongly and significantly predictive of invasive cervical cancer risk. This association also reflects folate inadequacy and genetic polymorphisms affecting one carbon metabolism. These findings provide a theoretical basis for deciding on folate and B12 supplementation as part of cervical cancer prevention programs.

TH-20
Molecular Markers for the Early Detection of Preneoplastic and Neoplastic Lesions of Uterine Cervix

Prishla Varghese*, Bhupesh K Prusty, M. Subbarayan, P Sodhani[2], S Batra[3] and BC Das

Division of Molecular Oncology, [2]Division of Cytopathology, Institute of Cytology and Preventive Oncology (ICMR), I-7, Sec-39, Noida 201301, India. [3]Department of Gynecology, Lok Nayak Hospital, new Delhi, India. *prishlaprishla@rediffmail.com

Background: Cancer of the uterine cervix is the most common malignant tumor in Indian woman and accounts for almost 12% of all cancers in woman world wide, and represents the second most frequent gynaecological malignancy in the world. Human papillomaviruses infections are considered to be the main causative factor for widespread cervical cancers. Although considerable data is available both from India as well as International literature on the role of HPV in the development of cervical cancer. The critical role played by HPV in altering tissue kinetics during cervical cancer progression is not known. **Objective:** The present study focuses on the role of the viruses in modulating cell cycle and apoptotic parameters, and its role in gross genetic alterations. **Methods:** 100 cervical tissues of varying malignant and premalignant phenotypes are immunohistochemically analyzed for the NF-kB family members c-fos, c-Jun, fra-1, AP1 members p65 and p50 and cell cycle markers p53, cyclin D1 and apoptotic marker Bcl2. Immunocytochemical analysis was performed using the streptavidin-biotin method. **Results:** A significant increase in the expression of c-fos, p65 and p50 protein has been noted in invasive cancers and high-grade squamous intraepithelial lesions. Expression of Fra-1 has been substantially down regulated in premalignant and malignant cases. c-Jun expression has been found to be similar in normal mucosa and both neoplastic and preneoplastic lesions. Cell cycle regulatory proteins p53 and cyclin D1 over expression has been noted in all the premalignant and malignant phenotypes. HSIL and LSIL cases showed mild to moderate Bcl2 protein expression whereas invasive carcinoma cases showed intense overexpression. **Conclusion:** Overexpression of proteins of the NF-kB and AP-1 family in preneoplastic lesions suggest that these antibodies can be used as molecular markers for early detection of malignant tumors. Similarly lesions showing increased expression of p53 and cyclin D1 in preneoplastic stages can be taken as suggestive indication that these lesions are forerunners of malignant transformations. Thus these proteins can also be used as markers for early detection. The presence of bcl-2 protein in the cancerous cases seems to be an overexpression due to its genetic alteration, which may play critical role in loss of cell

differentiation in the process of malignant transformation and progression.

TH-21

Analysis of Chromosome 11 Deletion and Bcl-1/Cyclin D1 Alteration in Uterine Cervical Carcinoma of Indian Patients

Ratnesh Kumar Singh*, Nilanjana Bhattacharya, Neelanjana Chunder, Ranjit Mondal, Syamsundar Mandal, Susanta Roychowdhury[1], Chinmay Kumar Panda

Department of Oncogene Regulation, Chittaranjan National Cancer Institute, Kolkata 700026, India.[1]Genetics and Genomic Division, Indian Institute of Chemical Biology, Kolkata 700032, India. * ratnein@yahoo.com

The aim of this study was to understand whether there is any association between specific deleted regions in chromosome 11 (chr.11) and alteration (amplification/rearrangement) of Bcl-1/Cyclin D1 locus, located at 11q13, in uterine cervical carcinoma (CA-CX). The deletion mapping of chr.11 was studied using 17 highly polymorphic microsatellite markers in 65 primary uterine cervical lesions. The Bcl-1/Cyclin D1 alterations were analysed by Southern blot and / or Polymerase chain reaction (PCR) method in respective cervical lesions. Chr.11 deletion was found to be significantly associated with progression of CA-CX. High frequency (48 - 65%) of deletion was found in 11p15.5 (D1), 11q22.3-23.1(D2) and 11q23.3-24.1(D3) regions, and significant association was seen among deletions in D2 and D3 regions. Bcl-1/Cyclin D1 locus alteration was observed in overall 27% cervical lesions. Co-amplification of Bcl-1/Cyclin D1 locus was seen in 10% samples. However, no association was found between the deleted regions and Bcl-1/Cyclin D1 locus alterations. Our study suggests that there is no co-operativity between the deleted regions (D1- D3) in chr.11 and Bcl-1/Cyclin D1 alterations, but these alterations may provide cumulative effect in progression of the tumor. The D1 - D3 regions may harbor candidate tumor suppressor gene(s) (TSGs) associated with the development of CA-CX.

TH-22

Expression of Momordica Charantia Lectin Specific Glycoconjugates in Premalignant and Malignant Lesions of Uterine Cervix

Smitha L. S, Laija S. Nair, Rekha A. Nair, Sujathan K and Remani P.

Regional Cancer Centre, Thiruvananthapuram. 695 011, India. *remanipr@yahoo.com

Alterations in glycosylation during carcinogenesis lead to differences in specific glycocojugate expressions on cell membranes. Lectins are a heterogenous group proteins and glycoproteins sharing a common ability to recognize and combine with specific carbohydrate moieties on cell surfaces. Due to these properties lectins are widely used to study the expression of surface glycoconjugates on normal and malignant cells. Some plant lectins are reported to have significant role in predicting the malignant potential of precancerous lesions. In this study we have used Momordica charantia lectin (MCL) to study the expression of cell surface glycoconjugates in premalignant and malignant lesions of uterine cervix. MCL was isolated from the seeds of Bitter gourd (*Momordica charantia*) and purified using immobilized column of D-Galactose. This purified MCL was then conjugated with Horse-radish peroxidase (HRP) and used to evaluate the glycoconjugate expression on various dysplastic and carcinomatous tissues of uterine cervix using diaminobenzidine as substrate. Formalin fixed paraffin embedded tissues from 38 cases of dysplasia, 52 cases of carcinoma and 24 normal tissues were used for the lectin histochemical studies. It was observed that lectin binding pattern and staining intensities were different in various types of cervical lesions. Normal epithelium showed mild membrane staining whereas in

carcinoma the intensity of staining was high particularly in the membrane of differentiated cells. Different dysplastic lesions showed varying intensities of staining. Intensity of staining was found to be increased with the severity of the dysplasia. Correlation analysis between different groups revealed that intensity of staining had a positive correlation with various stages of tumour progression. Thus the present study suggests that MCL may be used as a probe for further elaboration of detection and grading of precancerous and cancerous lesions of uterine cervix.

TH-23

To Study the Prevalence of High-risk Human Papillomavirus in Women with Benign Cervical Cytology using Polymerase Chain Reaction: A Hospital Based Study from North India

Ritu Aggarwal*, Sachin Gupta, R Nijhawan[1], Vanita Suri[2], S K Arora

Department of Immunopathology, Cytology & Gynecpathology[1] and Obstetrics & Gynecology[2], Post Graduate Institute of Medical Education and Research, Chandigarh, India. *deepakritu@yahoo.com

Introduction: Cervical cancer is the commonest cancer among Indian women. High risk HPV detection holds the potential to be used as a tool to identify women, at risk for subsequent development of cervical cancer. **Objectives:** There is a pressing need for identifying prevalence of asymptomatic cervical Human Papillomavirus infection in local population. With this aim, the present study was undertaken to determine the prevalence of HPV DNA in women with benign cervical cytology. **Methods:** The subjects were recruited from the gynecology outpatient and were subjected to Pap smear. Two hundred and sixty samples were subjected to Polymerase chain reaction, using consensus primers for low and high risk HPV (types 6, 11, 16, 18, 31, 33). The samples positive for HPV DNA were further assessed for high-risk consensus primers for HPV (types 16,18,31,33) as well as using type specific primers for HPV 16 and 18. **Results:** Seventy six (29.2%) women tested positive for low and high risk HPV. Six (2.3%) of the entire cohort tested positive for high risk HPV. Three samples were positive for type 16, 2 for type 18 and one tested positive for both type 16 and 18. The prevalence according to demographic and socioeconomic variables will be presented. **Conclusion:** The study generates epidemiological data of prevalence of subclinical HPV in comparatively good socioeconomic and literate population. The data generated will be useful for setting guidelines for mass screening of HPV, treatment and prophylaxis in India.

TH-24

Assessment of the Expression of Apoptosis and Proliferation Related Proteins in Cervical Cancer

Sayani Dasgupta*, Himani Sharma, Palika Datta, Amit Kumar Dinda[1], Neerja Bhatla[2], Sunesh Kumar Jain[2] and Neeta Singh

Department of Biochemistry, Pathology[1] and Obstetrics & Gynecology[2], All India Institute of Medical Science, New Delhi 110029, India. *capri_181@rediffmail.com

Introduction: Cervical cancer is one of the major causes of female mortality in the world, especially in developing countries. In India, 132,000 new cases are detected annually, with more than 65% diagnosed in stage 3 and 4, mainly due to inadequate facilities for screening and detection. 98% of invasive cervical carcinomas are associated with Human Papillomavirus infection, particularly the high risk types 16 and 18. **Objective:**

Aim of this study is to analyze HPV infection and progression of cervical carcinoma in relation to apoptosis, its related proteins (p53, p73), transcription factor (NFkB) and proliferation markers (Ki-67, PCNA). Materials and **Methods:** Protein expression was assessed by immunohistochemistry of 30 histopathologically confirmed cervical tumor biopsy samples and 10 normal cervix samples. HPV-16 infection was detected by DNA isolation and PCR. TUNEL assay was performed to evaluate the apoptotic index. **Results:** All the tumor samples were HPV-16 positive. Significantly higher expression was seen in tumor samples for Ki-67 (26/30; 86.87%; p=0.004), PCNA (24/30; 80%; p=0.015) and p73 (11/30; 36.37%; p=0.032) as compared to normal controls (Ki-67: 3/10;30% and PCNA: 3/10;30%). Higher expression was seen for p53 (7/30; 23.33%) and NFkB (7/30; 23.33%) also but it was not statistically significant. Apoptotic index was significantly lower in tumors as compared to controls (p=0). **Conclusion:** Higher expression of proliferation markers and apoptosis related proteins suggests that these may serve as adjuncts for conventional diagnostic and prognostic markers. The lower apoptotic index in tumors as compared to controls also suggests that the apoptotic pathway is deregulated in cervical cancer.

TH-25
Prevalence of HPV 16 Variants in Cervical Cancer in India

Shailja Pande*, Neeraj Jain, and Bhudev C Das

Division of Molecular Oncology, Institute of Cytology and Preventive Oncology, Noida. *shailjapande@yahoo.com

Introduction: Cancer of the uterine cervix has emerged as the second most malignant tumor in the world, but the number one cancer in Indian women. Infection of high-risk Human Papillomavirus (HPVs) types 16 and 18 has been considered as the principal sexually transmitted causal agent in the development of cervical cancer. Although HPV 16/18 E6 and E7 prototype are considered to be the most important transforming genes, a large number of sequence variants have been observed in invasive cervical cancer worldwide. Analysis of high risk HPV variants is important in view of recent development of HPV vaccine and its efficacy trial in different ethnic population, particularly in India where prevalence of HPV in cervical cancer is one of the highest in the world. **Objectives:** To analyze the HPV 16 E6 variants in cervical cancer in North Indian population. **Methods:** We have analyzed HPV16 E6 variants in 50 cervical squamous cell carcinomas using PCR and direct nucleotide sequencing. **Results:** Out of 50 patients' 21(42%) showed variant 350G whereas rest 29(58%) revealed prototype sequence i.e., nucleotide T at position 350 in the E6 region of HPV 16 using PCR-based 350G/T specific primers. Direct nucleotide sequencing of E6 region was performed on only 17 samples, which confirmed PCR findings, as well as revealed additional nucleotide changes G145T, T286A, A289G and C335T, which were observed exclusively in the sequence showing 350G variant. **Conclusions:** The study reveals no major difference in distribution of 350G/T variants in squamous cell carcinoma of cervix. But the presence of covariants in 350G sequences is interesting which needs to be investigated in detail with larger sample size, since the identification of HPV16 variants would be important for generation of specific immune responses in the context of vaccine development and treatment strategies against HPV and cervical cancer.

TH-26
p53 Codon 72 and p21 Codon 31 Polymorphisms and Cervical Cancer Susceptibility in Indian Women

Paramita Bhattacharya and Sharmila Sengupta*

Human Genetics Unit, Indian Statistical Institute, 203, B.T. Road, Kolkata-700108, India.*sharmila@isical.ac.in

Introduction: The role of genetic cofactors in transformation of HPV infected cervical epithelium is well established, where cell cycle control is crucial. Hence identifying host genetic determinants of susceptibility to cervical cancer (CaCx) may help to identify women at risk. **Objectives:** The hypothesis tested was whether polymorphic variants of p53 (Arg72Pro) or p21 (Ser31Arg) were associated with enhanced susceptibility to CaCx either independently or jointly.). p53 is a transcription factor for p21, a cyclin dependent kinase inhibitor. **Methods:** Fresh cervical biopsy samples (n=90; 58 HPV16/18 positive) were collected from subjects diagnosed with squamous cell carcinoma, from a referral cancer hospital. The controls samples (cytologically normal), cervical scrapes (n= 191; 115 HPV negative and 76 HPV16/18 positive), were collected from unrelated individuals through population screening. These were genotyped by PCR and RFLP. Age-adjusted OR, 95% CI and p-values were determined. **Results:** The association of p53 proline homozygosity at codon 72 with CaCx ($OR_{adjusted}$ = 4.11; 95%CI= 1.08-15.55; p= 0.038) was reconfirmed among individuals harboring HPV16/18 infection. There was significant association between the p21 arginine allele (rare allele with frequency of 0.1) at codon 31 and CaCx, compared to HPV negative controls ($OR_{adjusted}$= 2.01; 95%CI= 1.00-4.06; p= 0.05). The two risk factors jointly failed to show statistical interaction towards susceptibility to CaCx. The rare p21 arginine allele was significantly associated with CaCx in the p53 codon72 heterozygous (proline/arginine) group of subjects ($OR_{adjusted}$= 2.91; 95%CI= 1.12- 7.56; p= 0.028). **Conclusion:** Variants of p53 and p21 genes appear to enhance susceptibility to CaCx, in Indian women. The two risk factors, although part of a common causal pathway, appear to act in a mutually exclusive manner.

TH-27

In-Situ Immune Profile in Invasive Cervical Cancer and Chronic Cervicitis - A Pilot Study Using Immunohistochemistry

***Sreenivas A*[1]*, *Sudhir Krishna*[2], *Geetashree Mukherjee*[1], *Jayshree RS*[1]**

[1]Kidwai Memorial Institute of Oncology, Bangalore-29. [2]National Center for Biological Sciences, Bangalore-65. *adurthisree@hotmail.com

The effector phase of the immune response can generally be divided into three types: cellular (type 1 or Th1), humoral (type 2 or Th2) and regulatory (Treg) responses. Th1 type of an immune response is thought to be required for the regression of Human Papillomavirus (HPV) lesions. We examined the insitu immune profile in cases of Invasive Cervical Cancer (ICC), and compared it with that of HPV positive and HPV negative Chronic Cervicitis. Thirteen cases of paraffin embedded tissues of histologically proven ICC, and thirty cases each of HPV positive and HPV negative Chronic Cervicitis were examined by Immuno Histochemistry using antibodies to CD3, CD4, CD8, IL2R, IL2, gIFN, CTLA4, TGFb, TNFa, IL10, and IL4. The number of cells with positive signals in five different fields of 100 cells each were counted. The mean averages and the standard deviations in the ICC group was as follows: CD4: 92.4 + 6.7, CD8: 53.1 + 19.9, IL2R: 85.2 + 16.7, IL2: 25+ 9.4, IFNg: 76.6 + 24, CTLA4: 38+ 17.3, TGFb: 49.6 + 24.7, TNFa: 44 + 29.6, IL10: 39.3 + 15.2, IL4: 38.5+ 25.4. Thus, the lesions of ICC showed a marked lymphocytic infiltrate in the stroma: both in terms of CD4 and CD8 cells. IL2R was also well expressed in the tumor infiltrating lymphocytes. The expression of IL2 however, was poor. Expression of both IL2R and IL2 in CD4+ cells is an indication of activated T cells. However, in our results we found CD4+,

IL2R+ in the absence of IL2, which could be indicative of Treg cells. CTLA4 expression also substantiated our interpretation of Treg cells.

TH-28

Selective Suppression of Activator Protein-1 by Berberine in Human Cervical Cancer Cells

Sutapa Mahata*[1], Bhupesh K. Prusty[1], Ramdasan Kuttan[2] and Bhudev C. Das[1]

[1]Division of Molecular Oncology, Institute of Cytology and Preventive Oncology, Noida and [2]Amala Cancer Research Centre, Amala Nagar Thrissur, Kerala. *sutapamahata@yahoo.co.in

Introduction: Cervical Cancer is one of the most common malignant diseases of women, caused by infection of specific types of high risk human papillomaviruses (HPVs) such as HPV type 16 and 18. Constitutive expression of two early genes E6 and E7 of high-risk HPVs, responsible for the development of cervical cancer, is mainly dependent on the availability of AP-1 transcription factor of the host cells. Since several antioxidants, such as Pyrrolidine-dithiocarbamate (PDTC), curcumin etc., have been found to have modulatory effects on the transcriptional regulation of AP-1 and HPV, we have selected Berberine (5, 6-Dihydro-9, 10-dimethoxybenzo [g]-1, 3-benzodioxolo [5, 6-a] quinolizinium; 7, 8, 13, 13a-tetradehydro-9,10-dimethoxy-2, 3-methylenedioxy berbinium), a plant alkaloid derived from *Berberis* and *Coptis*, which has a wide range of pharmacological effects including antimicrobial, anti-inflammatory effects and anti-proliferative activity in cancer cells. **Objectives:** To examine the effect of Berberine on transactivation and binding activity of AP-1. **Methods:** We have used HPV 18 positive human cervical carcinoma cell line, HeLa and HPV 16 positive human cervical carcinoma cell line, Siha for the present study. Electrophoretic mobility shift assay was performed to investigate the effects of Berberine on AP-1 binding activity. **Result:** Berberine has been found to inhibit AP-1 binding activity in a dose and time dependent manner at concentration higher than 10 microgram per ml in Siha cells and at 1 milligram per ml in HeLa cells. Berberine inhibited AP-1 activity almost completely at 1 milligram per ml after 24-hrs of treatment in both the cell lines. **Conclusion:** Berberine has a strong inhibitory effect on AP-1 binding activity in cervical cancer cells and is suggestive of its possible utility in the treatment of cervical cancer.

TH-29

Role of RhoC in Motility and Cervical Carcinoma

Sweta Srivastava* and Sudhir Krishna

National Centre for Biological Sciences, Bangalore 560065, India. *sweta@ncbs.res.in

Introduction: RhoC, a member of the Ras related small GTPase protein family, regulates actin cytoskeletal organization. Recent reports implicate RhoC in metastasis of numerous carcinomas including breast and lung. However, the clinical significance of RhoC in cervical cancer is yet unknown. **Objective:** Progression of carcinogenesis involves epithelial to mesenchymal transition (EMT) accompanying increased motility and invasiveness. RhoC is regulates actin organization and cell motility. Expression and regulation of RhoC activity was thus investigated. **Method:** The expression of RhoC was analyzed by reverse transcription-polymerase chain reaction (RT-PCR) and immunohistochemical analysis (IHC). Wound healing assay was employed to study EMT in cervical cell lines. **Result:** IHC analysis of 30 patient samples showed increased cytosolic staining of RhoC in invasive tissue sections in contrast to very mild expression levels in normal tissue samples. Semi-quantitative RT-PCR on cervical cancer cell lines including HeLa, SiHa and CaSki showed higher RhoC levels as compared to RhoB and RhoA. In vitro wound healing assay with atorvastatin, an HMG CoA reductase inhibitor, which prevents Rho geranylgeranylation, dominant negative RhoC and PI3K inhibitor (LY294002)

reduced wound closure as compared to untreated cells. However the effect of atorvastatin and LY294002 on EMT was observed to be different in each cell line. It has also been observed that TGF-beta induced EMT, in CaSki and SiHa, may be blocked by expressing dominant-negative RhoC and addition of atorvastatin, suggesting the presence of RhoC downstream of TGF-beta signaling in EMT. **Conclusion:** These results collectively indicate that RhoC may be a potential metastatic factor in cervical carcinoma. It may also be suggested that RhoC may be regulated in these cell lines by different signaling pathways.

TH-30
Role of TGF-Beta in Inducing Epithelial to Mesenchymal Transition (EMF) in Cervical Cancer Cells

Tessy T. Maliekal* and Sudhir Krishna*

National Centre for Biological Sciences, Bangalore 560065, India. *tessy@ncbs.res.in

Introduction: Although different studies have explored the role of TGF-beta in cervical cancer, there are several questions that remain unanswered. It appears that the effect of TGF-beta is context dependent; it can act as a growth inhibitory signal, but for invasive neoplastic cells, it may promote invasion and metastasis. TGF-beta can induce EMT, which is essential for invasion and metastasis. Recent evidences suggest that the jagged induced notch signaling and NF-kappaB are involved in EMT. **Objective:** If TGF-beta can play a dual role, what are the factors that regulate two different pathways? The roles of NF-kappaB and Notch are also evaluated in the present study. **Methods:** The TGF-beta induced EMT was analyzed in cervical cancer cells. The induction of EMT was evaluated by immunofluorescence. Growth inhibitory effect of TGF-beta was analyzed by MTT assay and FACS analysis. The inductions of possible intermediates in EMT were analyzed by real time RT-PCR at different time intervals. The activation of NF-kappaB, Smads and Notch will be evaluated by reporter assays. The role of NF-kappaB in TGF-beta induced EMT will be evaluated by overexpressing NF-kappaB, and then inhibiting it with IkappaB construct. **Results:** TGF-beta induces EMT at the lower concentrations and growth inhibition at higher concentrations. At 2ng/ml, the induction of EMT is high and the growth inhibition is negligible while at 10ng/ml EMT is negligible and growth inhibition is high. The role of notch and NF-kappaB in EMT will be discussed. **Conclusions:** During cervical cancer progression, the TGF-Beta levels goes down and this may result in increased EMT and invasion.

TH-31
Synergistic Role of Human Papillomaviruses (HPV's) and Epstein Barr Virus (EBV) in Oral Cancers

V. Gopalkrishna, R.S. Tomar, M.G.. Pol and A. Jamkar*[1]

National Institute of Virology, 20-A, Dr.Ambedkar Road, Pune-411001 and [1]Sassoon General Hospitals, Pune. *gopalvk58@hotmail.com

Association of viral infections in the development of malignancies has been established to some extent. Human Papillomaviruses and Epstein Barr viruses were found to be associated in oral cancers. However, synergistic role of these viruses and role of transforming gene (BNLF-1) of EBV is not clearly understood. Present investigation has been undertaken in order to rule out the possible role of these viruses, any co-infections and transforming genes involved in the study group. Forty five surgically operated oral tumor tissues specimens and thirty normal mucosal specimens as controls were recruited for the study. HPV genotyping and EBV detection (BamHI-W, BNLF-1) was carried out by Polymerase chain reaction

(PCR) using type specific primers. Our results indicates, HPV 16 was the high risk type found in 31.2% (n=45) of oral cancers, HPV 18, 6, 11 were detected only in 6.6%, 2.2% of cases respectively. Total HPV DNA positivity in the study group was found to be 42.2%. A high prevalence of EBV DNA positivity, 44.5% (n=45) was also observed in the same subjects when BamHI-W repeated region of EBV was screened. However, when BNLF-1 transforming gene was used, only 13.3% of cases were found positive for EBV. Co-infections of both HPV, EBV were also observed in 11.2% of males (n=27) and 22.2% of females (n=18) respectively. Interestingly, EBV BNLF-1 gene was also detected in HPV negative oral tumors in both males/female patients. Only in 10-13.3% of the normal mucosal controls contained either of the viruses. Correlation of the viral infections and clinical significance of the disease will be discussed.

TH-32

Jagged1/Notch Mediated PI3K Activation is Deltex1 Dependant and Promotes Epithelial to Mesenchymal Transition in the Context of Human Cervical Carcinogenesis

Vanitha K. Subbaiah*, Karthikeyan Veeraraghavalu and Sudhir Krishna

National Centre for Biological Sciences, TIFR, UAS-GKVK Campus, Bangalore-560065. India. *vanitha@ncbs.res.in

Introduction: Deregulated Notch signaling has been linked to the induction of anoikis resistance through a PI3K-PKB/Akt dependant pathway and complements the functions of papillomavirus oncogenes in cervical carcinogenesis. Parallel observations have also shown a role for Notch signaling in mediating epithelial to mesenchymal transition (EMT), a suggested key component of tumour progression. **Objectives:** Here, we have addressed three questions: 1. Does PI3K mediate Notch1 driven EMT? 2. What is the mechanism of activation of PI3K by Notch1? 3. What does the heterogeneity in cervical tumor derived cell lines mean for our understanding of the Notch1-PI3K-EMT process? **Method:** Immunohistochemical analysis, wound healing assay and immunofluorescence analysis and soft agar was employed to achieve the following results. **Results:** Our results show that Notch signaling drives EMT through a PI3K pathway. This process does not appear to involve the canonical de-repression of CSL and instead utilized Deltex1, a poorly characterized Notch effector. Finally, we show Notch1-PI3K mediates EMT in cervical tumor derived lines that retain the expression of Jagged1. **Conclusion:** We thus show that cervical tumour derived cell lines may fall into two categories that: 1) exhibit Notch-dependent EMT and 2) do not exhibit features of EMT. Given that a major proportion of human cervical tumor show features of Jagged1 dependant signaling, it is important to try and understand what stages of tumor evolution these cell lines represent?

TH-33

E-cadherin and NM23-H1: Molecules with Suppressive Roles in HPV Associated Epithelial Cell Tumors

Veronica S. Jeyadoss*[1,2], Smriti M. Krishna[2], Devaraj Halagowder[1], Devaraj Niranjali[3], Prabha Balaram[2]

[1]Unit of Biochemistry, Dept. of Zoology, University of Madras, Chennai, [2]Div. of Cancer Research, Regional Cancer Centre, Trivandrum, [3]Dept. of Biochemistry and Molecular Biology, University of Madras, Chennai, India. *smritisunil@yahoo.co.in

Introduction: Tumor invasion and metastasis are the major causes of treatment failure in cancer. The

Ancient medicinal plants viz., *Hemidesmus indicus* R. Br., *Ocimum sanctum* Linn., *Tinospora cordifolia* (Willd.) Miers ex Hook.f & Thoms. have also been found to act as potential anticancer plants.

Bioinformatics, Molecular Epidemiology and Clinical Cancer Research (Saturday)

S-1
Flow Cytometric Analysis of TH1 and TH2 Cytokines in PBMCs as a Parameter of Immunological Dysfunction in Patients of Superficial Transitional Cell Carcinoma of Bladder

***Abhilasha Agarwal*[1]*, Saurabh Verma[1], Usha Burra[1], NK Mohanty[2] and S. Saxena1**

[1]Institute of Pathology, Safdarjung Hospital Campus, New Delhi. [2]Department of Urology, Safdarjung Hospital, New Delhi. *sunita_saxena@yahoo.com

Transitional cell carcinoma (TCC) is the commonest cancer of the bladder and constitutes about 80-90% of urothelial tumors with recurrence rate of 30-90%. Adjuvant chemotherapy and immunotherapy has been used recently as a prophylactic measure to reduce the frequency of recurrences. This study is aimed to evaluate TH1 and TH2 balance in TCC patients using flow cytometry and to assess immunological and cellular factors influencing the anti-neoplastic activity of immunomodulating agents like BCG and IFN-a2b in combination with chemotoxic drugs administered based on the results of *in vitro* cytotoxicity assay on autologous tumor cells. The immune dysfunction was analyzed prior to intravesical therapy in 41 TCC patients and in 21 normal healthy individuals to get baseline data of surface antigen and cytokine expression. The percentages (mean ± SD) of T-cells producing IFN-g IL-2 and TNF-a were significantly reduced in patients (19.17 ± 4.94, 52.31 ± 20.86 and 12.83 ± 4.49 respectively), as compared to healthy controls (23.38 ± 3.67, 67.56 ± 12.0 and 17.63 ± 5.96 respectively), ($p<0.01$, 0.018, 0.001). On the contrary, the mean levels of IL-4, IL-6 and IL-10 in patients (63.84 ± 17.01, 60.46 ± 14.79 and 65.79 ± 14.84 respectively) were significantly higher as compared to healthy controls (24.43 ± 8.77, 26.51 ± 5.28 and 20.61 ± 3.81 respectively), ($p<0.001$). Out of 41 patients, 18 were recurrent whereas 23 were non-recurrent cases. The mean percent expression of CD3 and CD4 were significantly lower in patients showing recurrence (47.03 ± 16.01 and 23.99 ± 9.84 respectively) than those showing non- recurrence (51.18 ± 17.02 and 31.12 ± 12.27 respectively). In patients showing recurrence, the expression of IL-2 was significantly lower (47.70 ± 21.55) whereas the levels of IL-4 and IL-10 were significantly higher (66.29 ± 13.59 and 68.70 ± 11.82 respectively) than patients in the non-recurrent group (55.40 ± 18.80, 61.62 ± 19.42 and 62.66 ± 16.60 respectively). The cell surface markers CD3, CD4 and CD8 along with NK cells were found to be significantly lower in patients than healthy controls ($p<0.01$). Patients with bladder cancer seem to develop a TH2 dominant status with a deficient type1 immune response. The lymphocyte evaluation along with cytokine measurement can provide a sensitive and valuable tool for evaluating the function of cell-mediated immunity in these patients and find application in diagnosis and therapeutic monitoring of bladder cancer patients.

S-2
Differential Expression of 14-3-3 Zeta in Human Oral Squamous Cell Carcinoma

***Ajay Matta*[1], Sudhir Bahadur[2], Siddartha Datta Gupta[3], Ranju Ralhan[1]**

Department of Biochemistry[1], Department of Otorhinolaryngology[2] Department of Pathology[3], All India Instiute of Medical Sciences, Ansari Nagar, New Delhi-110029, India. *matta_ajay@rediffmail.com

Introduction: 14-3-3 proteins are phosphoserine-binding proteins that regulate activities of a wide array of targets via direct protein-protein interactions. Primarily these interactions are mediated by phosphorylation at specific sites on target protein. 14-3-3 proteins bind and regulate key proteins in various physioclogical processes such as intracellular signaling (eg. Raf, MLK, MEKK, PI-3kinase, IRS-1), cell cycling (eg. Cdc25, Weel, CDK2), apoptosis (eg. Bad, ASK-1) and transcription regulation (eg FKHRL1, DAF-16) In contrast to SH2 and PTB domains, which serve mainly to mediate protein-protein interactions, 14-3-3 proteins in many cases alter the function of the target protein, thus allowing them to serve as direct regulators of their targets. Using Differential Display, we recently reported increased expression of 14-3-3 zeta in oral cancer. Aim: The aim of the study was to determine the clinical significance of the expression of 14-3-3 zeta in human oral squamous cell carcinomas (OSCCs). **Methods and Results:** Immunohistochemical analysis of 14-3-3 zeta protein in paraffin embedded sections of human oral squamous cell carcinomas (OSCCs) and matched histologically normal oral tissues showed intense cytoplasmic immunoreactivity observed in 44 of 50 (88%) of OSCC tissues, while no detectable 14-3-3 zeta immunostaning was observed in any of the matched histologically normal oral tissues. **Conclusion:** Differential expression of 14-3-3 zeta in OSCC and normal oral tissues warrants an in-depth analysis of its potential as a candidate molecular target in oral cancer.

S-3

Differential Expression of Retinoic Acid Receptors in Normal and Malignant Esophageal Tissues

Anupam Kumar*[1], Jatinder Kaur[1], Tushar Kant Chattopadhyay[2], Meera Mathur[3], Ranju Ralhan[1]

[1]Department of Biochemistry, [2] Gastrointestinal Surgery, [3] Pathology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi-110029, India.*anupam_biochem@hotmail.com

Introduction: The chemopreventive and chemotherapeutic activities of retinoids may be attributed to their ability to modulate growth, differentiation and apoptosis of epithelial cells, suppress or reverse epithelial carcinogenesis. Many of these effects of retinoids result from modulation of genes by two distinct classes of retinoid receptors: RARs and RXRs, alterations in their expression may lead to tumorigenesis. **Objectives:** To determine whether alterations in expression of retinoid receptors are related to the development of esophageal squamous cell carcinomas (ESCCs), Methodology: The expression of RARa, b, g and RXR was studied in 50 untreated primary esophageal carcinomas and 19 distant normal tissues by immunohistochemistry. **Result**: RARb expression was observed in 18/50 (36%) ESCCs, while 16/19 (84%) of matched histologically normal esophageal tissues displayed RARb immunopositivity (p=0.001, OR=3.405). Significant increase in RAR immunopositivity was observed in ESCCs (40/50; 80 %) as compared to normal tissues (9/19 cases; 47%) (p=0.008; OR=2.77). RAR expression was observed in ESCCs (37/50cases; 74%) as compared to normal tissues (16/19; 84%); without significant difference. However, poorly differentiated esophageal cancer showed marked decrease in RARg immunopositivity (p=0.017; OR=6.0). Interestingly, increased expression of RXRa was observed in 43/50 (86%) ESCCs in comparison with (10/19; 53%) normal tissues (p=0.003; OR=3.09). Logistic regression analysis revealed RARg /RXRa$^+$ phenotype as most significantly associated with dedifferentiation of the tumor (p=0.014; OR=11.0). **Discussion & Conclusion:** The hallmark of the study was the significant increase in expression of RAR and RXR proteins and loss of expression of RAR protein in ESCCs in comparison with the distant normal epithelia.

S-4

Functional IFNG Polymorphism in Intron-1 in Association with an Increased Risk to Promote Sporadic Breast Cancer

Anjana Saha, Ashish Dhir*, Anand Ranjan, Vibhuti Gupta, Narendra Bairwa, Ramesh Bamezai

National Centre of Applied Human Genetics, School of Life Sciences, Jawaharlal Nehru University, New Delhi-110067, India. *bamezai@hotmail.com; bame0200@mail.jnu.ac.in

IFNG is an important Th1 cytokine, which plays a role in immune-surveillance and anti-tumor activity. A case-control study involving 54 sporadic breast cancer patients and 144 healthy controls to explore if the genotype variation of a proposed non-specific enhancer element with a dinucleotide (CA) repeat in Intron-1 had a role in the susceptibility to promote sporadic breast cancer was carried out. Genotype analysis carried out by SSLP and confirmed by sequencing showed an increased frequency of $(CA)_{12}$ allele ($p<0.001$) and decreased frequencies of $(CA)_{15}$ ($p<0.01$) and $(CA)_{>15}$ ($p<0.001$) alleles in sporadic breast cancer patients as compared to controls. Further, in-vitro reporter assays for $(CA)_{12}$ and $(CA)_{15}$ alleles suggested these to be associated with decreased and increased expressions, respectively, suggesting the $(CA)_{12}/(CA)_{12}$ background to act as one of the factors which could lead to low production of IFNG. The study concludes that such genetic background for a proposed non-specific enhancer element with $(CA)_n$ repeat within intron-1 of IFNG gene might put the individuals with this genotype at higher risk to promote the development of sporadic breast cancer due to a resultant compromised immune surveillance.

S-5

Homology Modeling of Lactate Dehydrogenase of Trichomonas vaginalis by Using SWISS-MODEL and WHAT IF

Ashok Kumar**, Jagan Mohan Rao[1]

National Center for Human Genome Studies & Research Chandigarh-160 014. [1]CIST-University of Mysore and E.C.I.L-Delhi-110028. * karans06@yahoo.com

The present study aims at proposing three-dimensional structure of LDH from its protein sequence. X-ray Crystallography or NMR Studies appears to be the preferred choice in determining structural details of proteins. However, situations where proteins fail to crystallize and/or protein solubility limited both the above mentioned method goes into vogue. Comparative modeling could be an alternative method for structure information of such proteins. The process consists of Fold assignment, template selection, target-template alignment, model building and model evaluation. The target sequence and structure templates were taken, considering 40% identities and retrieve using "BLAST". Target sequence was manually aligned with set of template structures. The alignment was guided by conserved residues as identified by multiple sequence alignment of related protein, a model was constructed by threading the target sequence on template and submitting to "SWISS MODEL" & "WHAT IF" server for optimization. Finally, both Models were evaluated using "PROCHECK". This was used to assess the geometry of residues in the target structure obtained by comparing with stereo chemical parameter derived from well-defined high resolution structure. The secondary structural features seen in "RASMOL" for both models of "SWISS MODEL" & "WHAT IF" were similar. Both had almost the same number of helices, strands and turns. Thus the model obtained is correct and "WHAT IF" helps to model a protein structure better.

S-6

Conventional Cytogenetic and FISH Studies in Diagnosis of Hematological Malignancies: Two Case Reports

Bani B. Ganguly** and M.B. Agarwal[1]

MGM Centre for Genetic Research and Diagnosis, MGM's New Bombay Hospital, Navi Mumbai, India. [1]Hematology Department, Bombay Hospital and Research Centre, Mumbai, India. *sg2707@vsnl.com

Malignant disorders are no longer considered as "killer disease" owing to continuous refinement of cytogenetic and molecular technologies. Chronic myeloid leukemia (CML) is the most known established example with the presence of Philadelphia chromosome in 95% cases. Philadelphia chromosome, the first marker chromosome detected in malignant disease, is a reciprocal translocation between chromosomes 9 and 22 resulting in rearrangement of abl and bcr genes and production of a new bcr-abl chimeric gene. Besides cytology and immunophenotyping, cytogenetic diagnosis has established its independent role in differentiating leukemias and their prognostic evaluation accordingly. In the modern era, most of the diagnostic laboratories are well equipped for FISH or RT-PCR for speedy generation of result on interphase nuclei for facilitating the earliest possibility of treatment-intervention. However, these techniques are restricted to extract genetic information only for the target genes located on specific chromosomes. Two cases diagnosed as CML and studied for bcr-abl gene or Philadelphia chromosome are discussed. FISH study detected presence of the genetic marker on the second day after sampling in both the cases, whereas conventional chromosomal study has obviously detected the same marker but in complex forms showing t(9;22;11) and t(5;9;22) respectively. Both the cases had no consistent abnormality confirmed by genomic karyotyping. Apart from CML-marker gene, there were further genes involved, including MLL (11q23) and (?) gene (5q22) in case 1 and 2 respectively, indicating the need for aggressive treatment to combat such variant CML. FISH and DNA-based technologies could be considered in conjunction with chromosomal study. The information obtained from the present study strongly advocates the use of conventional gold-standard techniques for better understanding of the molecular mechanism and management of malignant diseases.

S-7

Polymorphism in DNA Repair Gene, XRCC1 and Drug Metabolizing Enzyme, GSTM1 in Patients with Oral Leukoplakia

***Binod Kumar Yadav*[1], *Jatinder Kaur*[1], *Anurag Srivastava*[2] *and Ranju Ralhan*[1]**

Department of [1]Biochemistry, [2]Surgical Discipline, All India Institute of Medical Sciences, Ansari Nagar, New Delhi-110029, India. *binod3aug@hotmail.com

Introduction: An individual difference in susceptibility to chemical carcinogen is one of the most important factors in the estimate of risk of human cancer. GSTM1 is polymorphic member of the mu class gene family of the glutathione-s-transferases (GST) that aid in detoxifying electrophilic carcinogens. Depletion of cellular glutathione levels sensitizes cells to the toxic effects of these carcinogens. Polymorphism in GST genes, causing variations in enzyme activities, may influence susceptibility to oral cancer and leukoplakia. Inter-individual differences in DNA repair capacity have been demonstrated among patients with squamous cell carcinoma of the oral cavity. The XRCC1 DNA repair gene facilitates DNA strand break and base excision repair. The XRCC1 399Gln polymorphic allele has been indicated to have a contributive role in DNA adduct formation, sister chromatid exchange, and an increased risk of cancer development. **Objectives:** The goal of the study was to determine the association of genetic polymorphism in XRCC1 and GSTM1 genes with the risk of development of oral leukoplakia in tobacco consumers. Materials and **Methods:** 100 leukoplakia patients and 100 age and gender matched healthy controls were included in the study to investigate the polymorphism in GSTM1 and XRCC1 399Gln by PCR and PCR-RFLP, respectively. **Results:** Polymorphisms in GSTM1 and XRCC1 (at codon 399) genes were studied in leukoplakia patients and age and gender matched controls. 43/100 (43%) patients showed GSTM1 null genotype as compared to 36/100 (36%) controls. 46/100 (46%) of the patients were heterozygote for XRCC1 399Gln as compared to 34/100 (34%) in controls. GSTM1 null genotype together with XRCC1 variant allele was more frequently observed in patients who were consumers of tobacco for more than 10 years (OR=10.1). **Discussion & Conclusion:** This study demonstrates the association of polymorphism in XRCC1 399Gln and GSTM1 genes in tobacco consuming oral leukoplakia patients.

S-8

Retinoic Acid Receptors and Their Relationship with Cell Cycle Regulators in Oral Carcinogenesis

Chhavi Sharma*[1], Jatinder Kaur[1], Nitin Chakravarti[1], Sudhir Bahadur[2], Siddartha Datta Gupta[3], Ranju Ralhan[1]

[1]Department of Biochemistry; [2]Department of ENT; [3]Department of Pathology; All India Institute of Medical Sciences, New Delhi, India. *chhavi27@rediffmail.com

Introduction: Oral squamous cell carcinoma (OSCC) is the common malignancy in males and the third most common in females in India and is a major cause of cancer morbidity and mortality worldwide. Alterations in the expression of retinoid receptors are implicated in human malignancies. The altered expression of retinoic acid receptors (RARa, b and g) and retinoid X receptors RXRa and their relationship with cell cycle regulators (p53, p16 and p21) is associated with development, progression and prognosis of oral cancer. **Materials and Methods:** The expression of the Retinoic acid receptors 9RARs) was analyzed using immunohistochemistry in 200 cases of oral squamous cell carcinomas, 100 potentially malignant lesions and 50 histologically normal oral tissues using monoclonal antibodies. These were then correlated with expression of cell cycle regulators as well as with clinicopathological parameters. **Results:** Expression of retinoic acid receptors RAR b, RAR g and cell cycle regulators p16 and p21 was decreased in majority of oral SCCs as well as in potentially malignant lesions. The multivariate stepwise logistic regression analysis indicated that the most significant predictor of transition from normal to potentially malignant stage was $RARa^{+}/P21^{-}$(Odd's ratio, OR=4.4); hyperplasia to dysplasia was $RARa^{+}/p53^{+'}$ (OR=4,7) and potentially malignant to malignant phenotype was RAR a^{+} (OR=2.1). **Discussion and Conclusion:** Alterations in the expression of retinoic acid receptors occur early in oral carcinogenesis. Deregulated expression of RARs and cell cycle regulators can serve as potential predictive markers in the multistep process of development and progression of oral cancer.

S-9

Prevalence of Risk Factors of Cancer Cervix in Married Women of Aboriginal Community 'Baiga' a Primitive Tribe in Madhya Pradesh

Dolla C.K.*, Dash A.P.

Regional Medical Research Center for Tribals, Nagpur Road, P.O. Gahra, Medical, Jabalpur. Madhya Pradesh. *ckdolla@yahoo.com

Introduction: Cervical cancer is the second most common malignancy among women worldwide behind breast cancer and the most common cause of cancer-related morbidity and mortality in the developing world. Cervical cancer screening provides early detection of cervical cancer and is a preventative health care measure to all women at risk. Selective screening using risk assessment strategies is potentially useful, particularly in resource-poor settings. As the population of ethnic women increases, it is important to assess whether these women are at risk. Baiga women were selected from a area known as 'Baiga chak' in Dindori district, Madhya pradesh. High level of illiteracy and high prevalence of unhygienic genital practices was correlated with significant level of genital infections. This study highlights prevalence of risk factors in Baiga ethnic women. **Objectives:** The prevalence of the risk factors associated with cancer cervix. **Methods:** A community based, cross-sectional and observational study carried out in Baiga chak villages, Dindori district of Madhya pradesh. The study was undertaken from October 1999 to April 2000. 249 married Baiga women in the

reproductive age group were included in the study. Data was collected by house-to-house survey by direct interview method. We obtained information on age at marrieage, first child deli vary and parity. Symptoms of reproductive tract illness recorded on pretested schedules. **Results:** More than two-third (73.6%) of the study population belonged to the vulnerable age group (25-45 years) for this disease while 71.6% were married before they attained 18 years of age, and 28% of the married women gave birth to their first child before they were 19 years of age. 48.6% of the study population had parity more than three. 79.5% of the women study group were not practicing any methods of family planning and 20.5% of the same population were permanently sterilized, Only 3.5% of the women had satisfactory genital hygiene (during menstruation) practice and 52.9% had symptoms of reproductive tract infection (PID). **Conclusion:** Although the Baiga women not using hormonal Contraceptives, which has been associated with an increased risk of cervical cancer, there was a high prevalence of other important risk factors associated with cancer cervix like age, age of marriage, age of first childbirth, parity, genital hygiene and reproductive tract infections in the study population. Therefore, screening and early detection can be directed specifically to the group at risk.

S-10
Biomedical-Informatics: Its Implications in Oral Cancer

Rakesh M. Rawal[1], Jalpa N. Mehta*[*1], Shilin N. Shukla[2], Pankaj M. Shah[3]

Department of Cancer Biology[1], The Gujarat Cancer & Research Institute, Asarwa, Ahmedabad-16, Gujarat, India. Research & Education[2], The Gujarat Cancer & Research Institute, Asarwa, Ahmedabad-16. [3]The Gujarat Cancer & Research Institute, Asarwa, Ahmedabad-16. *jalpa12@hotmail.com

Introduction: Bioinformatics, in general, and medical informatics, in particular, are disciplines encompassing a variety of research areas, from molecular biology to medical practice and public health surveillance. Biomedical-informatics is a discipline that has become an essential part of the biomedical research community. This convergence will eventually play a pivotal role in creating a bridge of opportunity by integrating scientific and clinical specialties to promote the advances in treatment, risk assessment, diagnosis, therapeutics, and health-care outcome. Its application to oral disease, termed "oral genomics", can aid in the molecular understanding of the genes and proteins, their interactions, pathways, and networks that are responsible for the development and progression of oral cancer. **Objectives:** The application of oral genomics share common methodological challenges to help us understand how the enormous amount of data is translated into an improved overall understanding to oral cancer. **Method:** The methodology involves survey of literature, meta-analysis. Its role involves deciphering genomic, transcriptomic, and proteomics data generated by high-throughput experimental technologies and organizing information gathered from traditional biology. **Conclusion:** This area has created a steady stream of large and complex genomic data, which has transformed the way a clinical or basic science researcher approaches genomic research. In addition, a better integration will facilitate the discovery of genes for novel biomarkers and targets for new therapeutic interventions.

S-11
Abstract Withdrawn

S-12
Role of 'AAAT' Repeats on Human Cathepsin L Expression
Lokesh Kumar Sharma* and Shyam S. Chauhan

Department of Biochemistry, All India Institute of Medical Sciences, New Delhi - 110029, India. *lokeshaiims@rediffmail.com

Cathepsin L, a lysosomal cysteine protease, is over expressed and secreted by malignant cells. It has been established to play a crucial role in tumor invasion and metastasis. The cloning and genomic organization of human cathepsin L revealed the presence of 'AAAT' repeats in it's first intron. To investigate the role of these repeats on cathepsin L expression, we cloned this repeat region from a variety of human tumor cell lines and assayed cathepsin L activity spectrofluorimetrically. We found this 'AAAT' region to be repeated 8 times in five cell lines and 10 times in two other cell lines. The cell lines with 10 repeats exhibited significantly higher cathepsin L activity as compared to those with 8 repeats. These results suggest that there is a direct correlation between human cathepsin L expression and number of repeats in it's first intron. However, some other additional factors affecting cathepsin L expression cannot be ruled out. We have also observed that the activity of cathepsin L in the leucocytes of CML patients is significantly higher (31 fold) as compared to the normal controls and there is a decrease in this activity in response to treatment with Gleevac. This decrease has been found to be parallel with the observed clinical improvement in the treated CML patients. However no such dramatic difference in cathepsin L activity was observed in a limited number of AML and ALL patients as compared to normal controls.

S-13

Psycho-Social and Behavior Disorders in Patients with Reproductive Tract Infections

M.A. Khan*

Division of Behavioral Oncology, Institute of Cytology & Preventive Oncology (ICMR), I-7, Sector - 39, Noida - 201 301, India. *madrikhan@hotmail.com

Introduction: Reproductive tract infections have assumed an epidemic proportion accounting for a high morbidity and mortality among males and females of reproductive age groups. Many of these patients display high risk behaviour including promiscous sexual practices, needle sharing and drugs intake .The association between high risk behaviour of patterns and reproductive tract infections including HIV infection has been reported in many studies and also there have been a high prevalence of psycho-social disorders in patients with STDs & RTI's. **Objective:** The objectives of the present study was to assess the psycho-social disorders following HIV infection in patients with STDs and RTIs. **Methods:** The study was conducted at Lok Nayak Hospital, attached to Maulana Azad Medical College, New Delhi. The total sample was 133; 96 were males and 37 females. The age range of males was 16-60 years (27.35 ± 7.69) and of females was 15-45 years (26.94 ± 6.82). The cases comprised of all consecutive prospective STD patients attending skin & veneral diseases clinic. Psycho-social problems and information regarding their STD & RTI's and HIV perception, condom use, and partner relationship was obtained on a specially prepared proforma. **Results:** STD's were detected in 36.4% male and RTI's in 37.5% female patients through case reports, clinical examination and microbiological investigations. HIV positivity was detected in 5.2 % of males and 8.1% females using ELISA kits (for HIV_1 & HIV_2) and confirmed by western blot hybridization. Before initiation of the study, only 10% patients used condoms occassionally but after imparting health education using information technology (IT) & counseling, 42% started using condoms on a regular basis. **Conclusions:** The present study shows that counselling and IT could play a crucial role in modifying the high risk behaviour of individuals.

S-14

An Indirect Approach of Finding Novel Transcribing Coding Sequences Related to Cancer by Study

of Allelic Loss Coupled with Insilico Gene Prediction and Experimental Validation

Narendra K. Bairwa, Madhuri Bhuvanagiri*, Katayoon Darvishi and Ramesh Bamezai

National Center of Applied Human Genetics, Human Genetics Section, School of Life Sciences, Jawaharlal Nehru University, New Delhi-110067. *bamezai@hotmail.com; bame0200@mail.jnu.ac.in

An in-silico approach with molecular biology methodology was combined to map un-annotated potential regions of putative tumor suppressor gene(s) in patients with sporadic breast cancer. Our background studies had shown maximum LOH in the regions surrounded by D17S934 and D17S787 markers in most of the tumors. In-silico transcript map of the region was generated on Build 35.1 Sequence map information provided by NCBI. It was observed that approx 50kb region which included D17S934 marker showed sparse UNIGENE information. Annotation was carried out for the informative marker regions using in-silico gene prediction tool GENSCAN. Three genes (BK000637, BK000979-RNKBS1, RNKBS2) were annotated in D17S787 and a gene (BK000585) with three exons was predicted in D17S934 region. A similar approach was followed for prediction of novel genes on chromosome 3 and 15. Further, the experimental validation of one of the predicted exons revealed the presence of a novel EST (Accession no. AY343912) in the D17S934 locus region. The predicted coding regions when analysed showed presence of both point mutations and deletions in a substantially higher frequency in sporadic breast cancer patients as compared to controls.

S-15
Deregulated Expression of Cylcooxygenase-2 in Oral Carcinogenesis

Meenakshi Sawhney*[1]*, Nidhi Rohatgi[1], Siddhartha Datta Gupta[2], Anurag Srivastava[3], Nootan K. Shukla[4], Suryanarayana S. V. Deo[4], and Ranju Ralhan[1]

[1]Department of Biochemistry; [2]Department of Pathology; [3]Department of Surgical Disciplines; [4]Department of Surgical Oncology, Institute of Rotary Cancer Hospital; All India Institute of Medical Sciences, New Delhi, India.*sawhney_meenu@rediffmail.com

Introduction: Oral squamous cell carcinoma (OSCC) is the sixth most common malignancy and is a major cause of cancer morbidity and mortality worldwide. Paucity of well-defined diagnostic molecular markers severely hampers prediction of the clinical course of the disease. Establishment of an early and reliable biomarker for oral carcinogenesis will enable early diagnosis of cancer. Cyclooxygenase-2 (COX-2) is an inducible enzyme that is not found in normal conditions, but is induced by a variety of patho-physiological conditions. It is a key regulatory enzyme in the production of prostaglandins from archidonic acid. Studies show increased levels of cyclooxygenase-2 in premalignant and malignant lesions, and genetic evidence also implicates COX-2 in tumorigenesis. The therapeutic efficacy of specific COX-2 inhibitors is being evaluated. Materials and **Methods:** The expression of COX-2 was analyzed using immunohistochemistry in 102 cases of oral cancer and 30 histologically normal oral tissues using polyclonal antibody. **Results:** Microscopic evaluation of COX-2 from different progressive stages of oral tissues was found to display cytoplasmic immunoreactivity. COX-2 overexpression was observed in 66/102 (64.7%) oral cancer tissues, whereas 19/30 (63.3%) normal oral tissues analyzed did not show any detectable staining for COX-2 protein. **Discussion and Conclusions:** Significantly higher expression of COX-2 in oral squamous cell carcinomas compared to histologically normal tissues ($p=0.006$) suggests its association with oral carcinogenesis. COX-2 may serve as a potential diagnostic marker and target for developing molecular therapeutics for intervention in oral tumorigenesis.

S-16

Apoptotic Cell Death in Squamous Cell Carcinoma of Oro-pharyngeal Region

Navneet Saini and Ashish Kumar Mandal*

Department of Pathology, Maulana Azad Medical College, New Delhi-110002. *ruby_saini@rediffmail.com

I**ntroduction:** Apoptosis is a highly regulated process. Defect in apoptotic pathway leads to human diseases from neurodegenrative disorder to malignancy. Apoptosis may be induced through multiple pathways. One of the major pathway of apoptosis is through caspase-3. Thus, our aim was to study the expression of caspase-3 and its correlation with apoptosis in squamous cell carcinoma of oro-pharyngeal region. Materials and **Methods:** 1. Squamous cell carcinoma n=150, 2. Controls n=128. All the cases were collected from department of Pathology Maulana Azad Medical College.Control cases were mucosal biopsies from patients complaining of pain or stained mucosa, though histologically these were normal. 1. Caspase-3 expression was studied by immunohistochemistry (Avidin and Biotin method). 2. Apoptosis was studied by Tdt mediated UTP nick end labeling (TUNEL) staining method. **Results:** The immuno expression of caspase-3 was significantly less in tumor ($p<0.001$) as compared to controls. But the apoptotic index was significantly high. There was no correlation between caspase-3 and apoptosis. **Conclusion:** A significant pathway of cell death in squamous cell carcinoma of oro-pharynx is by apoptosis. Though it happens through activation of caspase-3, but does not appear to be main or sole pathway.

S-17

Role of Oxidative Stress and Transcription Factor AP - 1 in Pre and Post Operative Cases of Breast Carcinomas

***Priyanka Verma*[1], *Bhupesh K Prusty*[2], *M.L. Sherpa*[1], *J Bhattacharjee*[1], *Bina Ravi*[2], *Bhudev C Das*[3]**

[1]Department of Biochemistry, [2]Department of Surgery, Lady Hardinge Medical College & Smt. Sucheta Kriplani Hospital, New Delhi. [3] Department of Molecular Oncology, Institute of Cytology & Preventive Oncology; ICMR, Noida. *bhupesh_prusty@yahoo.com

Background: AP-1 (Activator Protein) is a composite transcription factor which is composed of either homodimers of heterodimers of Jun and Fos families.Free radicals like ROS, RNS and lipid peroxidation products such as Malondialdehyde (MDA) have been shown to induce pressure of c-fos and c-jun oncoproteins. **Objectives:**The aim of this study was to measure the levels of nitric oxide, glutathione, glutathione peroxides, MDA, SOD levels in pre and post-operative cases of breast carcinomas (after three months) and c-fos, c-jun expression. **Methods:** Quantitation of NO, glutathione, GPx, MDA, SOD was done spectrophotometerically in blood samples taken from 24 breast carcinoma patients (both before and after operation) and 5 patients with benign breast disease. Cancerous tissue of breast carcinomas was compared with healthy tissue obtained from mastectomy in same patients and from breast biopsies in benign breast disease cases. The level of expression of transcription factor AP-1, c-fos, c-jun was measured. The data was analyzed using t-test/ANOVA. **Results:** Levels of NO, MDA, SOD, glutathione showed a significant increase and GPx levels decreased significantly in the pre operative group (n = 24) in comparison to post operative cases in the same patients and in normal healthy controls. The expression of c-fos and c-jun in the control group ranged from nil to moderate ($p<0.001$).

S-18

Mutational Assessment of a Breast Cancer Susceptibility Gene, *BRCA1*, in Breast Carcinomas in Kashmiri Population

Rafiqa Eachkoti*[*1], Ishraq Hussain[1], Sheikh Aejaz Aziz[2], B.C. Das[3], Mushtaq A. Siddiqi[4]

[1]Departmentof Clinical Biochemistry, Sher-i-Kashmir Institute of Medical Sciences, Soura, Srinagar, Kashmir. [2]Department of Medical Oncology, Sher-i-Kashmir Institute of Medical Sciences, Soura, Srinagar, Kashmir, India-190011. [3]Institute of Cytology & Preventive Oncology (ICMR),1-7, Sector-39 Noida-201301, India. [4]Department of Immunology and Molecular Medicine, Sher-i-Kashmir Institute of Medical Sciences, Soura, Srinagar, Kashmir, India-190011. * sishraq@yahoo.com

Germline mutation analysis of *BRCA1* gene has demonstrated significant allelic heterogeneity. These differences represent historical influence of migration, population structure and geographic or cultural isolation. However, till date no attempt has been made to estimate the contribution of *BRCA1* gene mutations to breast cancer incidence in patients from Kashmir (Indian) valley. In order to investigate the role of *BRCA1*, we analyzed 43 breast cancer patients, 30 sporadic and a group of 13 high risk cases with either early onset or familial association of disease from Kashmir valley. Mutation screening in selected coding exons of *BRCA1* and their flanking Intronic regions by PCR-SSCP and DNA sequencing, led to the identification of two novel Intronic variants, c.199+67T>C and c.5396+187T>C, in exon 2 and 20 in two patients, one of which harbour both the variants. The absence of *BRCA1* mutations and low frequency of intronic variations in our study, suggest a limited role of *BRCA1* as a breast cancer susceptibility gene to our population.

S-19

Isolation and Screening of Anticancer Metabolites from *Boerhaavia diffusa*

Rakhi Srivastava*, Daman Saluja and Madhu Chopra

Dr. Br. B. R. Ambedkar Center for Biomedical research, University of Delhi, Delhi-110007, India. *dsalujach@yahoo.com

Introduction: Nature as a potential source of useful drugs has been recognized since ancient times. The drug discovery process increasingly requires the availability of large numbers of compounds. High-throughput screening has become an important tool in drug discovery. However, this technique requires a large number of compounds to be effective; these cannot be supplied by traditional organic synthesis, so two other sources are used: combinatorial chemistry and chemodiversity from nature. Chemodiversity in nature offers a valuable source, for e.g. secondary metabolites, previously regarded as waste products are now recognized for their resistant activity against pests and diseases. It resulted in the use of a large number of medicinal plants to treat various disease and some drugs in western medicine are based on the traditional use of such drugs. Some are used as pure compounds from the traditional medicinal plants, such as atropine, morphine, quinine & digitoxin and other modifications of such compounds, such as aspirin and local anesthetics. *Boerhaavia diffusa*, is a plant of the family of Nyctaginaceae and is widely used in traditional medicine for treatment of dyspesia, jaundice, enlargement of spleen, pain, rheumatism, snakebite etc. This plant also has hepatoprotective,

antilymphoproliferative and immunomodulatory effects. **Objectives:** To test the cytotoxic activity of ethanolic extract of *Boerhaavia diffusa*. **Methadology**: Ethanolic extract of *Boerhaavia diffusa* (roots and leaves) were prepared. Hela and U-87 cell line was maintained in MEM media supplemented with 10% FCS. Cytotoxic activity of crude ethanolic extract of Boerhaavia diffusa was evaluated by MTT assay and with trypan blue dye exclusion method. Methotrexate was used as a positive control. **Results:** In-vitro screening of the extract of Boerhaavia diffusa indicated that the crude fraction appeared to be cytotoxic against tumor cells. A dose dependent cytotoxic effect of root and leaf extract was compared with a standard anticancer compound methotrexate. The methotrexate was showing almost 40% cell death at a concentration of 200nM, whereas crude ethanolic extract of root was showing almost 30% cell death at concentration of 200 µg/ml; alkaloidal fraction showing 40% cell death at 300µg/ml and leaf extract was showing 40% cell death at 300µg/ml. These preliminary results showed that this plant could be further explored for its cytotoxic components.

S-20

Expression of Tumor Suppressor Proteins BRCA1 and p53 in Breast Carcinoma

***S.A. Raju Bagadi*[1]*, *Chandraprakash Prasad*[1], *Richa Tripathi*[1], *Siddharth Dutta Gupta*[2], *Anurag Srivastava*[3], *Rajinder Prasad*[3] *and Ranju Ralhan*[1]**

[1]Department of Biochemistry, [2]Department of Pathology, [3]Department of Surgical Discipline, All India Institute of Medical Sciences, Ansari Nagar, New Delhi-110029, India. *bsaraju@yahoocom

Introduction: Breast carcinoma is the second leading cause of cancer mortality in women and accounts for 20% of the total cancer-burden in India. Germline mutations in BRCA1/2 have been associated with increased susceptibility to familial breast cancer. However, the role of these genes in sporadic breast carcinomas remains to be delineated. While most of the studies have focused on analysis of gene mutations, the clinical significance of alterations in expression of these proteins remains to be determined. **Objective:** To determine the alterations in expression of tumor suppressor gene products BRCA1 and p53 in breast carcinomas; their relationship with clinicopathological parameters and estrogen receptor (ERa) status. **Methods:** Tissue samples were collected from Department of Surgical Disciplines, AIIMS, New Delhi. Expression of BRCA1, p53 and ERa was analyzed in paraffin embedded breast carcinoma tissue sections by immunohistochemistry (100 cases) using specific monoclonal antibodies and correlated with clinicopathological parameters by Chi-square test using SPSS10.0. **Results:** BRCA1 protein expression was not detectable in 40/100(40%) breast carcinomas. Accumulation of p53 protein was observed in 39/100 (39%) cases. ER expression was observed in 41/100(41%) patients. No association was observed between alterations in BRCA1 or p53 protein and any of the clinicopathological parameters or ERa status of the patients. **Conclusion:** Alterations in expression of BRCA1 and p53 are frequent events in sporadic breast carcinomas.

S-21

p53 Mutation Profile in Squamous Cell Carcinomas of the Esophagus in Kashmir (India) - A High Incidence Area

Mohammad M. Mir*[1], *Nazir A. Dar*[1], *Sailesh Gochhait*[4]*, *Showkat A. Zargar*[2], *Abdul G. Ahangar*[3] *and Ramesh Bamezai

Departments of [1]Clinical Biochemistry, [2]Gastroenterology and [3]Cardiovascular Thoracic Surgery, Sher-I- Kashmir Institute of Medical Sciences, Soura, 190011, Srinagar (J&K), and 4National Centre of Applied Human Genetics,

School of Life Sciences, Jawaharlal Nehru University (JNU), New Delhi-110067, India. *bame0200@mail.jnu.ac.in; bamezai@hotmail.com

Esophageal squamous cell carcinoma (ESCC) has been reported to show geographical variation in its incidence, even within areas of ethnic homogeneity. Kashmir valley, in north of India, has been described as a high-risk area for ESCC. Here, we make a preliminary attempt to study mutations in exons 5-8 (the DNA binding domain) of the tumor suppressor gene, p53, in 55 ESCC patients from Kashmir. Polymerase chain reaction followed by direct sequencing analysis revealed the presence of mutations in 36.36% (20/55) tumors, assessed for the extent of allelic instability. The 20 mutations, found in 20 patients, comprised of 17 single base substitutions (11 transitions + 6 transversion) and 3 deletions. The seventeen single base variations represented 12 missense mutations, 2 nonsense mutations and 3 variations located in intron 6, one of which resulted in a splicing variant. The patients when compared, for the incidence of p53 mutation with various demographic features revealed females to be at increased risk (p=0.016, OR=4.13, 95% CI 1.26-13.46). Comparison of mutation profile with other high-risk areas reflected both differences and similarities indicating co exposure to a unique set of risk factors. This might be due to the special dietary and cultural practices of Kashmir that needs validation; also for the gender based difference in the incidence of p53 mutation observed in this study.

S-22

Role of Complementary and Alternative Therapies in Cancer Management in India

Sanjoy Kumar Pal*

Department of Gastroenterology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Raebareli Road, Lucknow UP 226 014, India. *sanjoypal@yahoo.com

Introduction: Throughout the world and in India a large number of patients with cancer try various complementary and alternative medicines/therapies (CAM). The reasons for adopting these therapies most certainly are complex viz. cultural, ignorance, socioeconomics, inadequate access to mainstream medical facilities etc. The subject of alternative therapies for cancer stirs quite a debate within the field of oncology. Viewpoints range from an enthusiastic acceptance of complementary therapies to the belief that they are all quack medicine. **Objectives:** To collect information about the various CAM tried by India by India cancer patients. **Methods:** Information was collected by searching the Internet, scientific journals and news papers etc. **Results:** Homeopathic approaches developed by Prasanta Banerji Homeopathic Research Foundation and 'Psorinum' developed by New Horizon Center for Cancer Research & Treatment are very popular in Kolkata. Ayurvedic approaches developed by Vaidya Chandra Prakash Cancer Research Center, Dehradun; Huma Cancer Society, Lucknow and CARCTOL are very popular in northern part of India. The other population CAM in this region includes dietary regimen Sarvapisti developed by Daya Sankar Research Center, Varanasi; Kromba an herbal mixture in Rishikesh and Tibetan herbal cancer medicine from Dharamshala, Kangra. Herbal medicine SJ-29 and Muthu Marunmthu is popular in South India. Other approaches include nutritional therapy, Tulsi, Haldi, acupuncture, auto urine therapy, Reiki, hypnosis, religious therapy, meditation, yoga, laughter therapy and black magic. **Conclusion:** Various CAM are popular in India, but scientific information on them are limited. CAM may play an important role in cancer management in our country and thus should be thoroughly investigated.

S-23

Bcl6 Somatic Hypermutation - a Marker for Germinal Center Transit B Cell Non-Hodgkin's

Lymphomas

Sasikala P.S.*, Nirmala Nancy K., Mahji U., Shirley S and Rajkumar T.

Dept of Molecular Oncology, Cancer Institute (WIA), India. *ps_sasikala@yahoo.co.in

Introduction: Non-Hodgkin's Lymphoma is the 7th most common type of cancer seen in India. Diffuse large B cell lymphomas (DLBCL), the most prevalent NHL subtype in our population represents a clinically and histologically diverse group of neoplasms. More than 50% of the patients are either resistant to the current treatment or succumb to the disease. Nearly 70% of DLBCL were reported to undergo Bcl6 somatic hypermutation (SHM) at the 5'end of the non-coding region. Our aim in this study is to determine the distribution and the frequency of Bcl6 SHM in different subtypes of NHL in Indian patients. **Materials and method:** Genomic DNA extracted from lymphocytes from 20 healthy donors, one placental tissue and lymph node biopsies from 95 NHL patients was used in this study. The mutational status of the Bcl6 gene was screened by radiolabelled PCR-SSCP analysis and confirmed by direct sequencing using ABI 310 genetic analyzer. The expression pattern of BCL6 protein in the corresponding paraffin embedded sections was studied by IHC analysis. **Results:** Bcl6 SHM was observed in 54% of DLBCL and 65% of FL in our patients and was found to be a rare event in other NHL subtypes. No significant correlation was observed between the Bcl6 SHM and its protein expression. **Conclusions:** Our study corroborates the fact that the Bcl6 SHM is very specific for B cell malignancies. Since the Bcl6 SHM had similar properties to that of IgG SHM, it may be used as a marker for germinal center transit B cell lymphomas. Our finding shows that some of the mutations resulted in the generation of new binding site for strong transcription factors. Though their implication is presently unknown it might be involved in the deregulation of BCL6 protein expression and lymphomagenesis.

S-24
Bioinformatics in Cancer Research

Seema Mishra*

Supercomputing Facility for Bioinformatics and Computational Biology, IIT-Delhi 110016, India. *seemamishra7@yahoo.co.in

Cancer bioinformatics entails using and analyzing the information from the HGP, as well as genes from several organisms, through the application of bioinformatics tools in order to help develop a cure. It also entails taking genetic variation studies and proteomics into account. Different bioinformatics tools as applied to cancer research are: microarray analysis (MGED, microarray gene expression data; microarrays.org, from UC, San Francisco), protein structure prediction and modeling tools (Expasy proteomics server; PDB; Swiss-PDBViewer), pathway analysis (BioCarta; KEGG) etc. Basically, in order to search for a target protein, genes that are expressed abnormally in cancer cells are identified by microarray analyses. Genetic variations or SNPs (Single Nucleotide Polymorphisms) which may predispose individuals to cancer are also identified and analysed (NCI Genome Anatomy Project SNP Index). Proteins encoded by the abnormal genes are then identified and their interactions studied by pathway analyses and probable functions deduced. Further, structure prediction is done using either comparative modeling (SWISS-MODEL) or threading (LOOPP) or ab-initio tool (Rosetta). After proper identification of a good target structure, Computer-Aided Drug Design (CADD) is used to design a good lead (compound with drug-like properties). Binding of the lead to the target is analysed using docking algorithms, and further evaluations using molecular modeling techniques (Hyperchem, InsightII) and optimizations are done. Tests such as QSAR (Quantitative-Structure-Activity

Relationship), ADME/T (Absorption, Distribution, Metabolism, Excretion, Toxicity) etc. are further conducted to deduce oral bioavailability and potency before being finally declared as a drug.

S-25

Post-transcriptional Regulation of Human Cathepsin L Expression by the 5' Untranslated Region of its mRNA Species

Shivani Arora* and Shyam S. Chauhan

All India Institute of Medical Sciences, Ansari Nagar, New Delhi-110029, India, * shivani_aiims@yahoo.com.

Cathepsin L, a cysteine protease, is over expressed and secreted out of the cells in various cancers. Human cathepsin L is encoded by multiple mRNA species namely hCATL A, AI, AII and B. HCATL A, AI and AII differ only in the lengths of their 5' untranslated regions (UTRs). We describe here the identification of a novel splice variant, namely AIII, which has the shortest 5'UTR. This species was found to be the most abundant splice variant in various tumor cell lines detected by RNAse protection assay. Comparison of the relative efficiencies of translation by *in vitro* transcription coupled translation assays demonstrated that the AIII variant was translated with the highest efficiency. Also, in vivo stability assays showed that AIII was also the most stable splice variant. To identify whether the 5'UTR was both necessary and sufficient for the observed translational difference among these variants, the UTRs of A and AIII were cloned upstream to luciferase reporter gene and then in vitro transcribed and translated. Again, the UTR of AIII was able to translate the luciferase gene with significantly higher efficiency as compared to that of A. This suggested the presence of some negative cis acting regulatory element/s in A that could suppress the translation of a heterlogous gene also. To identify this element, various deletion constructs were made and their relative translational efficiencies were assessed. Deletion analysis suggested the presence of more than one negative regulatory element in the 5'UTR of hCATL A. In summary, our results demonstrate that the 5'UTRs of hCATL mRNA species regulate their translational efficiency and stability, which may play an important role in the overexpression of cathepsin L in malignant cells.

S-26

BRCA1 and BRCA2 Genes Mutations in Indian Breast Cancer Families

Singh S.K.[1], Valarmathi T.M[2]. , Shukla N.K.[2] and Das S.N.[1]

[1]Department of biotechnology and [2]BRA-IRCH, All India Institute of Medical Sciences, New Delhi, India. *satya_n_das@hotmail.com

Introduction: Mutation in BRCA1and BRCA2 genes has been widely implicated in the development of breast/ breast and ovarian cancer syndromes well as certain other tumors of familial origin around the globe. However, there is paucity of data in Indian patients with familial breast cancer. Therefore, the present study was planed to analyze mutation in BRCA1 and BRCA2 genes in Indian breast / breast and ovarian cancer families. **Objectives:** This study was conducted to determine the frequency and types of BRCA1 and BRCA2 mutation in Indian familial breast cancer patients. **Methods:** Screening of BRCA1and BRCA2 coding sequences, and intron-exon boundaries and their flanking intronic regions by PCR, PCR-mediated site-directed mutagenesis (PSM), SSCP, CSGE (Conformation Sensitive Gel Electrophoresis) and direct DNA sequencing techniques. **Results:** 19 families of breast / breast and ovarian cancer and 140 normals were screened for BRCA1 and BRCA2 gene mutations. Total 26 mutations were found, including 25 missense, 5 frameshift, 5 nonsense and 1 polymorphism

were detected in breast cancer families while no mutation was observed in normal / sporadic breast cancer patients. **Conclusion:** Indian breast / breast and ovarian cancer families showed a distinct mutation pattern in BRCA1 and BRCA2 genes. Some of the founder mutation such as del 185 AG was also seen in Indian subjects.

S-27

Inherited Predisposition and Environmental Exposure in the Causation of Familial or Multiple Primary Cancer

[1,2]Sunita Shankar*, [1,3]Rajiv Sarin, [1]Neena Bhatnagar, [2]Ashwin Kotnis, [1,2]Jayant Sastri, [3]Supriya Chopra, [2]Rita Mulherkar and [1, 2]Shyam Agarwal

[1]Cancer Genetics Unit, [3]Radiation Oncology and [2]Genetic Engineering Department, Tata Memorial Hospital and [2]ACTREC, Mumbai. *rmulherkar@actrec.res.in

Background: There is scant data on the incidence, pattern and genotype of Familial Cancers or Multiple Primary Neoplasms (MPN) from India. Epidemiological and initial genotyping data of families seen in a Cancer Genetics Clinic (CGC), over a 2 year period (2003-2004) is presented here. **Results:** In 222 families, scrutiny of medical records and pedigree analysis suggested possible autosomal dominant syndromes (157 families); autosomal recessive syndromes (13 families: XP-10; albinism with cancer-3) or MPN (52 cases). Autosomal dominant conditions included Hereditary breast / breast ovarian cancer-76 families, hereditary colorectal cancers (HNPCC / FAP)- 10, Li-Fraumeni Like- 9, Neurofibromatosis- 6, Tuberous Sclerosis- 3, VHL- 2, non-syndromic familial cancers- 46 and various other rare syndromes such as Gorlin's, MEN, familial Wilms' or testicular cancer in 5 families. **Discussion:** While the entire spectrum of hereditary cancer syndromes was seen, in 46/170 families no specific cancer syndrome could be identified. In patients with upper aero-digestive tract MPN, significant excess of GSTM1/T1 null genotype, tobacco habits (95%) and a family history of cancer (40%), suggest gene-environment interaction in MPN genesis. Specialized clinics such as this CGC and DNA banking / cell lines provide a unique opportunity for genetic, epidemiological and psycho-social research in a large number of informative kindreds with a potential for identifying new genes and understanding the determinants of penetrance and anticipation or gene environment interaction.

S-28

Cytomorphological alterations following Human Papillomavirus Infection correlated with HPV DNA Types

Veena Kashyap*, Uma Kailash, Suresh Bhambhani and B.C. Das

Institute of Cytology & Preventive Oncology (ICMR), Sector-39, Noida. *sureshib@yahoo.com

Infection with human papillomavirus is known to be a major risk factor for squamous intraepithelial lesions and persistent HPV infection is precursor to cervical cancer. Pap smear is a routine screening method in gynecologic pathology for detecting cytologic changes including those due to human papillomavirus infection of the uterine cervix. Koilocytosis has been regarded as most pathognomic features of HPV infection. On the basis of cytomorphological peculiarities of HPV infection 250 smears were divided into two groups. Group I included 60 smears with classical koilocytotic changes and Group II consisted of 190 smears with non-koilocytotic changes. Cells from these smears were processed for PCR amplification of HPV DNA type status 6,11 and 16. It was observed that 40 (66.6 %) of 60 Group I cases and 30 (15.8%) of 190 Group II were positive for

HPV DNA type 16 and 20 (33.3%) of 60 Group I and 95 (50 %) of 190 of Group II cases showed positivity for HPV DNA types 6 &11. Remaining 65 (34.2%) were negative for any HPV DNA type.

S-29

Concomitant Association of Lower Genital Tract Infections with Human Papillomavirus Infection of Cervix

Veena Kashyap and Suresh Bhambhani*

Institute of Cytology & Preventive Oncology (ICMR), Noida. *sureshib@yahoo.com

The Papanicolaou stained smear is a routine screening method in gynecology for detecting cytologic changes of precancerous and cancerous changes including those due to human papillomavirus. Most, if not all precancerous and cancerous lesions of the cervix are accompanied by a HPV infection. It is also claimed that the cytomorphologic identification of cellular changes is currently the most convenient rapid, economic and sensitive available procedure for detection of HPV infection in the genital tract. The paper presents the co-existence of other lower genital tract infections in HPV infected cases versus non-HPV (control) inflammatory smears. From the data it was observed that in HPV infected smears the commonest associated infections were of Garderenella vaginalis followed by Trichomonas vaginalis and others, however in non-HPV inflammatory smears, the commonest infections were of Trichomonas vaginalis followed by candida and others. Associated infections in HPV group of patient was about 8% higher than non-HPV patients.

S-30

Hodgkin's Lymphoma in North Indian Children: Prevalence and Significance of Epstein-Barr Virus Latent Membrane Protein-1 Detection in Hodgkin's and Reed Sternberg Cells

Veronique Dinand*[1], Laxman S. Arya[2], Ramesh Dawar[3]

[1]Department of Pediatrics, All India Institute of Medical Sciences, New Delhi. [2]Department of Pediatrics, Apollo Hospital, New Delhi. [3]Department of Pathology, All India Institute of Medical Sciences, New Delhi. *vatsalya@touchtelindia.net

Background: One hundred and forty six cases of childhood Hodgkin's lymphoma seen at the All India Institute of Medical Sciences were studied in order to assess the prevalence of Epstein Barr virus (EBV) latent membrane protein-1 (LMP1) in the diagnostic lymph node biopsies. The association with clinical, epidemiological factors and prognostic significance of EBV detection were analysed. **Material and Methods:** Paraffin-emmbedded lymph node biopsies of children diagnosed with Hodgkin's lymphoma from 1991 to 2003 were stained immunohistochemically with primary monoclonal antibodies against CD45, CD20, CD45RO, CD15 and CD30 antigens for immunological classification, and against EBV-LMP1. Epidemiological factors, clinical staging, response to chemotherapy and survival were analysed in correlation with pathological subtype and immunostaining results. **Results:** Out of 146 cases, mixed cellularity subtype (MC) was seen in 72.6%, nodular sclerosis (NS) in 22.6%, lymphocyte depletion (LD) and nodular lymphocyte predominance (NLP) in 1.4% each, while 2.5% were unclassified. MC, NS and LD subtypes were significantly associated with advanced stage disease (stage III and IV) as compared with NLP ($p=0.04$). EBV-LMP1 was detected in 132 cases (91.1%). Nine bone marrow biopsies involved with HL at presentation proved concordant with EBV status in the lymph node. In

five out of six relapsed cases, EBV status of lymph node biopsy at relapse was concordant with the initial biopsy. There was a significant association at univariate level between EBV positivity and younger age (p=0.001), and between EBV and lower socio-economic level (p=0.04). There was a trend towards a poorer outcome in CD15 negative cases of classical HL (5-year event free survival 75.5% vs. 84.9%, p=0.12). EBV negative cases had a poorer 5-year event-free survival (78.7% vs. 85.2%), though the difference was not statistically significant. **Conclusion:** EBV was detected immunohistochemically in 91% of childhood HL in Northern India, more often in younger age and lower socio-economic status.

S-31

Prognostic Significance of Flt3 Mutations in Acute Non-Lymphocytic Leukaemia

Sunil K. Sidharthan[1], Praveenkumar B. Raveendran*[1], Sreejith Nair[2], Geetha Narayanan[2] and Ravindran Ankathil[1]

[1]Division of Cancer Research, [2]Division of Medical Oncology, Regional Cancer Centre, Thiruvananthapuram, Kerala, India. *praveenrcc@yahoo.com

Introduction: Activating mutations in FMS like tyrosine kinase receptor (flt3) gene are detected in approximately 30% of Acute Non-Lymphocytic Leukaemia (ANLL) cases. Studies have demonstrated that flt3 ITD are associated with significantly worse clinical outcome, although converse reports are also available. Controversy still exists as to the prognostic significance of flt3 mutations in ANLL. **Objective:** To identify the prevalence and clinical significance of flt3 gene mutations in ANLL patients. **Methods:** 134 ANLL patients from Regional Cancer Centre, Thiruvananthapuram were analyzed for flt3 mutations. Exon 14 and 15 of flt3 gene were PCR amplified using primers and length mutations were detected by PAGE. Point mutations were detected employing SSCP analysis. Survival status was analysed using Kaplan-Meier method. **Results:** Flt3 mutation was detected in 30 out of 138(22%) samples analysed. The frequencies of different mutant/wild type ratios obtained were ratio = 1(10%), ratio < 1(8%) and ratio > 1(3.6%). Haematological variables demonstrated a positive correlation with the length mutations of flt3. flt3 mutations were significantly associated with decreased remission rate, (26.7%) for patients with flt3 ITD when compared to patients with wild type flt3 (56%), and elevated risk of relapse. The overall survival rate post therapy was 70% vs. 40% for patients with wild type flt3 and flt3-ITD respectively. **Conclusion:** To conclude, flt3 mutation status might be a valuable tool for accurate risk stratification of ANLL patients and might allow the identification of a subset of patients with higher risk of therapeutic failure who could potentially benefit from more intensive therapeutic approaches.

S-32

Ras p21 and its Expressional Variations in the Development of Oral Squamous Cell Carcinoma

Prishla Varghese*[1], Prabha Balaram[1] and BC Das[2]

Division of Cancer Research, Regional Cancer Centre[1], Trivandrum-695011, Kerala. Division of Molecular Oncology, ICPO[2], Noida. *prishlaprishla@rediffmail.com

Introduction: Cancer of the oral cavity is a disease of acquired occupational, nutritional and environmental insults. Chronic exposure of the oral cavity to a variety of mutagens/carcinogens present

in the betel quid (a common chewing habit in Indian population), Bidi/cigaratte smoking and alcohol consumption results in the development of clinically distinct premalignant lesion which got a 5-10% chances of progress towards malignancy in future. Oncogenic alteration has been marked as one of the main causative factor for carcinogenesis. **Objectives:** To understand the biological events mediating the multistep process of oral tumorigenesis, in the present paper we report the results of our investigation on the occurrence of ras p21 protein in HNSCC presenting with various degree of dysplasia and the different subsets of cancer. In addition, we investigated whether occurrence of demonstrable Ras p21 protein in these cases show any particular expression pattern that could predict the future behavior of the lesion. **Methods:** 409 oral lesions of varying normal, premalignant and malignant phenotypes are immunohistochemically analyzed for rasp21 antibody. Immunocytochemical analysis was performed using the streptavidin-biotin method. **Results:** Rasp21 expression was up-regulated when compared with normal mucosa in 86.6%(180/209) of cancer patients and 83.5%(146/176) of premalignant patients. Significant differences in expression pattern of Rasp21 ($p=0.003$) were observed between various histopathological groups of oral cancer by one-way ANOVA test. Staining was noted only on the cell membranes in normal mucosa while cytoplasmic and membrane staining with varying intensity was noted in premalignant and malignant lesions of the oral cavity. Expressional variation of Ras p21 in severe dysplastic lesion (absence of staining in both membrane and cytoplasm of basal layer of cells) from the other dysplastic and hyperplastic lesion suggest that ras p21 can be used as a marker of early detection of oral cancer along with other histological changes like the intracellular distribution of Ras p21, positive cell ratio and staining intensity.

S-33

Preventive Role of Ascorbic Acid against PPAR- Ligand Induced Hyalinizing Trabecular Adenoma of Thyroid Follicles in Mice

A Nath and <u>Mridula Sharma</u>*

Cell Biology and Toxicology Laboratory, Department of Zoology, Patna University, Patna - 800 005.

In the present study preventive role of Ascorbic acid against PPAR- ligand induced *Hyalinizing trabecular adenoma* (*Adenocarcinoma*) of thyroid follicles has been observed in Swiss albino mice. Animals were provided with 160ppm PPAR-g ligand as Pioglitazone, a hypoglycemic drug alone in one group and a another group they were administered with 200 ppm Ascorbic acid as Celin through drinking water for 12 weeks. Control and treated animals were sacrificed after 8^{th}, 10^{th} and 12^{th} week for histopathological, ultrastructural and hormonal study. After treatment with PPAR-g ligand alone, histopathological features of thyroid follicles were similar to Hyalinizing trabecular adenoma (i.e. Adenocarcinoma) showing Acinus formation. Follicles appear as *Large pleomorphic follicles or Cohesive expensile masses*. It shows total autolysis under electron microscope. Significant elevation in Serum TSH level is observed during hormone study. Hyalinizing trabecular Adenoma seems as a precursor for follicular thyroid carcinoma in the present study. But after treatament with PPAR-g ligand along with Ascorbic acid its ultrastructure of thyroid follicles reveals *hydropic degeneration*. Its histopathological features show failure of normal differentiation into adenocarcinoma with scattered inflammatory infiltration and enormous vascularization. In hormone study TSH level of serum gets decreased. The result demonstrates that Ascorbic acid act as preventive measures against PPAR-g ligand drug induced thyroid carcinoma.

S-34

Detection and Genotyping of Human Papillomavirus in Invasive Cervical Cancers by the Reverse Line Blot Assay

Neerja Bhatla*[1], Lalit Dar[2], A. Raj Kumar Patro[2], Alka Kriplani1, Gulati Arti[1], Sangita Rani[1], Kusum Verma[3], Keerti V. Shah[4], Patti E. Gravitt[5]

Departments of [1]Obstetrics and Gynaecology, [2]Microbiology and [3]Pathology, All India Institute of Medical Sciences, New Delhi, India. Departments of [4]Molecular Microbiology and Immunology and [5]Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA.*nbhatla@aiims.ac.in

Introduction: Cervical cancer is the commonest among Indian women, with 126,000 new cases and 76,000 deaths annually. Several high-risk HPV types are known to cause the disease, but Indian data is mostly limited to study of a few genotypes. **Objectives:** To evaluate HPV genotypes in invasive cervical cancers. **Methods:** Eighty women aged 25 to 65 years with histologically confirmed cervical cancer were included. The control group comprised 150 women with other gynaecological problems who were normal on Pap smear and colposcopy. HPV DNA was determined by PCR using L1 consensus primers. Genotype was determined by a reverse line blot hybridization assay (Roche) which detects 22 high and 15 low risk genotypes of HPV. **Results:** 94.2% cases had squamous cell carcinoma and 5.8% had adenocarcinoma. 78.6% were in Stage II/ III; 60.9% were over 50 years age. HPV DNA was detected in 78 (97.5%) cervical cancer samples and 23 (15.3%) of controls. One squamous cell and one adenocarcinoma were negative for HPV. High-risk HPV was found in all 78 positive cervical cancer cases, with multiple infections in 10 cases, and in 14 (9.3%) controls. Ten high-risk HPV types were detected of which HPV 16, 18 and 45 were the commonest among the cancers (74.4%, 15.8%, 10.5%), irrespective of histopathology. HPV 16, 39 and 52 were the commonest among controls (26.1, 13.0 and 8.2%). **Conclusions:** The pattern of HPV infection seen in India seems similar to that reported worldwide. A vaccine against HPV 16 and 18 could protect against 75% of cancers.

S-35

Regulation of Multidrug Resistant Cancer with A Novel Metal Complex

Ananda Mookerjee*[a], P. Dutta[a], S. Majumder[a], R.N. Baral[b], S.K.Choudhuri[a]

Department of Environmental Carcinogenesis and Toxicology, Chittaranjan National Cancer Institute, 37, S.P. Mukherjee Road, Calcutta-700 026, India. *anandamookerjee@yahoo.com

Multidrug Resistance (MDR) in cancer and other diseases like tuberculosis, malaria and leishmaniasis a.e. major impediment to modern drug therapy and have already reached to a demonic dimension. Several mechanisms operate behind the phenomenon of MDR in different diseases. In the context of cancer the three major mechanisms of MDR are perhaps over expressions of different efflux pumps for driving out chemotherapeutic drugs, gene amplification and hyper expressions of growth factors to induce strong survival signals. MDR in cancer is sometimes associated with over production of growth factors like fibroblast growth factor that provide survival signals to neoplastic cells to overcome the detrimental effects of chemotherapeutic drugs. Sometimes gene amplification neutralizes the effect of drugs those are analogues of substrates of various enzymes. Moreover, MDR in cancer in many cases is associated with over expressions of various efflux pumps that reduce the amount of drugs inside the cells. These efflux pumps often sequester the drug to organelle like Golgi apparatus causing subsequent efflux of drugs. These efflux pumps are mostly ATP dependent and are classified as ATP-binding cassette (ABC) transporters.

Some of these ABC transporters are P-glycoprotein (P-GP), multidrug resistance associated protein (MRP), ATP 7B and so on. MRP over expressing resistant cells often have elevated level of reduced glutathione (GSH) and glutathione S-transferase (GST) and anticancer drugs are effluxed either as conjugate with GSH (drug-GS conjugate) or cotransported along with GSH by MRP transporters (like MRP 1). The work has been carried out in drug resistant Ehrlich ascites carcinoma cells (EAC/Dox) and drug resistant sarcoma 180 cells (Sar 180/Dox) in vivo. Interestingly the anticancer drug doxorubicin (Dox) failed to induce protein phosphatase (PTPase) activity as well as reactive oxygen species (ROS) production in the resistant cells as compared to Dox-sensitive counterparts. The MDR cells also inhibited nitric oxide (NO) production by splenic mononuclear cells (SPMC) of mice inoculated intraperitoneally with these cells. The GSH levels and GST activity of these cells were also very high that explains the lack of ROS in these cells. Interestingly, several kinases including protein tyrosine kinase (PTK) is found to be activated in these drug resistant cells. The present study indicates that these kinases are related to stronger survival signal and drug resistance. The present study describes the development of a novel chemotherapeutic drug viz., copper (II) (N-2-hydroxy acetophenone)glycinate (CuNG) that modulates the signaling events to resolve MDR cancer. CuNG is a copper coordination complex and is a strong depletory of GSH, CuNG, on intramuscular (im) administration initially induces NO response of SPMC and increases ROS production in the cancerous cells. The activation of splenic macrophages was also evident by their distinct , morphological changes. This NO response is not found 7 days following treatment. However, CuNG is found to modulate activities of several kinases including PTK and thereby induce caspase activity and apoptosis of neoplastic cells to trigger their apoptosis. Nuclear degradation becomes evident within 7-10 days of CuNG treatment. By 2 weeks following CuNG treatment, the basal proliferation rate of SPMC and lymph node cells (LNC) reduces while response to mitogenic stimuli with concanavalin A (Con A) or with a combination of phorbol-12 -myristate-13-acetate (PMA) and ionomycin (I_0) increases. On the other hand, the proliferation of neoplastic cells decreases drastically and the number of apoptotic cancerous cells increases to a large extent. Since apoptosis, rather than necorsis is perhaps the preferred mode for resolving cancers by CuNG, the possibility of necrotic shock remains distant. However, no drug so far has been reported to induce preferential apoptosis of MDR cells. The novel drug CuNG causes preferential apoptosis of MDR cells in vivo that may be a gigantic step towards successful chemotherapy of MDR cancers. Further works are in progress to decipher whether the drug action is due to an activation induced cell death (AICD) process. The study of the interaction of CuNG on ATP7B is also warranted.

S-36

Role of 9-O-Acetylations of Sialic Acids in Children with Acute Lymphoblastic Leukemia toward Immune-Surveillance

Chitra Mandal**, S. Bandyopadhyay, S. Ghosh, K. Mukherjee, S. Chowdhury, A. Mallick, C. Mandal and S. Ray

Immunobiology Division, Indian Institute of Chemical Biology, 4, Raja S. C. Mullick Road, Kolkata 700032, India. *cmandal@iicb.res.in

Childhood acute lymphoblastic leukemia (ALL) is characterized by overexpression of 9-*O*-acetylated sialoglycoconjugates (9-*O*AcSGs) on hematopoietic cells concomitant with high titers of anti-9-*O*AcSGs. The present study was aimed to evaluate whether this high induction of anti-9-*O*AcSGs at disease presentation contributes toward immune-surveillance. Accordingly, anti-9-OAcSGs were affinity purified from sera of ALL patients and normal individuals and their specificity towards the glycotope having terminal 9-O-acetylated sialic acid linked sub terminal N-acetyl galactosamine in 2-6 manner (9-*O*AcSA 2-6GalNAc) was established. Subclass distribution of anti-9-*O*AcSGs revealed a predominance of IgG2 in ALL. Analysis of glycosylation profile of anti-9-*O*AcSGs purified from sera of ALL patients (IgG_{ALL}) and normal (IgG_N) demonstrated disease

specific antibodies differ in content and nature as compared with normal controls. Enhanced amount of 9-*O*AcSA specific IgG2 induced in ALL was unable to trigger activation of FcgR, the complement cascade and cell mediated cytotoxicity, although its glycotope binding ability remains unaffected. Interestingly, only $IgG1_N$ emerged as the potent mediator of cell mediated cytotoxicity, complement fixation and activator of effector cells through FcgR. In ALL, the observed subclass switching of anti-9-*O*AcSGs to IgG2, alteration in their glycosylation profile along with impairment of a few Fc-glycosylation-sensitive effector functions hint towards a disbalanced homeostasis thereby evading the host defense. These findings justify further evaluation of the mechanism for functional unresponsiveness of antibodies and production of 9-*O*AcSA specific chimeric antibodies with normal Fc domain for therapeutic applications.

S-37

Detection and Genotyping of Human Papilloma Virus in Cervical Swab Samples by Reverse Line Blot Assay

Lalit Dar*[*1], Neerja Bhatla[2], Shobha Broor[1], A. Raj Kumar Patro[1], Alka Kriplani[2], Sunil Kumar Pati[1], Sangeeta Rani[2], Arti Gulati[2], Keerti V. Shah[3], Patti E. Gravitt[4]

Departments of [1]Microbiology and [2]Obstetrics and Gynecology, All India Institute of Medical Sciences, New Delhi, India and Departments of [3] Molecular Microbiology and Immunology, and [4]Epidemiology, Johns Hopkins University, Baltimore, USA. *lalitdar@yahoo.com

Introduction: Cervical cancer, caused by human papilloma virus, is the second most common cancer in the world, accounting for 280,000 deaths and 510,000 new cases each year. It is the commonest cancer in Indian women. More than 100 HPV genotypes have been identified in humans of which at least 40 HPV types are found in the genital tract and are categorized as being either "high risk" or "low risk" types. **Objective:** To detect the carriage of HPV in cervical swabs by polymerase chain reaction and to type the virus detected. **Methods:** One hundred ninety six women aged 30 to 65 years attending the gynecology OPD, AIIMS with complaint of vaginal discharge, post coital bleeding, inter-menstrual bleeding or unhealthy cervix with no obvious tumor were included in the study. The presence of HPV DNA in the cell samples was determined by polymerase chain reaction (PCR) using L1 consensus primers. The genotype was determined by a reverse line blot hybridization assay (Roche, USA) capable of detecting 37 genotypes of HPV. **Results:** HPV DNA was detected in cervical swab samples from forty six (23.5%) women by the PCR reverse line blot detection method. Twelve high risk and 7 low risk types were detected. The commonest types were HPV-16 in 17(37%), HPV-18 in 1(2.2%), HPV 52 in 4(8.7%) and CP6108 in 9 (19.6%). Eleven (24 %) of the positive samples showed presence of DNA from multiple HPV types. **Conclusion:** HPV-16 was identified as the predominant type in cervical swab samples in our patients.

S-38

Human Papillomavirus Infection in Women with Different Clinical Manifestations

***Jayanti Mania-Pramanik*[*1], Shilpa C. Kerkar[1], Vinita S. Salvi[2]**

[1]National Institute for Research in Reproductive Health, Indian Council of Medical Research, [2]Seth G.S. Medical College and KEM Hospital, Parel, Mumbai 400012. *jayantimania@rediffmail.com

Human Papillomavirus (HPV) is sexually transmitted and causally related to cervical cancer. Almost all

cervical cancers and high-grade cancer precursors are caused by specific high-risk types of HPV. Hence diagnosis of this infection has gained a priority area of research while dealing with cancer of the cervix. Our objective is to evaluate the use of an in-house PCR to diagnose HPV infection in women with different types of clinical manifestations. Cervical specimens, Pap smear and specimens for gram's stain were collected from 243 women attending the Gynecology OPD of KEM hospital. PCR was carried out using consensus primers MY09 and MY11 to amplify 450 bp DNA of L1 open reading frame of HPV. PCR results were confirmed by southern hybridization using specific in-house synthesized probes. Typing was done by restriction digestion as well as using a commercial kit. HPV was detected in 15 (6.2%) women and 12 (80%) of them were infected with high risk HPV. Symptoms like leucorrhoea, burning, micturation, pain in abdomen or discharge with foul smell was observed in 11 (73.3%) of these women. Among the women with high risk pregnancy (n=73), 5.5%; 3.1% of infertile women (n=64) and 4.9% of pregnant women (n=41) were with this infection. Association of any other infections like *C. trachomatis*, Bacterial vaginosis or Candida was observed in 4 (26.7%) of these women. But these infected women were with normal cervical cytology as observed by Pap which suggests the inclusion of HPV testing along with cervical cytology particularly in women who present some form of signs and symptoms. Use of commercial kit for HPV typing and results of sequencing was also evaluated.

S-39

GSTP1 Gene Polymorphism in Lung and Oesophageal Cancers in North Indian Population

Sobti RC*[*1], Kaur P[1], Singh J[1], Janmeja AK[2], Bindra HS[3] and Jindal SK[4]

[1]Department of Biotechnology, Panjab University, Chandigarh, [2]Department of Chest and Infectious Disease, GMCH, Sector 32, Chandigarh, [3]Department of Gastroenterology, Oswal Cancer Hospital, Ludhiana, [4]Department of Pulmonary Medicine, PGIMER, Chandigarh. *rcsobti@pu.ac.in

Mammalian cells are constantly exposed to a wide variety of genotoxic agents from both endogenous and exogenous sources. These genotoxic agents are detoxified by a variety of enzymes so that these can be eliminated from the human body. The impact of polymorphism in detoxifying enzymes coding genes has received particular interest in cancer research. These detoxifying enzymes are classified into phase I and II enzyme systems. Phase I enzymes involves mainly Cytochrome P450 and Phase II involves Glutathione-S-transferase, beside others. One of the major Glutathione-S-transferase is *GSTP1* which plays an important role in detoxification of major classes of tobacco-carcinogens. *GSTP1* codes for an enzyme, which is a major metabolizer of the activated product of benzo(a) pyrene. But little is known about the possible risks attributable to variant forms of *GSTP1* in individual responses to environmental carcinogens such as cigarette smoke. We have studied *GSTP1* exon 5 codon 105 polymorphism in 170 cancer (90 of lung and 80 of oesophageal cancer) cases and 224 age and gender matched control subject In lung cancer the genotype frequency of *GSTP1* Ile/Ile, Ile/Val and Val/Val genotypes was 52.2, 45.5 and 2.2 percent in cases and 43.2, 51.9 and 4.8 percent in controls respectively. The genotype frequency of Ile/Ile and Ile/Val +Val/Val in oesophageal cancer was 49.5 and 56.6 percent as again 49.1 and 50.5 percent in controls respectively. No association for *GSTP1* gene polymorphism with both lung (OR=0.7, 95% CI= 0.29-1.23, p value = 0.27) and oesophageal cancer (OR = 1.28, 95% CI= 0.73-2.23, p value= 0.46) has been observed.

S-40

Mutation and Expression Analysis of BRCA2 Gene in Breast Cancer Patients from India

Neema Goel[*1], Neeraj Jain[1], Suresh Hedau[1], Syed A Husain[2] and Bhudev C Das[1]*

Division of Molecular Oncology, Institute of Cytology and Preventive Oncology (ICMR), I-7, Sector 39, Noida 201 301. Department of Biosciences, Jamia Millia Islamia, Jamia Nagar, New Delhi 110 025. *neema_jalma@rediffmail.com

Introduction: Breast cancer in India is the second most common cancer in women after the cancer of the uterine cervix. The *BRCA2* gene is implicated in approximately 30 to 45% familial breast cancer. However, BRCA2 gene has not been shown to play a significant role in sporadic breast cancer. **Objectives:** To detect and characterize the BRCA2 gene mutation and the level of its protein expression in sporadic as well as familial breast cancer patients compared to normal breast tissue. **Methods:** The frequently mutated BRCA2 exons 2, 9, 11 (for 6174 delT), 18 and 20, were analyzed in tumor DNA from 105 sporadic breast cancer patients for BRCA2 gene mutation using PCR, SSCP and nucleotide sequencing. The blood DNA from 24 familial breast cancer patients of two ethnically and geographically different population of India (North India and Goa) were analyzed for BRCA2 gene mutation in exons 10A, 11A, 11K, 11L, 11Z, 14A, 14B, 15, 22 and 27A in addition to above exons. The level of BRCA2 protein expression in hundred sporadic tumors was compared to normal controls using western blotting. **Results**: Only 1 (0.9%) *BRCA2* gene mutation, in the form of missense mutation in exon 2, was detected out of 100 sporadic breast carcinoma patients. In familial breast cancer too, no significant BRCA2 gene mutation was detected except for few common population polymorphism. Also, no significant change in BRCA2 protein expression was detected since approximately 70% of the breast cancer tumors showed BRCA2 protein expression equivalent to normal controls whereas only 15% of the tumor samples showed no expression and rest exhibited low BRCA2 protein expression. **Conclusions:** The absence of any significant BRCA2 mutation or alteration in the level expression of the gene indicates that the BRCA2 gene may not be playing an important role in the sporadic breast as well as familial breast carcinogenesis in Indian women.

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LATE ABSTRACTS

LA-1
Cytokine Profile in Advanced Cancer Cervix Patients under Neoadjuvant Chemoradiation

Alpana Sharma*[1], Medha Rajappa[2], Alpana Saxena[2] and Manoj Sharma[3]

[1]Department of Biochemistry, AIIMS, New Delhi-110 029; [2]Department of Biochemistry and [3]Department of Radiotherapy, Maulana Azad Medical College and Lok Nayak Hospital, New Delhi-110 002.

Cervical cancer is the most common cancer in Indian females. Host factors are critical in regulating the tumor growth and cytokines that modulate immunological control may be of particular importance. We analyzed the production of cytokines by peripheral blood mononuclear cells (PBMCs) in women with advanced cancer cervix, before and after neoadjuvant chemoradiation. Fifty patients with advanced cancer cervix (FIGO stage IIIa-IVb on histopathology) were enrolled in this study, along with fifty healthy controls. The samples were collected as follows: Firstly, before the start of any kind of therapy (S1), Secondly, after second course of chemotherapy (S2) and Thirdly, two weeks after completion of external and brachy radiation. Single blood samples were taken from controls. The PBMCs were cultured and the culture supernatant was collected at 48 hours for IL-2 and IL-4 levels and at 72 hours for IFN-γ and IL-10 cytokine levels. The levels of all cytokines were measured by ELISA kits, from Diaclone Research, France. The levels of IL-2 and IFN-γ showed a significant decline in all cancer whereas those of IL-4 and IL-10 showed a significant elevation in all cancer patients prior to treatment, in comparison with normal controls. After chemotherapy, a mild increase in IFN-γ and IL-2 levels was observed ($p<0.05$), which became highly significant after chemoradiation ($p<0.01$). After chemotherapy, a slight decline in IL-4 and IL-10 levels was seen (statistically not significant) and even after chemoradiation, this decrease remained insignificant in case of IL-10. Improved immune status in patients attaining remission after chemoradiation could results from improvement in their general condition, from reduction of tumor burden or due to effects of treatment on any of the various humoral or tumor derived factors described in malignancy. The above data reinforce the role of immunotherapeutic agents like IL-2 and IFN-γ as adjuvant to chemoradiation in order to maintain or potentiate the observed immunological rebound to sustain the long term effects of chemoradiation and hence survival even in advanced cervical cancer patients.

LA-2
Buchnania Lanzan Extract Administration Increases the Life Span of Rats with Hepatocellular Carcinoma

G.D. Reddy*, Ch. V. Rao, R. Kartik, P. Pushpangadan

Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute (Council of Scientific and Industrial Research), Rana Pratap Marg, Post Box No. 436, Lucknow-226 001, Uttar Pradesh, India. *dayanandr@yahoo.com

The effect of *Buchnania lanzan* bark extract administration after induction of hepatocellular carcinoma (H.C.C) by N-nitrasodiethylamine (NDEA) was studied in wistar rats. Administration of ethanolic extract of *B. lanzan* was found to significantly increase the survival of H.C.C. harbouring animals. All untreated rats died of tumor burden by 37.4 ± 1.9 weeks. Administration of *B. lanzan* extract (200 mg/kg b.w) after tumor development increased the survival of animals to an average of 52 ± 2.5 weeks. Serum gama glutamyl transpeptidase activity which was elevated to 185 ± 20 u/l by NDEA administration was lowered to 110 ± 19

u/l by the administration of *B. lanzan* extract. Similarly elevated glutathione S- transferase activity (1445 ± 113 nmol/ min per mg protein) and glutathione (24.3 ± 2.0 nmol/mg protein) levels in the NDEA administered group were found to be lowered to 1001 ± 80 nmol/min/protein and 12.5 ± 2.5nmol/mgprotein respectively. *B. lanzan*.

LA-3

Detection of Human Papillomavirus (HPV) Types 16 and 18 by In Situ Hybridization in Precancerous and Cancerous Lesions of Cervix

Smita Singh**, Malik Ankur, M Choudhury

Department of Pathology Lady Harding Medical College & Smt. Sucheta Kriplani Hospital, New Delhi-110001.*pannachoudhury@hotpop.com

The present study was done to evaluate the role of high risk human papilloma virus HPV16, HPV18 in preinvasive and invasive cancer cervix. 50 cases of preinvasive and invasive cancer cervix and 10 normal cervical biopsies were studied. In situ hybridization was done for HPV type 16 and 18 on tissue sections of all cases. The overall positivity of HPV DNA was 48% (24/50).The positivity of HPV 16 DNA for low grade squamous intraepithelial lesions (LSIL), High Grade Squamous intraepithelial lesions (HSIL) and squamous cell carcinoma (SCC) were 33.3% (3/9), 45.5% (5/11), 42.3% (11/26) respectively. The positivity for HPV 18 DNA for LSIL, HSIL and SCC were 0% (0/9), 18.2% (2/11), 30.8% (8/26) respectively 50% (2/4) cases of adeno carcinoma were positive for HPV 18 DNA only. A significant correlation between HPV 18 with higher stage and grade of tumor was found (P=0.00) while no such correlation was evident between HPV 16 with tumor stage and grade. Presence of HPV 16 and 18 DNA in a large number of preinvasive and invasive cervical cancers reaffirm the role of these high risk HPV types in the pathogenesis of cervical cancers in Indian patients and association of HPV 18 with higher stage and grade of cancer cervix.

loss of E-cadherin and Nm23-H1 proteins are associated with invasive cell types. It is also noticed that the presence of HPV alone is not sufficient for malignant progression, rather additional events are also required. **Objectives:** In the present study, the expression profiles of Nm23-H1 and E-cadherin proteins in HPV positive carcinoma of uterine cervix and nasopharyngeal carcinoma was evaluated to find out the impact of HPV infection on adhesion proteins. **Methods:** The expression pattern of E-cadherin and Nm23-H1 was assessed by Immunohistochemistry and the presence of HPV was assessed by PCR. Fifty-five cases of cervical tumors, Ten benign cervical lesions, One hundred and three NPC tumors and Twenty six benign lesions of nasopharynx were collected for the study. **Results:** A significant down regulation of E-cadherin in NPC and cervical carcinoma and its histological subsets was observed ($p = 0.009$). Expression of both proteins ranged from mild to moderate cytoplasmic expression in these tissues. HPV infection was associated with a down regulation of E-cadherin and Nm23-H1 in cervix cancer lesions, while no such down regulation was noticed in NPC lesions. Advanced disease stages of both cervical carcinoma and NPC showed a marked down regulation of E-cadherin and Nm23-H1 protein. Down regulation of the Nm23-H1protein was also evident in NPC ($r = -0.616$, $p= 0.000$) and cervical carcinomas ($r= -0.366$, $p=0.006$) with relation to cervical lymph node status, which suggests the potential use of Nm23-H1 as a biologic marker for nodal metastasis in both the epithelial tumors. **Conclusion:** The results thus indicate that the role of the adhesion proteins and the interaction of HPV in human neoplasia is most likely complex and is tissue specific and that different regulatory mechanisms may act in different tumors.

Transcriptional Regulation, Signal Transduction, Cancer Genomics & Proteomics (Friday)

F-1
Modulatory Influence of *Brassica compestris* and *Mentha piperita* on DMBA Induced Skin Papillomagenesis in Swiss Albino Mice

Abha Sharma and Ashok Kumar*

Radiation and Cancer Biology Laboratory, Department of Zoology, University of Rajasthan, Jaipur-302004, India. *guds@hotmail.com

Epidemiologic studies have provided initial leads for the identification of numerous naturally occurring chemopreventive agents in the dietary components, effective against multi-stage carcinogenesis. *Mentha piperita* is shown to have antioxidant ant antiperoxidant properties. *Brassica compestris* contains indole-3-carbinol and sulforaphane (glucosinolates) which are potent stimulators of natural detoxifying enzymes in the body. The present study evaluates the modulatory influence of the ethanolic extracts of Mentha piperita and Brassica compestris on skin papillomas in female Swiss albino mice. Random-bred female Swiss albino mice (8 weeks old), weighing 24±2 gm were used. 7,12- Dimethyl benz(a) anthracene and croton oil procured from Sigma Chemicals Co, USA. Two stage protocol consisting of initiation with a single topical application of a carcinogen, DMBA (100µg/50µl of acetone) followed by thrice weekly treatment till 16 weeks with a promoter (croton oil- 1% in acetone) were employed in Swiss albino mice. The animals were divided into 3 groups (Gr I, II & III). Gr I animals were administered orally Mentha and Brassica suspension (400mg/kg body weight respectively), Gr II were given Brassica suspension (800mg/kg body weight) and Gr III were given the Mentha suspension (800mg/kg body weight) respectively at pre, peri and post initiational stages. In DMBA treated animals (control group), increased frequency of bone marrow micronuclei was accompanied by enhanced lipid

peroxidation and antioxidant depletion. Gr II and Gr III showed a greater inhibition (83%) of DMBA induced bone marrow micronuclei compared to control group while GrI showed insignificant inhibition. Lipid peroxidation was significantly reduced in GrII and Gr III compared with control group ($p<0.01$ and $p<0.01$ respectively).The levels of GSH in liver and the activities of hepatic GPx and GST were significantly increased in Gr II and Gr III as compared to the control($p<0.01$ and $p<0.001$ respectively). Gr I significantly enhanced the activity of GST compared with the control ($p<0.01$) but the lipid peroxidation was also significantly increased as evidenced by the high levels of TBA- reactive substances (TBARS). Both GPx and GST play a pivotal role in the biotransformation of carcinogens into non-reactive metabolites that can readily be excreted. Thus the present investigation is suggestive of the chemopreventive activity of *Brassica compestris* suspension and *Mentha piperita* suspension, when administered alone, in Swiss albino mice against 7, 12- dimethyl benz(a) anthracene and croton oil. The chemoprevention may be due to modulation of detoxifying enzyme systems and pharmacological properties of *Brassica compestris* and *Mentha piperita*. However, no significant chemopreventive activity was observed when *Brassica compestris* and *Mentha piperita* were used in combination. This may be due to antagonistic interaction between Brassica and Mentha resulting in the activation of carcinogenesis.

F-2

Increased Expression of Matrix Metalloproteinase-2, Matrix Metalloproteinase-9 and their Inhibitors in Invasive Retinoblastomas

Adithi Mohan*, Nalini Venkatesan, Mahesh Palanivelu Shanmugam and Subramanian Krishnakumar

Departments of Ocular Pathology and Ocular Oncology, Medical and Vision Research Foundations, Sankara Nethralaya, Chennai, India. *adithi_mohan@vsnl.net

Introduction: Matrix metalloproteinases (MMPs) are known to play a central role in the cell migration during cancer metastasis. Tissue inhibitors of metalloproteinases (TIMPs) regulate MMP activity by controlling the breakdown of extracellular matrix components. **Objectives:** To study the expression of MMP-2, MMP-9, TIMP-1 and TIMP-2 in retinoblastoma. **Methods:** Sixty tumors and a human retinoblastoma cell line (Y79) were evaluated by immunohistochemistry for MMPs and TIMPs. Growth fraction of the tumors was studied by Ki-67 labeling index. Statistical analysis done using non-parametric testing. **Results:** Among the 30 non-invasive tumors, MMP-2 and MMP-9 negative in 24 tumors each. TIMP-1 and TIMP-2 negative in 28 and 19 tumors respectively. Among the 30 invasive tumors, MMP-2 was positive in 23 tumors, MMP-9 in 19 tumors, TIMP-1 in 23 and TIMP-2 in 21 tumors. Ki-67 > 50% was seen in 26 invasive tumors as compared to 17 non-invasive tumors. The Y79 cell line was also seen to express the four markers. There was statistically significant increase in the expressions of both MMPs and TIMPs ($P<0.0001$) in the invasive tumors as compared to the non-invasive tumors. There were moderate correlations between the expressions of Ki-67 and TIMP-1 (?= 0.355; $P< 0.01$), Ki-67 and MMP2 (?= 0.306; $P<0.01$) and between TIMP-1 and MMP-9 (?= 0.314; $P<0.028$). **Conclusion:** The expressions of MMP-2, MMP-9, TIMP-1 and TIMP-2 was higher in the invasive tumors. Both MMPs and TIMPs have a key role in extracellular matrix invasion in retinoblastoma, largely through their elaboration by tumor cells.

F-3

Therapeutic efficacy of Pomegranate (*Punica granatum* Linn.)

Ajaikumar KB*, Fijesh PV, Simona PS and Jose Padikkala

Amala Cancer Research Centre, Thrissur - 680 555, Kerala, India. *amalaresearch@rediffmail.com

Introduction: *Punica granatum* Linn. (Punicaceae) commonly called as 'Pomegranate' is a large deciduous shrub or small tree used medicinally in Europe, IndoChina, South Africa etc for the treatment of various diseases such as ulcer, hepatic damage, ascites, snakebite etc. Due to this high traditional medicinal use we selected this plant for the present study. **Objective:** To study the pharmacological activities of Pomegranate fruit rind and fruit juice. **Methods:** The present study evaluated the antioxidant, antiinflammatory (Carageenan, Dextran and Formalin induced), Hepatoprotective (Paracetamol and CCl4), Antiulcer (Ethanol and Aspirin induced), Antitumor (DLA induced solid tumor and EAC induced Ascites tumor), Radioprotective and Chemoprotective activities of fruit juice and 70% methanolic extract of fruit rind, separately. **Results:** The present study reports for the first time the antioxidant, anti-inflammatory, antiulcer, hepatoprotective, radioprotective, chemoprotective and antitumour activities of *P. granatum* fruit rind and fruit juice. The fruit juice and 70% methanolic extract of fruit rind showed potent in vitro superoxide, nitric oxide and hydroxyl radical scavenging activities and anti-inflammatory activities against carageenan, formalin and dextran induced inflammatory models in Swiss albino mice. The administration of fruit juice (2.5ml/kg b. wt and 5.0ml/kg b. wt) and the methanolic extract of fruit rind (250mg/kg b. wt and 500mg/kg b. wt) in rats significantly inhibited the chemically induced gastric and hepatic damage in a dose dependent manner. The administration of fruit juice (20ml/kg b. wt and 40ml/kg b. wt) and the methanolic extract (50mg/kg b. wt and 100mg/kg b. wt) inhibited solid tumor and ascites tumor in Swiss albino mice. Fruit juice and methanolic extract of fruit rind exhibited significant radioprotective and chemoprotective activities. The toxicity studies revealed that both fruit juice and fruit rind are non-toxic. The phytochemical analysis of the fruit juice and fruit rind showed the presence of alkaloids and anthocyanidines. **Conclusion:** In conclusion, the present study revealed the protective effect of Pomegranate fruit against various diseases.

F-4

Expression and Cellular Localization of NF- B Superfamily Members in Oral Carcinogenesis

Alok Mishra*[1], Alok C. Bharti[1], Daman Saluja[2], B.C. Das[1]

[1]Division of Molecular Oncology, Institute of Cytology and Preventive Oncology, I-7 Sector-39, NOIDA, UP-201301. [2]Laboratory of Medical Biotechnology, Ambedkar Centre for Biomedical Research, Delhi University, Delhi-110 007. INDIA. *alok_hpv@rediffmail.com

Introduction: Oral cancer is one of the most common cancers in India and South East Asian region consisting up to 50% of all malignant tumors. HPV infection, alcohol and tobacco abuse are the major etiological factors for oral carcinogenesis. HPV and tobacco carcinogens are known to modulate the expression and activation of transcription factors such as NF-kB and AP-1. However, their role in the process of oral carcinogenesis is yet to be confirmed. **Objectives:** To know the transactivation and expression pattern of NF-kB superfamily members in different stages of oral precancer and cancer. **Methods:** Fifty fresh oral tissue biopsies were collected which included 3 normal controls, 16 precancerous and 31 cancerous cases prior to chemotherapy / radiotherapy from the collaborating hospitals and stored at -70^{o}C. HPV diagnosis of samples was done by consensus and type-specific PCR primers. The transactivation status of NF- B in oral biopsies was determined by Electrophoretic Mobility Shift Assay. Western blots and Immunohistochemical analysis were performed to know the expression levels and localization of different NF-kB proteins components. **Results:** Fifteen percent of malignant oral biopsies have shown presence of HPV primarily of high risk type 16 and 18. Oral cancer biopsies have shown constitutive activation of NF-kB with preferential homodimerisation of NF-kB p50 subunits. However, the homodimerization pattern of p50/p50 was found to be independent of HPV infection in oral squamous cell carcinomas. At

protein level, both by western blotting and immunohistochemistry an over-expression of p50 as well as c-Rel was observed in cancer tissues while the p65 level remained similar in normal oral and in different grades of oral lesions including cancer. **Conclusion:** NF-kB p50 and c-Rel may play an important role in the constitutive activation of NF-kB during oral carcinogenesis.

F-5

Noscapine and Polycystic Ovary Syndrome: Towards a New Development

***Anjali Priyadarshani*[1]*, *Anju Katyal*[1], *Aseem Bhatnagar*[2], *and Ramesh Chandra* [1]**

[1]Dr.B.R.Ambedkar Center for Biomedical Research, University of Delhi, Delhi 110007, India. [2]Institue of Nuclear Medicine and Allied Sciences, DRDO, Delhi 1100054, India. *anjalinath78@yahoo.com

Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies in women of reproductive age. PCOS accounts for 75% of women with anovulatory infertility. The late consequences, such as risk of endometrial cancer, cardiovascular disease, and infertility warrant an early and effective diagnosis of the syndrome. The underlying cause of PCOS is an abnormality of ovarian androgen, which is the common final manifestation of the failure of Hypothalamus-Pituitary-Ovarian axis. We present the development of a rat model of PCOS using anti progesterone RU486 and evaluate the efficacy of noscapine for its treatment. RU486 administration induced ovulation blockade, persistent vaginal cornification, uterine ballooning and alterations in serum levels of LH, FSH, progesterone, estradiol and testosterone mimicking a classical PCOS in women. Administration of noscapine corrected these derangements at dose of 120 mg/Kg of body weight. Moreover the serum level of LH, FSH, testosterone, estradiol and progesterone reverted to the basal level on noscapine administration. Ovary showed follicles in different stages of development with no cystic manifestation. It also restored the reproductive potential when compared with the PCO induced rat. Radiolabelling, Scintigraphic and SPECT studies showed prominent localization of ^{99m}Tc-Noscapine in rabbit's ovary, implicating ovary as target organ in its corrective effect. Protein profiling studies indicated restoration of overexpressed proteins to normal levels in noscapine administered rat ovaries as compared to induced polycystic ovaries. These results represent the first identification of noscapine as a participant in the correction of polycystic ovary disease (PCO). Thus the correction of PCO by administration of Noscapine is a novel and significant observation, and opens new avenues in PCO treatment.

F-6

Silibinin Inhibits TNF -Induced NF- B Activation via IKK -I B Pathway in Human Colon Carcinoma Cells: Possible Implications in Colorectal Cancer Prevention and Intervention

Chapla Agarwal*, *Sivanandhan Dhanalakshmi and Rajesh Agarwal

Department of Pharmaceutical Sciences, School of Pharmacy, University of Colorado Health Sciences Center, Denver, CO 80262, USA. * chapla.agarwal@uchsc.edu

Colorectal cancer is the second most common invasive malignancy and cause of cancer-related deaths in the United States; however, there have been limited successes so far in its prevention and intervention. In this regard, transcription factor NF-kB has been implicated in the growth and survival of several human malignancies including colorectal cancer, suggesting that the agents that inhibit NF-kB activation could be effective in colorectal cancer therapy. Employing various human colorectal carcinoma cell lines, here we assessed the effect of

silibinin, the major active constituent in a widely consumed hepatoprotective dietary supplement milk thistle extract, on TNF alpha-induced NF-kB activation and upstream effectors in this pathway. Colorectal carcinoma cell lines were treated with silibinin followed by TNF alpha, and nuclear extracts were analyzed for NF-kB DNA binding activity by gel shift assay. Western immunoblotting was done for protein levels of p65, p50, and both phospho- and total IkB alpha in nuclear or cytosolic fraction, and in-beads kinase assay was done for IKK alpha activity.Treatment of HT29, LoVo and SW40 human colorectal carcinoma cells with silibinin resulted in a strong inhibition of TNF alpha-induced NF-kB activation, in a dose-dependent manner. Consistent with this, nuclear levels of p65 and p50 sub-units of NF-kB were also reduced concomitant with an increase in their cytosolic levels, following silibinin treatment of the cells. Additional studies showed that silibinin pre-treatment was more effective in inhibiting TNF alpha-caused NF-kB activation as compared to its post-treatment. In the studies assessing upstream molecular mechanism of this effect, silibinin treatment resulted in a significant increase in the level of I B with a concomitant decrease in phospho-IkB alpha. Kinase assays revealed that silibinin inhibits IKK alpha kinase activity, suggesting this to be a molecular target of silibinin effect in inhibiting NF-kB activation. Together, these results indicate that silibinin could be a useful agent for colorectal cancer prevention and intervention by inhibiting NF-kB-mediated cell survival signaling.

F-7

Black Tea Extract As Effective as Green Tea Polyphenols, in Prevention of BP Induced Lung Carcinogenesis

Prosenjit Saha, Chaiti Ganguly*, Sarmistha Banerjee, Sugata Manna, Chinmoy Panda, Sukta Das

Chttaranjan National Cancer Institute, Kolkata, India. *chaitig@yahoo.com

Introduction: Lung cancer is one of the leading causes of cancer deaths worldwide and the number of cases continues to increase. Green Tea, rich in antioxidant flavanols is considered to have chemopreventive and anticarcinogenic properties but efficacy of black tea and their compounds is yet to be fully explored. **Objectives:** The present study was designed to evaluate the effect of black tea extract (Theaflavins) in comperison to the active green tea compound, on Benzo(a)pyrene (BP) induced lung carcinogenesis in strain A mice. **Methods:** Treatment was initiated at the post initiation phase. TF was administered by ip injection and the same amounts of green tea polyphenol EGCG were used as positive control. Identification of apoptotic and proliferative cells was carried out by TUNEL method and BrdU incorporation. Expression of Caspase-3 and COX-II was done by Western blotting and expression of Bcl-2 and C-Myc protein localized by immunohistochemistry. **Results:** Immunohistochemical localization in the precancerous lung lesions have shown a reduction in proliferating epithelial cells and increased number of apoptotic cells in the bronchiole region. This is also reflected in the downeregulation of Cox-II, the molecular markers associated with proliferation and upregulation of Caspase-3. Suppression of Bcl-2 and C-Myc proteins expression were observed after treatment, which accounts for a significant reduction in incidences and delayed onset of hyperplasia, dysplasia and carcinoma *in situ* in BP treated lung. **Conclusion:** Treatments with black tea extract down regulate the expression of the marker proteins studied, which is as effective as green tea compounds. These findings were also supported by expression of the same proteins on NCI-H460 a human non-small cell lung cancer cell line.

F-8

Effect of Melatonin at Different Time Points on DNA Synthesis of HepG2 Cells in Culture: An Implication for Cancer Chronotherapy

Dakshayani K. Babu*, Rao K.V.K., Yuvraj N.[1] and Subramanian P.
Department of Biochemistry, Annamalai University, Annamalainagar - 608 002, Tamil Nadu. India. *Chemical Carcinogenesis Group, Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Tata Memorial Centre, Kharghar, Navi Mumbai - 410 208, India. *kb_dakshayani@yahoo.com

Circadian rhythms are known to be exhibited by all peripheral tissues and mammalian cells in culture. Recently chronotherapy of cancer has attracted attention, as the efficacy of most drugs including antineoplastics varies according to the time of administration. Melatonin, a pineal gland hormone holds the unique position of being the only known chronobiotic regulator of neoplastic cell growth. Accordingly, we have investigated the effect of melatonin on DNA synthesis in HepG2 cells for 24 h period. HepG2 cells were treated with 8 mM melatonin and [^{3}H] thymidine (0.1 mCi/ml) was added at 6 time points for 24 h period (00:00; 04:00; 08:00; 12:00; 16:00; 20:00; 24:00). DMSO treated HepG2 cells served as controls. DNA synthesis was measured by the method of Yusof and Edwards (Carcinogenesis, 11, 761, 1990). DNA synthesis in HepG2 cell lines varied over 24 h period. Maximum DNA synthesis in HepG2 control was at 23:31 h. Melatonin inhibited the DNA synthesis maximally at this time point (0:59 h). Our results support the fact that cancer cells are more susceptible to anticancer agents when they are exposed at a time point when DNA synthesis is maximum. This study would form the basis for cancer chronotherapy, which aims in drug administration at appropriate timing according to the circadian rhythms of cancer cell susceptibility which will avoid adverse effects of chemotherapy.

F-9

TGFb Estimation: Impact of Platelet Derived Fraction on Prognostication of Breast Carcinoma

Trivedi Sunil[1], Dave Heena[1], Trivedi Trupti[1], Shah Rohini[1], Shah Manoj[2], Shukla Shilin[3]

[1]Receptor & Growth Factor Laboratory, [2]Pathology Department, [3]Department of Medical Oncology, The Gujarat Cancer & Research Institute, Asarwa, Ahmedabad 380 016, India. *drsuniltrivedi@hotmail.com

Cellular growth & differentiation, an orderly process is controlled by a number of physiological networks including growth factors - the most widely studied. TGFb is a stable, multifunctional cytokine axis shows differential expression during breast tumorigenesis. High TGFbs is present in platelets which get released on degranulation. Most of circulating TGFbs is thus platelet derived and results into false elevation. Estimation of 'tumor derived' TGFbs thus is crucial in their evaluation as prognosticators in human breast carcinoma. An attempt here evaluates the correction of platelet derived TGFbs by 'normalization' with PF-4 (a known marker of platelet degranulation) in a series of 107 previously untreated breast cancer patients by comparison with clinico-pathologic criteria including survival.

F-10

Differential Expression of Sperm Protein 17 in Human Esophageal Squamous Cell Carcinoma

Garima Gupta*[1], Rinu Sharma[1], T.K.Chattopadhyay[2], S.Dattagupta[3], Ranju Ralhan[1]

Department of [1]Biochemistry, [2]G.I.Surgery, All India Institute of Medical Sciences, Ansari Nagar, New Delhi-110029, India. *garimagupta_aiims@yahoo.co.in

Introduction: Carcinoma of the esophagus is the eighth most common cancer worldwide and the fifth leading cause of cancer death in the world with a low five-year survival rate. Identification of genes that are

differentially expressed in tumor cells is important for understanding the molecular basis of cancer. Gene expression profiling of esophageal cancer using differential display led to the identification of an over-expressed cDNA fragment encoding Sperm Protein 17(Sp17). Sp17 is an antigenic protein highly expressed in spermatozoa whose known function is to bind sperm to zona pellucida. Recently, however, Sp17 expression was demonstrated in multiple myeloma and ovarian cancer, suggesting that it may be a novel cancer-testis antigen. Aim: The aim of the study was to analyze the expression of Sp17 in human esophageal squamous cell carcinoma (ESCC) and normal tissues and to determine the levels of circulating anti-Sp17 antibodies in ESCC patient sera in comparison to healthy subjects. **Methods and Results:** Sp17 transcript was detected in ESCCs but no expression was detected in normal tissues. To evaluate protein expression and localization immunohistochemical studies were done using mouse monoclonal antibodies. ESCCs showed positive nuclear and cytoplasmic staining while no expression was detected in normal tissues. Since Sp17 is immunogenic, the aberrant expression of this cancer testis antigen in esophageal tissues may lead to generation of antibodies. The expression levels of circulating anti-Sp17 antibodies were determined in sera collected from esophageal cancer patients and healthy controls using Enzyme Linked Immunosorbent Assay (ELISA). High levels of circulating antibodies were observed in ESCC patient's sera as compared to healthy subjects. **Conclusion:** Sp17, a highly immunogenic protein *in-vivo* might serve as a candidate molecular marker for diagnosis of ESCC, however further investigation of its role in esophageal tumorigenesis is warranted.

F-11

Clinical Usefulness of Alterations in Sialic Acid, Sialyltransferase and Sialoproteins in Breast Cancer

Gaurav H. Pancholi**, Jayendra B. Patel, Amisha A. Desai, Pankaj M. Shah, Prabhudas S. Patel

Biochemistry Research Division, The Gujarat Cancer & Research Institute, Ahmedabad - 380 016 Gujarat, India. *hellogp2002@yahoo.co.in

Sialic acid, the end moieties of the carbohydrate chains are biologically important and essential for functions of glycoconjugates and are reported to be altered in cancer patients. Two hundred and twenty five breast cancer (BC) patients, 100 patients with benign breast disease (BBD) and 100 healthy females (controls) were enrolled for the study. Eight hundred and twenty four follow-up samples of 225 breast carcinoma patients were also evaluated. The association of sialic acid forms, sialyltransferase and a-2-6 sialoprotein levels with presence and extent as well as prognosis of breast carcinoma was studied. Serum sialic acid forms and sialyltransferase revealed significantly elevated levels among untreated breast cancer patients as compared to the controls, patients with BBD as well as cancer patients in remission. Non-responders showed comparable levels of the markers with those found in breast cancer patients at the time of diagnosis. Higher levels of sialic acid forms at diagnosis were associated with poor prognosis. A positive correlation between serum levels of different forms of sialic acids and extent of malignant disease was observed. The changes in serum proteins with terminal a-2-6 sialic acid correlated well with alterations in the levels of sialic acid forms and sialyltransferase. Malignant tissues showed elevated levels of sialic acid and sialyltransferase as compared to surrounding normal tissues. The results suggested potential utility of these markers in evaluation of clinical outcome.

F-12

Effect of Naturally Occurring Sulphur Compounds and Isothiocyanate on the Cell Mediated Immune Responses of Metastatic Tumour Bearing Animals

Manesh Chittezhath and Girija Kuttan*

Dept. Immunology, Amala Cancer Research Centre, Amala Nagar, Thrissur, 680555, Kerala.*Dept.Pharmaceutical Sciences, School of Pharmacy, UCHSC, Denver, CO 80262. *amalaresearch@rediffmail.com

Introduction: Although various immune responses can be generated to tumour cells, the response frequently is not sufficient to prevent tumour growth. One approach to cancer treatment is to augment or supplement these natural defense mechanisms. Modulation or activation of immune system is important in cancer therapy. **Objectives:** The objectives of the present study was to analyze the immunostimulatory activities of naturally occurring organosulphur compounds such as DAS and DADS in metastatic tumour bearing C57BL/6 mice. **Methods:** Effect of DADS and DAS on NK cell activity, Antibody-dependent cellular cytotoxicity (ADCC), Antibody dependent complement mediated cytotoxicity (ACC) and their effect on anti and pro-inflammatory cytokines such as IL-2, TNF-a, Il-1b, IL-6 and GM-CSF were evaluated in metastatic tumour bearing C57BL/6 mice. **Results:** NK cell mediated cytolysis was enhanced maximally by the treatment of DAS (47%) and DADS (65.4%) on 4th day after tumour inoculation. Maximum NK cell activity in control tumour bearing animals was observed only on 15th day after tumour inoculation. Antibody-dependent cellular cytotoxicity (ADCC) was also enhanced by the treatment of DADS (50.3%) and DAS (34.3%), compared to metastatic tumour bearing control animals (21%). Antibody dependent complement mediated cytotoxicity was significantly increased by the treatment of DADS (31.3%). Treatment with DADS resulted in a significant upregulation of the serum concentration of IL-2 (47.1 pg/ml) compared to control animals (6.69 pg/ml). The serum concentration of pro-inflammatory cytokines such as TNF-a, Il-1b, IL-6 and GM-CSF, which were highly elevated in control animals were effectively reduced by the treatment of DADS. **Conclusion:** The above study revealed immunostimulatory effects of DADS and DAS on metastatic tumour bearing animals, and these activities may be due to the stimulation of cell mediated immune responses.

F-13

Effect of Biophytum sensitivum on B16F-10 Melanoma Cells Induced Metastasis

Guruvayoorappan.C* and Girija Kuttan

Dept. Immunology, Amala Cancer Research Centre, Amala Nagar, Thrissur, 680555, Kerala, India. *amalaresearch@rediffmail.com

Introduction: Metastasis remains to be the major cause of death in cancer patients. Although several drugs have been recommended for cancer therapy there are no drugs presently available which can specifically arrest the metastatic process. Plant drugs are frequently considered to be less toxic and more free from side effects than synthetic ones. The study of such medicines might offer a way to find novel medicines in oncology. **Objectives:** To evaluate the antimetastatic activity of *Biophytum sensitivum* on B16F-10 induced metastatic model in C57BL/6 mice. **Methods:** Metastasis was induced by injecting B16F-10 melanoma cells via, the lateral tail vein of animals. Biophytum methanolic extract was administered daily at a dose of 500 µg/dose/animal (10 doses). After 21 days animals were sacrificed and the number of tumor nodules were counted in the treated and untreated animals. Biochemical markers of metastasis such as lung hydroxyproline, uronic acid, serum sialic acid and gammaglutamyl transpeptidase were estimated. **Results:** Simultaneous administration of the extract produced a significant reduction (95%) in tumor nodule formation. Increased lung collagen hydroxyproline (23.71 µg/mg protein) and uronic acid (329.2 µg/100 mg tissue) content in the lungs of control

animals was significantly reduced in Biophytum treated animals. Similarly elevated serum sialic acid (118µg/ml) and GGT activity (117n mol p-nitroaniline/ml) in control animals was significantly reduced in animals treated with the extract. The lifespan of the Biophytum treated animals was also seen to be significantly increased. **Conclusion:** The observed results show that *Biophytum sensitivum* could inhibit metastatic growth of B16F-10 melanoma cells in the mice model.

F-14
Immunological Studies in Juvenile Laryngeal Papillomatosis

[1]H. Ramasubramaniyam*, [2]Bhudev C. Das, [3]R.G. Sukumar and [3]S. Pushkala
[1]Professor of ENT, Melmaruvathur Adiparashakthi Hospital and Research Institute Kancheepuram, [2]Division of Molecular Oncology, Institute of Cytology and Preventive Oncology, Noida, [3]Professor, Department Of Immunology, Dr MGR Medical University, Chennai, India. *pushvel@yahoo.com

Juvenile Laryngeal papillomatosis is a relative common benign tumour of the childhood causing life threatening complications. Laryngeal papillmatosis is reported to be associated with the infection human papilloma virus (HPV) types 6 and 11. The preliminary work was done during the period 1995 to 1999. A study of 75 children suffering from juvenile laryngeal papilloma, both primary and with repeated recurrences were managed and treated in Government Children Hospital Egmore, Chennai. Samples were typed and found to be HPV type 6 and 11. No detailed study has been undertaken in India as to the typing of HPVs and biology of the viruses and their correlation with the aggressiveness of the disease. Immunological mechanisms, which effect the course of laryngeal papillomamatosis not well, understood. To elucidate the role of immunogenetic risk factors investigations can be done regarding Human Leucocyte Antigen (HLA) associations of juvenile laryngeal papilloma patients by Polymerase Chain Reaction (PCR) based methods. So it is proposed to study: 1. The types of HPV by PCR, 2. Correlation between types of HPV and their aggressiveness, 3. Cellular and humoral immune response, 4. HLA associations.

F-15
Radioprotective and Anticlastogenic Activity of Phyllanthus amarus

Hari Kumar K. B* and Ramadasan Kuttan

Amala Cancer Research Centre, Amala Nagar, Thrissur, 680555, Kerala, India.
*amalaresearch@rediffmail.com

Introduction: Drugs that are capable of protecting normal host tissues from the lethal effects of radiation without compromising its tumor reducing activity is of considerable interest in radiation medicine. Radiation also produces clastogenic changes, which can cause genotoxicity to normal cells. **Objectives:** To evaluate the radioprotective and anticlastogenic activity of an extract of the plant *Phyllanthus amarus* (*P. amarus*) in BALB/c mice. **Methods:** *P. amarus* extract (250 & 750 mg/Kg B.wt) was administered orally to mice continuously for 5 days prior to whole body radiation (6Gy) and for one month after irradiation. The animals were sacrificed at different time points and various hematological parameters such as total WBC count, bone marrow cellularity and a-esterase levels were analyzed. Antioxidant parameters viz. Catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione-S-transferase (GST), lipid peroxidation (LPO) and reduced glutathione (GSH) were assayed in blood and the liver. For anticlastogenic study BALB/c mice

were treated with *P. amarus* five days before radiation and irradiated at dose of 1.5Gy for micronuclei study and 3Gy for chromosomal aberration study. **Results:** Irradiation decreased the levels total WBC, bone marrow cellularity, a-esterase, CAT, SOD, GPX, GSH and increased the LPO levels and GST. Administration of *P. amarus* was found to enhance the decreased levels of antioxidant enzymes and GSH and decreased the elevated lipid peroxidation levels. *P. amarus* treatment reduced the number of micronucleated polychromatic and normochromatic erythrocytes and decreased frequency of chromosomal gaps, breaks and other aberrations occurred in mouse chromosomes as a result of radiation. **Conclusion:** The results indicated the radioprotective as well as anticlastogenic activity of P. amarus.

F-16

Immunomodulatory and Anticancer Effects of *Pleurotus ostreatus* Mycelia Derived Proteoglycans

Itisam Sarangi**, Dipanjan Ghosh, Sujit K. Bhutia and T. K. Maiti

Department of Biotechnology, Indian Institute of Technology, Kharagpur -721302, India.
*tkmaiti@hijli.iitkgp.ernet.in

Introduction: Both edible and medicinal Basidiomycetes have been evaluated for their nutritional and pharmacological properties. Many mushroom polysaccharides are reported as immunomodulatory and antitumour agents. Owing to the structural variability and diversity, these mushroom polysaccharides are capable of modulating immune system. *Pleurotus ostreatus* is one of the widely cultivated edible mushrooms and is well studied for its anticancer properties. **Objective:** Study of immunomodulatory and anticancer activities of neutral mushroom proteoglycans isolated from cultured mycelia of *Pleurotus ostreatus*. **Methods:** Mycelia of Pleurotus ostreatus were cultured in the conical flasks. Water-soluble fraction of alcohol-precipitated mycelia was passed through anion and cation exchange columns followed by gel filtration (sephadex G-100) column. Three neutral fractions were found and they were tested for in vitro and in vivo immunomodulatory and anticancer effects in mice model. **Results:** All the three proteoglycans stimulated mice thymocytes and splenocytes proliferation and elevated NK cell cytotoxicity. In vitro killing of S-180 and Dalton cells were also observed in presence of three fractions. Mushroom mycelia proteoglycans stimulated macrophage to produce NO. In vivo injection of proteoglycans to S-180 bearing mice decreased the number of cells, fluid volume and packed volume of ascites. **Conclusion:** Thus, the three neutral proteoglycans derived from the mushroom (*Pleurotus ostreatus*) mycelia could be used as immunomodulators and anticancer agents.

F-17

Status of DNA methylation in liver and kidneys of rats during different stages of post natal development and as affected by 4-dimethylaminoazobenzene (4-DAB) administration

Jaspreet Kaur*

Department of Biochemistry, Panjab University, Chandigarh, India, 160014. *jaspreet_virdi@yahoo.com

DNA methylation is involved in regulation of gene expression and differentiation in a cell but its role in early stages of dedifferentiation and initiation of carcinogenesis is lesser understood. To study DNA methylation in preneoplastic lesions, 3 different age groups of rats were taken (2,14 and 90 days old) and 4-DAB (also called butter- yellow; a hepatocarcinogen) was administered for 7 days at a dose of

108ƒÝg /g body wt. DNA isolated from liver and kidneys of control and 4-DAB administrated rats was hydrolysed by the method which does not affect 5- methylcytosine content (Diala and Hoffman, 1982), and was quantitated by HPLC. There is age related demethylation of DNA in both the organs of control rats. However, there was no significant change in total DNA content /g tissue with age or 4 - DAB administration. Carcinogen administeration was found to increase the percent 5- methyl cytosine content in livers of 9 and 21 days old rats whereas there was no change in 90 days old rats. No significant change was found in kidneys in any age group rats upon carcinogen administration. Results indicate that differential gene expression during post natal development might be influencing the initiation phase of carcinogenesis in its target organ liver. Also the methyl moieties of 4 - DAB might play some role in causing methylation in liver.

F-18

Antiangiogenic Activity of *Boerhaavia diffusa In Vitro* and in Animals

Leyon Varghese and Girija Kuttan***

Dept. Immunology, Amala Cancer Research Centre, Amala Nagar, Thrissur, 680555, Kerala, India. *Dept.Pharmaceutical Sciences, School of Pharmacy, UCHSC, Denver, CO 80262. *amalaresearch@rediffmail.com

Introduction: Angiogenesis is a key early event in tumor progression and metastasis. Angiogenesis and inflammation is well associated and inflammatory modulators are known to affect the process of tumour angiogenesis. Several studies have shown that many of the dietary/medicinal phytochemicals possess strong Anti-inflammatory and antiangiogenic activity thereby inhibiting tumour progression. **Objectives:** The aqueous-methanol extract of *Boerhaavia diffusa* was used to study its antiangiogenic activity by affecting the inflammatory modulators using in vivo as well as in vitro models. **Methods:** B16F10 melanoma cell were used to induce neo vessel formation on the ventral side of animals. Serum cytokine level was evaluated in both the control and extract treated (20mg/Kg) the angiogenesis induced animals by using respective ELISA kits. For *in vitro* studies rat aortic ring assay was performed in the presence or absence of extract. **Results:** Administration of extract (20mg/Kg) significantly inhibited the tumour directed capillary formation. Analysis of the serum cytokine profile showed a drastic increase of proinflammatory cytokines such as IL-1b, IL-6, TNF-a, GM-CSF and the endothelial proliferating agent VEGF in the angiogenesis induced control animals, which were significantly reduced by *Boerhaavia* treatment. Rat aortic ring assay revealed that extract inhibited the production of proangiogenic factors from B16F10 melanoma cells as the conditioned medium from the treated cells showed reduced capillary outgrowth. **Conclusion:** *Boerhaavia* treatment to the animals showed a strong antiangiogenic activity. Since the treatment regulated the level of proinflammatory and pro/anti angiogenic cytokines in the serum, the observed antiangiogenic activity is attributed, at least in part, to the regulation of the levels of these cytokines and growth factors in the circulatory fluids of the angiogenesis induced animal. Moreover the mRNA expression studies in the same cell line showed a reduced level of expression by *Boerhaavia* treatment further confirmed this interpretation.

F-19

Anti-Inflammatory and Antitumor Activities of Water Soluble Polysaccharides Isolated from a Macrofungus, *Phellinus rimosus* (Berk) Pilat

Meera C Radhakrishnan and Janardhanan KK***

Amala Cancer Research Centre, Amala Nagar, Thrissur, 680555, Kerala, India. *amalaresearch@rediffmail.com

Introduction: Inflammation, a fundamental protective response, can be one of the factors to accelerate the development of cancer. Epidemiological studies have shown that chronic intake of non-steroidal anti-inflammatory drugs (NSAID) reduces the incidence of colon, prostate, lung and breast cancers. Mushrooms are used in traditional Chinese medicine to treat several disease conditions. *Phellinus rimosus* is a wood rotting macro fungus, found growing on jackfruit tree trunks in Kerala. Recent investigations in our laboratory have shown that extracts of the basidiocarps of this mushroom possess significant biological activities. The major constituent of the extract is polysaccharide. **Objective:** To isolate the water-soluble polysaccharide from the basidiocarps of *P. rimosus* and to evaluate its anti-inflammatory and antitumour activities. **Methods:** The water-soluble polysaccharide from basidiocarps of *Phellinus rimosus* was isolated by the method described by Mizuno et al (2000). The anti-inflammatory activity of the polysaccharide was evaluated using carrageenan induced acute and formalin induced chronic inflammatory models in mice. Antitumor activity of the polysaccharide was determined by solid tumor model in mice using Daltons Lymphoma Ascites cells (DLA). **Results:** The water-soluble polysaccharide isolated from *P. rimosus* showed significant anti-inflammatory and antitumour activities in a dose dependent manner. The polysaccharide showed 72.94% and 61.9% inhibition in acute and chronic inflammation respectively at a concentration of 10 mg/kg body weight when administered orally. Tumor volume was reduced by 97.7% and tumor weight by 82.2% when the polysaccharide was administered orally at a concentration of 10mg/kg body weight. **Conclusion:** The results of the present investigation reveal that the water-soluble polysaccharide isolated from the basidiocarps of Phellinus rimosus possessed profound antitumor and anti-inflammatory properties.

F-20

Transcription Factors Snail and Slug Downregulates Junction Components and Enhances Invasiveness of Epithelial Ovarian Cancer

Nawneet K Kurrey*, Amit Kunwar, S.A. Bapat

National Centre For Cell Science,Ganeshkhind, Pune, India. *nawneetkurrey@yahoo.co.in

Introduction: Epithelial Mesenchymal Transition (EMT) is vital for cell migration during development and is also implicated in tumor metastasis. During EMT, epithelial cells lose their polarity and cell-cell adhesion and acquire motile mesenchymal characteristics that enhances tumor invasiveness and metastasis. The Zinc finger transcription factors Snail and Slug have been reported to be involved in EMT by repressing different junction components. Here we hypothesized that pathological version of EMT is responsible for invasiveness of ovarian cancer, which is fascilated by Snail and Slug. **Objective:** 1. To examine the roles of transcription factors Snail and Slug in ovarian cancer. 2. To dissect out the individual roles of Snail and Slug during tumor progression. **Methods:** To prove our hypothesis, in vitro as well as *in vivo* experiments were conducted. For *in vitro* experiments, ovarian cancer cell line SKOV3 was taken as a model system. Stable and transient clones were isolated after transfection with an mSnail and mSlug. In selected clones, expression of junctions like adherens, tight and desmosome by RT-PCR and Western Blotting were also monitored. Similarly in the clones invasiveness and clonogenecity were examined by Matrigel assay and Soft agar assay. Tumorogenecity was confirmed in nude mice. **Results:** The ectopic expression of Snail or Slug resulted in EMT, enhanced motility, invasiveness and tumorogenecity in the cell line SKOV3.Snail suppress expression of adherent and tight junction components, while Slug suppresses expression of all three junction components viz adherens, tight and desmosome. The *in vivo* and *in vitro* experiment show increased invasiveness, clonogenecity and tumorogenecity property in clones. **Conclusion:** These results indicate that the Snail and Slug are involved in invasiveness of ovarian cancer. They may be putative targets in treatment of ovarian cancer.

F-21

Antioxidant, Anti-Inflammatory and Antitumor Activities of Cultured Mycelia of Morel Mushroom, *Morchella esculenta*

Nitha B* and Janardhanan KK

Amala Cancer Research Centre, Amala Nagar, Thrissur, 680555, Kerala *amalaresearch@rediffmail .com

Introduction: Molecular oxygen, while providing efficient energy production from ingested food, results in the free radical and peroxide by-products, which cause high intrinsic toxicity. Damage to DNA by oxygen free radicals is frequently postulated to cause initiation and progression of cancer. Chronic inflammatory diseases may promote the progression of neoplastic process. *Morchella esculenta* is an economically important excellently edible mushroom found growing in the Western Himalayan region. It is reported to be used in medicine and health care system by the traditional societies. Cultivation of this mushroom has not been successful till now and hence its mycelium is extensively used as a flavoring agent. **Objective:** To evaluates the antioxidant, anti-inflammatory and antitumor activities of ethanolic extract of *Morchella esculenta* mycelium grown in submerged culture. **Methods:** The antioxidant activity of 50%ethanolic extract was estimated by determining the superoxide, hydroxyl and nitric oxide radicals scavenging and inhibition of lipid peroxidation activities. Anti-inflammatory activity was determined by carrageenan induced acute and formalin induced chronic inflammatory models. Antitumor activity of the extract was determined by mouse solid tumor model induced by Daltons Lymphoma Ascites cells (DLA). **Results:** The extract showed significant antioxidant, anti-inflammatory and antitumor activities in a dose dependent manner. Oral administration of 500mg/kg body weight of extract showed 66.6% and 64.2% inhibition of acute and chronic inflammation respectively. Administration of 1000mg/kg body weight of the extract orally showed 74.1% inhibition in tumor volume and 79.1% decrease in tumor weight 30 day after tumor cell implantation. **Conclusion:** The present investigation thus reveal that the ethanolic extract of Morchella esculenta mycelium possessed profound antioxidant, anti-inflammatory and antitumor activities.

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Chemopreventive Effects of Vanadium in Chemically Induced Rat Colon Carcinogenesis: A Focus on DNA Damage and DNA Protein-Cross Links by Comet Assay

P. Suresh Kanna* and Malay Chatterjee

Division of Biochemistry, Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700032, India. *suresh_kannain@yahoo.co.in

Introduction: The trace element vanadium inhibits cancer development in variety of experimental animal models. **Objectives:** The present study was designed to investigate the chemopreventive effects of vanadium on 1,2 dimethyl hydrazine induced genotoxicity in rat colon preneoplasia. **Methods:** Male Sprague-Dawley rats were divided into four groups. Group A were designed as normal controls. Group B received DMH once a week (20mg/kg wt) intraperitoneally for 12 weeks. Group C rats received the same treatment of DMH as in group B, along with 0.5-ppm vanadium as ammonium monovanadate ad libitum in drinking water throughout the experiment. Vanadium alone was given to group D rats without any DMH injection. **Results:** The 12 week treatment with DMH resulted in significantly higher levels of DNA damage in rat colon as measured by the comet assay (higher mean values for length to width ratios (L:W) of DNA mass ($P<0.001$) and mean frequencies of cells with comets ($P<0.01$). The vanadium co-treatment reduced DNA damage in colon cells by 45% ($P<0.001$ and $P<0.02$ for L:W and tailed cells respectively). The comet assay also showed statistically higher

mean base values of DNA-protein mass (P<0.01) and mean frequencies of tailed cells (P<0.001) in the carcinogen-induced group after treatment with proteinase K. Treatment with vanadium for the entire period caused a significant (P<0.02) reduction (48%) in DA protein cross-links in colon cells. **Conclusion:** The results demonstrate that the early protective effect of vanadium in chemically induced rat colon carcinogenesis may be mediated by a reduction of carcinogen-induced DNA damage.

F-23

A Novel Anti-Cancer Lead Molecule from Curcuma Longa Induces Apoptosis In Human Laryngocarcinoma (HEp-2) Cells

Pathmanathan. K*, Venkatakrishnan.V, Ramadevi.S, Senthil.V, Giridharan.P and Arun Balakrishnan

Centre for Biotechnology, Anna University, Chennai-600 025, India. *paths_17@yahoo.com

Introduction: Natural products are the most consistently successful source of drug leads and they continue to provide structural diversity. A wide variety of natural products have been recognised to have the ability to induce apoptosis in various tumour cells of human origin and many of these substances are from herbal source. However, a large number of medicinal plants remain to be investigated further for their possible pharmacological value and also there is a need to develop new drugs to treat the increasing number of patients with different cancers. **Objectives:** To extract the active lead from *Curcuma longa* and validate its biological activity using *in vitro* bio screening in HEp-2 cells and also to understand the signalling pathway leading to apoptosis. Methodology: Active ingredient from *Curcuma longa* was pulled out by sequential solvent extraction and *in vitro* bioscreening using [^{3}H]- Thymidine incorporation assay. Characterisation of the lead molecule was done by NMR and Mass spectrometry. Apoptosis was confirmed by flow cytometry analysis. RT-PCR, nitric oxide production and colorimetric assay were performed to detect different molecular targets. **Results and Conclusion:** Bioassay guided purification has enabled us to obtain novel lead molecule with proven anti-cancer activity from *Curcuma longa*. Flow cytometry analysis has confirmed the induction of apoptosis by this molecule in HEp-2 cells. Activation of caspase - 3 was also confirmed using colorimetric assay. In order to prove the possible mechanism of induction of apoptosis by the lead molecule, RT-PCR analysis and nitric oxide (NO) assay has clearly demonstrated the activation of interferon (IFNg) followed by elevation of iNOS leading to production of nitric oxide (NO) in HEp-2 cells. NO in turn activated caspase -3 to induce apoptosis. Elaborate studies with this compound with respect to its efficacy to induce apoptosis in different cancer cell lines and understanding the mechanism of action may provide valuable information for its possible application in cancer therapy.

F-24

Curcumin Induces Apoptosis through the Impairment of Ubiquitin Proteasome Pathway

Priyanka Dikshit*, Anand Goswami Amit kumar Mishra and Nihar Ranjan Jana

Cellular and Molecular Neuroscience Laboratory, National Brain Research Centre, Manesar, Gurgaon - 122 050. *nihar@nbrc.ac.in

Introduction and **Objective:** Curcumin has long been used as a popular dietary spice and herbal medicine in several southeastern countries. Recent evidence suggests that the curcumin has chemopreventive and anti-tumor activities because of its ability to induce apoptosis. However, the molecular mechanisms through which curcumin induces apoptosis are not fully understood. **Methods:**

Mouse neuro 2a and HeLa cell lines were used for all studies in culture. Co-immunoprecipitation, immunoblotting and immunofluorescence staining were done using antibodies against various ubiquitin proteasome system components to delineate its pathway of action. Mitochondrial membrane potential was measured using confocal microscopy. **Results:** Here, we show that the curcumin-induced apoptosis is mediated through the impairment of ubiquitin-proteasome system. Exposure of curcumin to the mouse neuro2a cells causes dose-dependent decrease in proteasome activity and increase in ubiquitinated proteins. Curcumin exposure also decreases the turnover of the destabilized enhanced green fluorescence protein, a model substrate for proteasome and cellular p53 protein. Like other proteasome inhibitors, curcumin targets proliferative cells more efficiently than differentiated cells and induces apoptosis via mitochondrial pathways. Addition of curcumin to the neuro2a cells induces rapid decrease in mitochondrial membrane potential and release of cytochrome c into cytosol followed by activation of caspase-9 and caspase-3. **Conclusion:** Altogether, our result concludes that the curcumin-induced apoptosis is mediated through the impairment of UPP. Proteasome inhibitors are recently considered to be one of the promising groups of anticancer agent. Since, curcumin inhibits proteasome function and pharmacologically has been found to be safe, it has enormous potential in the prevention and therapy of cancer.

F-25

Bitter Fraction of *Swertia chirata* can Prevent Carcinogenic Risk Due to DMBA Exposure

Prosenjit Saha* and Sukta Das

Chittaranjan National Cancer Institute, Kolkata, India. *prosenjit_cnci@yahoo.co.in

Introduction: Prevention of cancer can be achieved either by avoidance of risk factors or by increasing the availability of protective factors that can minimize the chances of developing cancer. In recent times the importance of cancer protective factors are receiving much attention as because some but not all of the risk factors for cancer can be avoided. **Objectives:** The present study was an attempt to assess the cancer protective potential of the plant *Swertia chirata* that is used in Ayurveda as liver tonic. **Methods:** DMBA induced mouse skin carcinogenesis model was used for demonstrating the anticarcinogenic effect of purified extract of *Swertia chirata* (amarogentin). Liver enzymes (GST, GPx, SOD, CAT) were determined biochemically, immunohistochemical procedures were followed for detection of proliferating and apoptotic cells in skin lesion and expression of COX 2 and Caspase 3 proteins were analysed by Western blot. **Results:** It was observed that amarogentin could protect from DMBA induced skin carcinogenesis as revealed by reduction in incidence of skin papilloma. Liver Phase II detoxification enzymes were activated following treatment. The plant extract produced a reduction in proliferating epithelial cells and increased the number of apoptotic cells in the precancerous skin lesions. This is also reflected in the expression of the molecular markers associated with proliferation and apoptosis viz. downregulation of Cox-II and upregulation of Caspase-3. **Conclusion:** Relative Risk or Relative Protection and Attributable Risk, which regulate development of tumor can be assessed by determining the influence of test agent on these two cellular processes associated with the progression of carcinogenesis.

F-26

Tea Polyphenol Epigallocatechin 3-gallate Reverses the Anti-apoptotic Effects of Lowgrade Repetitive Stress through Inhibition of Akt and NF-kB Survival Pathways

Prosenjit Sen*, Prabir Kumar Chakraborty and Sanghamitra Raha

Crystallography & Molecular Biology Division, Saha Institute of Nuclear Physics, 1/AF, Bidhan nagar, Kolkata-700064, India. *prosen98@yahoo.com

Introduction: Exposures to low grade oxidative stress has become a common phemomenon, in these days of increasing environmental stress. Therefore, it becomes extremely relevant to investigate the factors determining the anti-apoptotic influence of repetitive stress and to document whether repetitive or chronic use of naturally occurring compounds influence the antiapoptotic outcome of repetitive stress. Upregulation of anti-oxidant defense by means of activation of catalase, superoxide dismutase, glutathionperoxidase has been identified as an underlying cause of the apoptosis inhibitory effects exerted by repetitive stress. Activation of MAPK family enzymes p42/44, JNK, p38MAPK has been implicated in both cell survival anddeath, p42/44exertcontrolover cell polliferation and survival, JNK associated with apoptotic death p38MAPK has been shown to influence both apoptosis and survival. Akt (protein kinase B-PKB) is another serine/threonine kinase which controls vital role in cell survival. Akt/PKB activation through phosphorylation on serine473 and threonine308 inhibits apoptosis by activating pro-apoptotic proteins BAD, FKHR or by activating cell poliferating gene NFkB. Overactivation of the Akt elevates the propensity of malignancy and has been identified as prime target of chemotherapy. Green or black tea polyphenols have recently been shown to possess growth inhibitory and pro-apoptotic effects especially in cancer cells. The tea catechin Epigallocatechin-3-gallate (EGCG) constitutes approximately 60% of the catechins in tea.The plasma/serum levels of tea polyphenols resulting from regular intake of tea are in the low micromolar range, peaking close to 1 mM. In this study. **Objective:** We try to identify the key upstream elements that promote survival in repetitively stressed cells and unravel the inter-relationships between the important regulators of survival. Furthermore, we aim at identifying a natural product, which through continuous exposure at low levels may negate the anti-apoptotic/pro-survival effects of repetitive stress. **Method:** For development of repetitive oxidative stress model V79 fibroblasts were exposed to 30mM H2O2 at 37 ^{o}C for 30 min in culture 5 days a week for a period of 4 weeks. The inhibitors SB-203580 (5mM) or LY-294002 (5mM) or EGCG (4.5mM) were added in the culture medium of both control or repetitively stressed cells. Untreated controls were subcultured normally and received no other treatment. After 4 weeks of repetitive stress, UVC radiation of 1 J/m2/s for 5 sec or 7.5 mM H2O2 for15 minutes were used to induce acute stress in previously treated or untreated cells. Activation of most of the proteins was observed by western blotting and, bands were identified by ECL.Capase-3 activity was monitored fluorometrically by using it's substrate AcDEVD. NFkB activity was measured by Mercury Pathway profiling SEAP system kit from Clontech, DNA fragmentation was quantitated from DNA of sub G0/G1 phase by FACs analysis. **Results:** Akt/Protein kinase B (PKB) became gradually phosphorylated at Serine436 and Threonine 308 during this period of repetitive stress. Exposure of the cells to LY294002 (5 mM), a phosphoinositide 3-kinase (PI-3-kinase) inhibitor and 4.5 mM Epigallocatechin 3-gallate (EGCG), a tea polyphenol almost completely blocked Akt activation by repetitive stress. Exposure to 5mM SB203580, a p38 Mitogen Activated Protein Kinase (p38MAPK) inhibitor during the repetitive stress period resulted in moderate inhibition of Akt phosphorylation. p38MAPK also became increasingly phosphorylated during the repetitive stress period and the phosphorylation could be prevented by exposure to SB203580. However, p38MAPK phosphorylation remained largely unaffected by the presence of either EGCG or LY294002. Transcriptional activity driven by Nuclear Factor kappa B (NFkB) was significantly (4 fold) enhanced by repetitive oxidative stress. This increase was largely abolished by simultaneous exposure to either SB203580 or EGCG. The repetitively stressed cells demonstrated a significant resistance to apoptosis by subsequent acute stress in the form of ultraviolet radiation (UVR) at 5J/m^2 or H2O2 (7.5 mM). The opposition to apoptosis conferred by repetitive stress was drastically reduced (>80%) by constant exposure to EGCG during the stress period. Similar exposure to SB203580 also resulted in considerable abrogation (>70%) of the anti-apoptotic influence of repetitive stress while the presence of LY294002 brought about a moderate reversal (about 50%). **Conclusion:** Our data indicate that activation of Akt and NFkB pro-survival pathways by repetitive low-grade stress results in a radical inhibition of the normal apoptotic response after subsequent acute stress. The tea polyphenol EGCG impedes the activation of both Akt and NFkB by repetitive stress and as a result aids in the preservation of the normal apoptotic response during subsequent acute stress.

F-27

Chemopreventive Effect of Diphenylmethyl Selenocyanate against Benzo (a) Pyrene Induced Lung Carcinogenesis in Strain a Mice

Rajat K. Das**, Sarmistha Banerjee and Sudin Bhattacharya

Department of Cancer Chemoprevention, Chittaranjan National Cancer Institute, 37, S.P. Mukherjee Road, Kolkata-700 026, India. *joydas_cncifeb01@yahoo.co.in

Introduction: Lung cancer has become the most common cause of cancer deaths due to increased tobacco habit and environmental pollutants especially automobile exhausts. Chemoprevention is recognized as a viable means to reduce cancer death in humans. Experimental evidences revealed that organoselenium compounds is an important chemopreventive agent, which possess antioxidative, antimutagenic and anticarcinogenic properties. Therefore chemopreventive intervention using synthetic organoselenium compounds may be a practical and cost effective approach for reducing lung cancer risk. Benzo (a) Pyrene (BP), one of the major polycyclic aromatic hydrocarbons present in tobacco smoke and in automobile exhausts is a risk factor for bladder, skin and lung cancer. **Objective:** In this study we evaluated the chemopreventive potential of diphenylmethyl selenocyanate against BP induced lung carcinogenesis in strain A mice. **Methods:** BP was injected subcutaneously at the subscapular region at a dose of 0.2mg/mouse in 1% aqueous gelatin as a suspension in strain A litter within 24 - 48hrs. after birth and the Se-compound was given orally after 5^{th} weeks of BP treatment at a dose of 3mg./Kg. b. w. upto 22^{nd} weeks. Progressive cellular and histological changes initiated by BP eventually resulting in formation of lung tumours were followed to identify the precancerous lesions, the targets for chemoprevention. **Results:** Hyperplasia and severe dysplasia were evident in carcinogen control group after 8^{th} and 22^{nd} weeks respectively, were effectively reduced after oral administration with diphenylmethyl selenocyanate. The Se compound can also significantly ($p<0.01$) reduce the hepatic microsomal lipid peroxidation and induce hepatic GST activity when measured after 8^{th} and 22^{nd} weeks of BP exposure. **Conclusion:** The result indicates that diphenylmethyl selenocyanate have the potential to modulate the lung carcinogenesis in mice exposed to BP an act as effective chemopreventive agent.

F-28

Management of Cancer Patients with Ayurvedic Therapy HUMA

S Hina Fatima** [1] and Sanjoy Kumar Pal[2]

Huma Cancer Society, Ganj Plaza, Hazratganj, Lucknow, UP 226001, India[1].Department of Gastroenterology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Raebareli Road, Lucknow UP 226014, India[2]. *humacancer@satyam.net.in

Introduction: The burden of cancer is increasing worldwide. According to WHO cancer patients in the developing world will double to 10 million new cases annually by 2015. Disturbingly, most of them will have limited access to the radiation therapy that could save or prolong their lives. Many patients with cancer are compelled to try various complementary and alternative medicines for cancer treatment and palliation. HUMA a poly-herbal Ayurvedic Rasayana formulated by Late Dr. S M Atiq in early 80's have gained tremendous popularity as an alternative cancer therapy (ACT) among patients in recent years. **Objectives:** To assess the effectiveness and side effectiveness and side effects of HUMA therapy in cancer patients with advanced disease. **Methods:** We retrieved and studied records of 200 cancer patients who tried HUMA. Out of which 47 (23.5%) were oral, 56 (28%) GI tract, 32 (16%)

haematological, 21 cervical (10.5%), and 46 patients had other types of cancer. Metastasis was present in 175 (87.5%), and 46 patients had other types of cancer. Metastasis was present in 175 (87.5%) patients. Eighty-seven (43.5%) patients had tried conventional therapy earlier. All patients trying HUMA therapy also received conventional life support care but without radio or chemotherapy. **Results:** Marked responses viz. decrease in pain, drying of pleural effusion & ascites and regression of tumor was observed in 73 (36.5%) of patients. Complete regression of tumors/lesion along with relapse free survival benefit with this ACT was observer in few oral and rectal tumor patients. No adverse side effects were observed in any patients.**Conclusion:** HUMA is tried primarily for palliation, however, marked remission of cancer observed in some patients deserves scientific attention.

F-29

Prooxidant Activity of Resveratrol in the Presence of Copper Ions: Implications For Anticancer Properties

S.M. Hadi**, Asfar S. Azmi and Aamir Ahmad

Department of Biochemistry, Faculty of Life Sciences, AMU, Aligarh-202002, India.*smhadi@vsnl.com

Several plant derived polyphenolic compounds are considered to possess anticancer and apoptosis inducing properties. Such compounds are recognized as naturally occurring antioxidants but also exhibit prooxidant properties under appropriate conditions particularly in the presence of transition metal ions such as copper. Over the last several years we have shown that various classes of plant polyphenols including flavonoids, curcuminoids, catechins and stilbenes are capable of catalyzing oxidative DNA cleavage in the presence of copper ions. Evidence in the literature suggests that the antioxidant properties of plant polyphenols may not fully account for their chemopreventive effects. On the basis of our own observations and those of others we have proposed a mechanism of DNA fragmentation and cytotoxic action by plant polyphenolics that involves mobilization of endogenous copper and the consequent prooxidant action. Resveratrol (3,4',5-trihydroxy stilbene) is a polyphenolic compound present in dietary material such as peanuts, grapes and red wine. It has been shown to be chemopreventive against various stages of chemically induced carcinogenesis. In our laboratory we have shown that resveratrol catalyzes the reduction of Cu(II) leading to oxidative DNA cleavage. In the present report we have studied the structure activity relationship between resveratrol and its structural analogs piceatannol (3,3',4,5' tetrahydroxy stilbene) and trans stilbene which does not contain any hydroxyl group. Piceatannol was found to be the most effective in the DNA cleavage reaction as well as a reducer of Cu(II) followed by resveratrol and trans stilbene which does not show any activity. The results indicate that the number and position of hydroxyl groups is important for the prooxidant action of resveratrol and piceatannol in the presence of copper ions. Further, we have also characterized the mutations induced by resveratrol-Cu(II) using plasmid Bluescript SK(+). Our studies indirectly support our hypothesis that prooxidant action of plant polyphenols may be an important mechanism of their anticancer properties.

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Anticancer Potential of Novel Synthetic Nucleoside Analogs

Sarika Sinha**[1], Richa Srivastava[1], Bhupesh K. Prusty[2], Bhudev C. Das[2] and Ramendra K. Singh[1]

[1]Nucleic Acids Research Laboratory, Department of Chemistry, University of Allahabad, Allahabad - 211002, India.
[2]Division of Molecular Oncology, Institute of Cytology and Preventive Oncology (ICMR), NOIDA - 201301, India.
*sarikasinha1@rediffmail.com

HPV is a DNA tumor virus and the causal agent of cervical cancer, the most common cancer in Indian women. HPV 16 is the most prevalent type and in India, more than 90% of the HPV infections are of HPV type 16. Two novel synthetic nucleoside analogs, viz. 9-(1'-â-D-arabinofuranosyl)-4-nitro-1,3-dideazapurine (1) and 5'-deoxy-5'-(mercaptopropionic acid)-adenosine (2) have been screened for their anticancer potential on HPV (Human Papillomavirus) 16 and 18 - positive cervical cancer cell lines, SiHa and HeLa, respectively. After a 24-hour treatment, compound 1 caused apoptosis in SiHa and necrosis in HeLa cells at 200µg/mL whereas compound 2 showed no effect on the morphology of these cells. Further studies were carried out to observe the effect of these nucleoside analogs on the binding efficiency of the transcription factor AP-1 to HPV-specific gene sequence, using Electrophoretic Mobility Shift Assay (EMSA). Compound 2 exhibited a downregulation of AP-1 binding to the HPV- specific gene sequence. Since AP-1 is an indispensable key regulator of epithelial cell-specific transcriptional activity of various HPV types, downregulation of its binding to the HPV genome may lead to interference with the expression of HPV genes, particularly the E6 and E7 genes which are responsible for tumorigenic transformation of cervical epithelial cells. However, compound 1 did not exhibit any effect on AP-1 binding. Western Blotting experiments were carried out to check the expression pattern of two of the constituent proteins of AP-1, c-fos and fra-1, in SiHa cells treated with compounds 1 and 2. Compound 1 was found to cause a down regulation in the expression of c-fos, which became almost nil in cells treated with 200µg/mL concentration. At the same time, fra-1 expression was found to gradually increase in cells treated with increasing concentrations of this nucleoside analog. Compound 2 downregulated c-fos expression at 10µg/mL concentration while fra-1 expression was found to be slightly upregulated with its increasing concentrations. Earlier workers have shown that as tumorigenic cervical epithelial cells traverse towards normalcy, c-fos expression is downregulated and fra-1 expression increases2. Hence, both these nucleoside analogs may be developed as anti-HPV molecules. A decrease in the expression of c-fos has also been shown to be associated with the onset of senescence in cells and an inhibition of DNA synthesis. Growth factors have been shown to cause induction of c-fos gene and protein expression. Since senescence and apoptosis have been proposed as possible mechanisms behind the antioncogenic properties of anticancer drugs, the compounds 1 and 2 may, in general, be considered as potential cancer chemotherapeutic agents

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Screening of Biological Properties of Medicinal Plants for Anticancer Activity Using In Vitro Techniques - An Approach towards Anticancer Drug Development

Senthil V .*, Jis C. Joy, Keerthivasan A., Giridharan P. and Arun Balakrishnan

Centre for Biotechnology, Anna University, Chennai-600 025, India. *senthilvee@rediffmail.com

Introduction: Medicinal plants are the most exclusive source of life saving drugs for the majority of the world's population. Medicinal herbs have been widely used for treatment of diseases in traditional way for several generations. An interaction between traditional medicine and modern biotechnological tools is to be established towards New Drug development. The interface between cell biology, *in vitro* assays and structural chemistry will be the best way forward to obtain valuable leads. **Objectives:** To determine the Anti-proliferative activity of Medicinal plants by *In-vitro* Bioscreens using cancer cell lines and Analysis of expression levels of Molecular signaling targets at Cellular event in Tumorigenesis. Methodology: 1. Medicinal plants were subjected to sequential solvent extraction and the active fractions were selected using [^{3}H]- Thymidine incorporation assay and column chromatography. 2. Human Cancer cell lines were used for screening of the target compounds. 3. Structural elucidation using NMR and Mass spectrometry. 4.The effect of the lead molecule in inducing apoptosis was confirmed using DNA fragmentation

assay, RNA extraction and amplification by RT-PCR, Western blotting for Protein level analysis. **Results and Conclusion:** Apoptosis or programmed cell death is a genetically regulated process occurring naturally in a response to a variety of signals. Many anticancer agents exert their cytotoxicity through DNA damage and induction of apoptosis. We found that the lead molecule obtained from *Andrographis paniculata* leaf, was able to induce apoptosis in human HeLa cells. In this work the extraction, purification and elucidation of active molecular lead based on the bioactivity directed based screening is described. We also explored the mechanisms through which this molecule induces apoptosis. This molecule activated p53 and subsequently resulted in the up-regulation of pro-apoptotic protein Bax followed by cytochrome c release from mitochondria leading to caspase-3 activation and then cells undergo apoptosis. DNA fragmentation assay further confirmed the apoptosis of the cells. This compound also suppressed Bcl-2 expression in HeLa cells. This work is an example of a natural product with interesting anti-proliferative activity, in which the basic skeleton can be further, used as a template for the production of New Chemical Entity.

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Antitumour and Immunomodulatory Activity of *Andrographis paniculata* Nees

Sheeja K* and Girija Kuttan

Dept. Immunology, Amala Cancer Research Centre, Amala Nagar, Thrissur, 680555, Kerala, India.
*amalaresearch@rediffmail.com

Introduction: Several immunomodulators are now being used in cancer therapy. Use of plants as a source of immunomodulators is still in a developing stage. **Objectives:** Methanolic extract of *Andrographis paniculata* (*A. paniculata*) was studied for its antitumour and immunomodulatory activity using animal models. **Methods:** Cytotoxicity of the extract was determined by trypan blue exclusion method and by MTT assay. Solid tumors were injected by injecting DLA cells (1×106 cells/animal, s.c) on the hind limb. For ascites tumors animals were injected with 1×10^6 EAC cells/animal to the peritoneal cavity. Animals were treated with methanolic extract of A. paniculata (10mg/dose/animal, i.p) for 10 consecutive days inorder to do immunomodulatory study. The levels of total WBC, bone marrow cellularity, a-esterase activity, the antibody titer and the number of plaque forming cells (PFC) were determined in normal as well as in tumor bearing animals. **Results:** Methanolic extract of Andrographis paniculata was found to be toxic towards both DLA cells EAC cells at a concentration of 500 µg /ml. The extract was also found to produce cytotoxicity towards L929, B16 F-10, and Vero cells at a concentration of 250µg/ml. Administration of the extract could inhibit the solid tumor development in mice induced with DLA cells and increased life span of mice bearing ascites tumor by 34.6%. *Andrographis paniculata* extract treatment was found to increase total WBC count. Bone marrow cellularity and a-esterase positive cells were also enhanced by the extract administration. Administration of the extract enhanced the antibody titre and PFC. **Conclusion:** The observed antitumour activity of *Andrographis paniculata* may be related to its immunomodulatory activity.

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Cancer Chemopreventive Potential of a Novel Organoselenocyanate Against DMBA-PMA Induced Skin Carcinogenesis in Mice

Sumitra Sengupta*, SK Ugir Hossain, Sudin Bhattacharya

Chittaranjan National Cancer Institute, 37, S.P.Mukherjee Road, Kolkata-700026, India. *zoom2sumi@yahoo.com

Introduction: Selenium is an essential dietary trace element required for mammals including humans to fight against various diseases including cancer. Extensive work is in progress to obtain suitable selenium compounds useful for prevention of cancer. It has been found that organoselenocyanates are far superior to other selenium compounds in terms of their chemopreventive efficacy. **Objectives:** In continuation of our anticarcinogenesis-drug development programme we have synthesized and evaluated the cancer chemopreventive potential of a novel organoselenocyanate 2-(2-Selenocyanato-ethyl)-benzo[de]isoquinoline-1,3-dione{Nap-(CH2)2SeCN} against DMBA-PMA induced skin carcinogenesis in Swiss albino mice. **Methods:** The test compound was administered orally in pre and pre+post treatment schedules at the dose of 3mg/Kg bw. Lipid peroxidation, activities of Phase II enzymes and GSH level in liver and skin tissues were estimated biochemically. **Results:** The compound was found to be non-toxic at the dose tested for the experiment. Significant ($p<0.01$) downregulation of lipid peroxidation and upregulation of Phase II detoxifying enzymes (GST, SOD, CAT) along with GSH level were observed following treatment, estimated on the 12th week of the first DMBA application, in comparison to the carcinogen control. Significant reduction in number of papilloma formed per mouse was noted after treatment (from 3.8 per mouse in carcinogen control to 2.3 per mouse). Papilloma incidence was also inhibited by 32-47%. **Conclusion:** The chemopreventive potential of the 2-(2-Selenocyanato-ethyl)-benzo [de]isoquinoline-1,3-dione{Nap-(CH2)2SeCN}seems clear at this stage of investigation.

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Green Medicine in Preventive Oncology

Sunita Garg[1], A.K. Jain, Renu Bala, A.K. Bhardwaj and Ashwani Goyal*

[1]National Institute of Science Communication and Information Resources, Dr K. S. Krishnan Marg, New Delhi -110012. [2]Department of Botany, Govt. P. G. College, Sector 39, NOIDA- 201 301. * goyal_ashwani@hotmail.com

Cancer is a major health problem worldwide which is likely to assume alarming proportions in the next two decades. Conventional therapies cause serious side effects and, at best, merely extend the patient's lifespan by a few years. In the last few decades, there has been probably an upsurge in the use of green medicine (herbal-based drugs) because of the miraculous success of them and no side effects like that of synthetic medicines. Keeping this into view research is being carried out to evaluate the potential anticancer activities of various vegetables, fruits and others plants. A general screening of papers published during last 2-4 years revealed that in addition to earlier reports, recently more than 65 plants have been found to possess anticancer activity on different cell lines and forms of cancer. It is further interesting to note that not only medicinal herbs or trees, some fruits and vegetables also have potential anticancer property. In this paper such reports have been summarized for the purpose of dissemination of information as well as to encourage further research and developing useful drugs and formulations in preventive oncology. Vegetables, which are reported to be anticancerous are: Onion (*Allium cepa* Linn.), Bitter gourd (*Momordica charantia* Linn.), Potato extract, Garlic (*Allium sativum* Linn.), Broccoli (*Brassica oleracea* Linn.) etc. Coffee(*caffeine*), green tea and cocoa (*Theobroma cacao* Linn.) are also anticarcinogen. Fruits, which have been found as anticancerous, include: Cashew kernel oil, Soursop (*Annona muricata* Linn.), *Actinidia chinensis* Planch. (Kiwi fruit), *Citrus sinensis* (Linn.) etc. The herbs and trees that were investigated by various researchers on different cancer cell lines and shown positive Results are about 45 including already reported ones like *Withania somnifera* Dunal, *Emblica officinalis* Gaertn., *Psoralea corylifolia* Linn., *Curcuma longa* Linn., *Catharanthus roseus G.* Don, *Taxus brevifolia* Linn., etc. Some weeds found to be anticancerous are: Ageratum conyzoides Linn., *Blumea lanceolaria, Achyranthes bidentata, Croton oblongifolius* Roxb., *Tribulus terrestris* Linn. etc.