INVITED SPEAKERS' ABSTRACTS

PL 1 Promises and challenges of RNAi therapeutics

Anil K. Sood, M.D.

M.D. Anderson Cancer Center, Houston, TX, USA.

Since the discovery of RNA interference (RNAi), there has been an explosion of interest and knowledge in using this technology for clinical applications. Although small molecule inhibitors and monoclonal antibodies have led to many successful therapies for cancer, many important cancer therapy targets are difficult to inhibit using these strategies. Use of short interfering RNA (siRNA) as a method of gene silencing has rapidly become a powerful tool in protein function delineation, gene discovery, and drug development. The promise of specific RNA degradation has also generated much excitement as a possible therapeutic modality, but *in vivo* siRNA delivery has proven difficult. Moreover, many physiological obstacles stand in the way of successful and efficient delivery. To overcome these limitations, we have developed a number of biocompatible nanoparticle strategies for highly efficient delivery of siRNA. We have used a novel complex of siRNA with a neutral nanoliposome (DOPC) for *in vivo* siRNA delivery. This delivery platform has shown substantial efficacy with regard to target modulation and anti-tumor effects in many different tumor models, and is now entering clinical trials. To target the tumor microenvironment, we have also developed delivery methods (e.g., chitosan nanoparticles) that allow highly efficient delivery of siRNA into both tumor cells as well as tumor associated endothelial cells. Therefore, the chitosan nanoparticles allow silencing of genes that play a functional role in tumor angiogenesis. In addition, we have also utilized these nanoparticles for targeted delivery by attaching peptides to the nanoparticles, which permits increased delivery into the tumor microenvironment. Collectively, these approaches offer new opportunities for therapeutic gene silencing, especially for otherwise undruggable targets.

IS 1 Perspectives on occupational carcinogenesis

Paul Brandt-Rauf

UIC School of Public Health, University of Illinois, Chicago, USA

Workplace carcinogen exposures remain a significant and preventable cause of cancer globally. This presentation will explore some of the advances in understanding the molecular mechanisms of occupational carcinogenesis and the molecular epidemiologic approaches to occupational cancer analysis, treatment and prevention.

IS 2 Epigenome deregulation in cancer

Zdenko Herceg, PhD

Head, Section of Mechanisms of Carcinogenesis; Head, Epigenetics Group, International Agency for Research on Cancer (IARC), 150 Cours Albert Thomas, F-69008, Lyon, France

Recent advances in epigenetics and epigenomics has a tremendous impact on our thinking and understanding of biological phenomena and importance of environmental stressors in complex diseases, notably cancer. Environmental and lifestyle factors are thought to be implicated in the development of a wide range of human cancers by eliciting epigenetic changes, however the underlying mechanisms remain poorly understood. The epigenome can be viewed as an interface between genome and environmental influence, therefore aberrant epigenetic events associated with environmental exposures (epimutagens) and factors in cell microenvironment are likely to play an important role in the onset and progression of different human malignancies. At the cellular level, aberrant epigenetic events influence critical cellular events (such as gene expression, carcinogen detoxification, DNA repair, and cell cycle), which are further modulated by risk factor exposures. However, there is little understanding about whether epigenetic changes in cancer and surrogate tissues can be used as biomarkers for exposure assessment, early detection and an intermediate biomarker for different health outcomes. Remarkable advances in epigenomics and the advent of powerful technologies for analyzing epigenetic patterns in both cancer tissues and normal cells indicate that the next few years will be fundamental for the identification of critical cancer-associated and exposure-associated epigenetic changes and for their evaluation as biomarkers. Here I will discuss recent progress in our understanding of epigenetic mechanisms through which environmental factors may promote tumour development and progression as well as its implications for biomarker discovery, risk assessment and cancer control.

IS 3 Mutant p53 confers chemo-resistance in human cancer through ephrin-B2-mediated bi-directional signalling pathways

Sk. Kayum Alam and Susanta Roychoudhury

Cancer Biology and Inflammatory Disorder Division, CSIR - Indian Institute of Chemical Biology, Jadavpur, Kolkata, India. Email: susanta@iicb.res.in

Chemo-resistance represents a major obstacle in the successful treatment of human cancer. Almost half of the human tumours harbour mutations in the p53 gene and these mutations are positively correlated with increased incidence of chemo-resistance. We performed a bio-informatic analysis to identify potential gene(s) that may contribute to the chemoresistance property of mutant *p53* bearing cancer cells. Trans-membrane protein Ephrin-B2 encoded from *EFNB2* gene interacts with Eph family of receptors which is the largest known subfamily of Receptor Tyrosine Kinase. The Eph/Ephrin interactions are important in development, especially in cell-cell interactions involved in nervous system patterning (axon guidance), angiogenesis, and possibly in cancer. The ephrin-B2 has been found to be over-expressed in many human primary tumor tissues. We provide evidences that mutant p53 transcriptionally activates *EFNB2* expression in the chemo-resistant cancer cells upon addition of chemo-therapeutic agents. Moreover, we show that sensitivity of mutant p53 cell lines to many DNA damaging drugs can be restored by knockdown of EFNB2. Detail mechanism of ephrin-B2 mediated drug resistance has been worked out. Thus, targeting ephrin-B2, has the potential to eradicate drug-resistant cells when applied in conjunction with other effective treatments.

IS 4 Osteopontin, a chemokine like protein acts as therapeutic target covering all hallmarks of cancer

Remya Raja, Smita Kale, Rosalin Mishra and Gopal C. Kundu

National Center for Cell Science, Pune 411007, India. Email: kundu@nccs.res.in; gopalkundu@hotmail.com

Substantial advances in breast cancer treatments have resulted in a significant decrease in mortality. However, existing breast cancer therapies often result in high toxicity and nonspecific side effects. Therefore, better targeted delivery and increased efficacy of drugs are crucial to overcome these effects. Osteopontin (OPN), a pro-inflammatory and chemokine like protein plays crucial role in regulating the oncogenic and angiogenic potential of various cancers. Several groups have demonstrated the role of OPN in regulating the cell signaling that ultimately controls tumor progression and metastasis covering all the hallmarks of cancer. During last several years, we have

demonstrated that both tumor and stroma-derived OPN regulate tumor growth and angiogenesis through induction of COX-2, uPA and VEGF expressions and activation of matrix metalloproteinase (MMP) in breast and other cancers. Our data revealed that OPN regulates p70S6 kinase dependent ICAM-1 expression and JAK/STAT3 signaling leading to tumor growth in breast cancer. Our recent data showed that OPN controls HIF-1alpha dependent VEGF expression and breast tumor angiogenesis. In addition, we have demonstrated that OPN activated macrophages play crucial role in melanoma progression and angiogenesis. Thus targeting OPN and its regulated signaling network could be novel therapeutic strategy for the management of cancers.

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IS 5 Unraveling the intercellular signaling networks that lead to tumor stroma formation

Colin Jamora, Tuan-Lin Tan, Manado Nakasaki

IFOM-inStem Joint Research Laboratory, Centre for Inflammation and Tissue Homeostasis, Institute for Stem Cell Biology and Regenerative Medicine, Bangalore, India

The cells and proteins surrounding a tumor, collectively known as the tumor stroma, are increasingly being appreciated for the crucial roles they play in tumor growth and metastasis. Tumorigenesis and dissemination are therefore dependent upon a network of signals exchanged between cancer cells, inflammatory cells, blood vessels, activated fibroblasts and extracellular matrix (ECM) proteins. Despite the emergence of the tumor stroma as the next battleground in the fight against cancer, surprisingly little is known about the biology of this microenvironment. This is of particular significance in light of recent observations that interactions between the tumor and its microenvironment are the basis for anti-cancer drug resistance. A central player in the reactive tumor stroma is the carcinoma associated fibroblasts (CAFs) that orchestrate the behavior of immune cells, vasculature, and carcinoma cells as well as the organization of the ECM. The etiology of the CAFs is still a matter debate and the mechanism of their activation is only superficially understood. We address this open question using a mouse model engineered to overexpress the transcription factor Snail in epidermal keratinocytes. Interestingly, this transgenic mouse model recapitulates the cardinal features of squamous cell carcinoma of the skin, including the activation of dermal fibroblasts into cells resembling CAFs. Contrary to its role in embryonic development, the Snail transgene stimulates the crosstalk between epidermal keratinocytes and underlying dermal fibroblasts rather than inducing an epithelial-mesenchymal transition (EMT). We found that mindin/spondin-2 is one mediator of this intracellular crosstalk and is responsible for the initiation of cutaneous inflammation and the development of the tumor stroma.

IS 6 Prostate cancer stem cells: molecular regulation and clinical relevance

Dean G. Tang, Ph.D.

The University of Texas M.D Anderson Cancer Center, Department of Molecular Carcinogenesis, Science Park, 1808 Park Rd. 1C, Smithville, TX 78957, USA. Email: dtang@mdanderson.org

Recent work suggests that most human tumors harbor self-renewing stem cell-like cancer cells functionally termed cancer stem cells or CSCs (1). The abundance of CSC varies in different tumors and at different stages. There is strong evidence that CSCs are relatively quiescent and intrinsically resistant to stand-of-care anti-cancer therapies. Our lab has been employing prostate cancer as a model to elucidate the basic principles that govern CSC development. Our work (2-9) has revealed that human prostate CSCs (PCSCs) largely reside in the undifferentiated (i.e., $PSA^{-/lo}$) PCa cell population, and are intrinsically resistant to androgen-deprivation therapy as well as chemotherapeutic drugs. RNA-Seq analysis demonstrates that the $PSA^{-/lo}$ PCa cells preferentially express genes associated with DNA damage repair, stem cell functions, epigenetic regulation, and neural development. 5-20% $PSA^{-/lo}$ PCa cells can undergo authentic asymmetric cell division generating differentiated PSA^{+} cells. The $PSA^{-/lo}$ PCSC pool is heterogeneous containing many subsets of more tumorigenic cells. Unbiased miRNA library screenings have uncovered multiple tumor-suppressive microRNAs, including miR-34a (6), let-7 (9), miR-128 (10), and miR-141 in different subpopulations of PCSCs. Systemic delivery of some of these miRNAs inhibited tumor regeneration as well as metastasis and extended the lives of tumor-bearing animals. These results suggest that several key tumor-suppressive miRNAs are coordinately downregulated in CSCs to regulate their biological properties and these tumor-suppressive miRNAs can be explored as novel replacement therapeutics targeting CSCs (7).

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IS 7 Proteasome activity is dispensable for the degradation of PML-RARA: efficacy of bortezomib along with arsenic trioxide in the treatment of ATO sensitive and resistant acute promyelocytic leukemia

Vikram Mathews

CMC Vellore, India

Acute promyelocytic leukemia (APL) is characterized by a reciprocal translocation, t(15;17) that leads to rearrangement of *PML* and *RARA* genes such that chimeric onco-protein PML-RARA is generated which is leukemogenic. Arsenic trioxide (ATO) has proven efficacy in the management of APL even as a single agent. Degradation of PML-RARA onco-protein upon treatment with arsenic trioxide (ATO) is predominantly mediated by the proteasome complex. Reports suggest that in relapsed APL patients who were treated upfront with ATO, mutations in the B2 domain of *PML* in *PML-RARA* gene are involved in resistance to ATO. These mutations predict a poor clinical outcome in spite of subsequent combination of ATO with chemotherapy (NEJM 2014). We had previously reported that Bortezomib (Bo), a proteasome inhibitor, was able to synergize with ATO by inducing apoptosis through increased levels of ROS and up regulation of the UPR pathway. The degradation of PML-RARA when Bo combined with ATO correlated with the level of up regulation of the autophagy pathway (*Blood*. 2012;120 ,3552). We further evaluated the mechanism of degradation of PML-RARA when ATO was combined with Bo and the effect of this combination on resistant cell lines, a mouse model and in relapsed patients. We generated in-house ATO resistant NB4 cell lines (NB4EV-ASR1, ASR2 and ASR3). In NB4EV-ASR1 we confirmed the presence of A216V mutation in the *PML B2* domain (previously reported to be involved in ATO resistance) while the other 2 clones did not have this or any other mutation in *PML-RARA*. We noted an increase in the baseline proteasomal activity in all the resistant cell lines when compared to naïve NB4 cells (n=3; data not shown). The combination of ATO and Bo induced a significant apoptosis in all the resistant cells similar to naïve NB4 cells (Figure 1A: n=4; Combination Index = 0.02).The mechanism of inducing apoptosis in the resistant cell lines was similar to naïve NB4 cells, as previously reported by us, and involved an increased level of ROS, decreased mitochondrial membrane potential, induction of UPR and activation of caspase-3 (Figure 1B). We next evaluated PML-RARA degradation in NB4 naive cells treated with a combination of ATO+Bo. At 24 hours, there was an evidence of induction in autophagy as shown by LC3II formation using western blot technique which increased at 48 hours; this time point coincides with time at which maximum PML-RARA degradation occurred (Figure1C). Similar results were seen in the resistant cell lines (with and without mutation A216V). Blocking autophagy by 3-methyl adenine showed a partial inhibition in the degradation of PML-RARA. We have also observed that there is an accumulation of p62 (ubiquitin binding protein) at 24 hours and this was degraded by 48 hours suggests that accumulated ubiquitinated products were cleared by autophagy via p62 (Figure1D). At the transcript level we observed an increase expression of p62 associated proteins such as Alfy and NBR1 in the ATO+Bo treated cells (data not shown). In a co-immunoprecipitation experiment, p62 and LC3II proteins precipitated along with PML-RARA (figure 1E), this was further validated by immunofluorescence microscopy (data not shown). Knock down of p62 transcript by siRNA showed an accumulation of PML-RARA in the treated cells. In an APL transplanted mice model, combination of ATO and Bo prolonged the life span of the mice as illustrated in Figure1F. In this group there was a significant decrease in the leukemia burden evidenced by decreased leukemic cells in bone marrow, peripheral blood and spleen by flow cytometry, RQ-PCR and decreased spleen size on day +20. A reduction in the LIC was demonstrated by secondary transplants. We also observed that transplantation of bone marrow cells from the long term surviving mice post ATO+Bo therapy did not induce leukemia (Figure1G) and no transcripts of PML-RARA were detected in the recipients. A phase II clinical study combining Bo with ATO and chemotherapy has been initiated for patients with relapsed APL (NCT01950611). In this ongoing study 11 patients have been enrolled. The median age was 32 years. 7 were males. All patients achieved hematological remission and the median time to complete molecular remission was 42 days. The addition of Bo was well tolerated. None of the cases had evidence of significant neuropathy, worsening of coagulopathy, IC bleed or a differentiation syndrome. Long term follow up is awaited to comment on the efficacy of this combination. In conclusion, the mechanism of ATO+Bo synergy is multi-factorial and appears to be predominantly due to increase in ROS activity and upregulation of UPR pathway leading to apoptosis. In spite of proteasomal inhibition by addition of Bo with ATO, PML-RARA continues to be degraded and this is mediated by up-regulation of autophagy pathway. ATO+Bo synergy was further confirmed

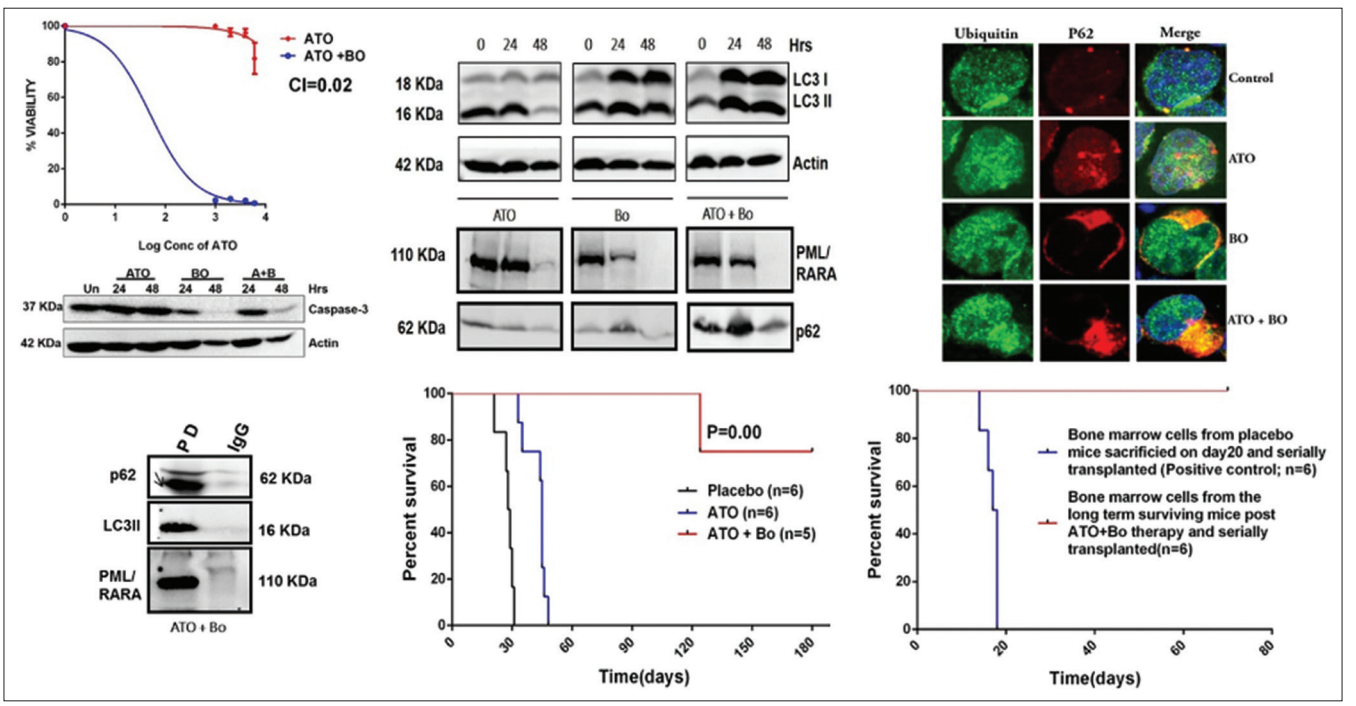

Figure 1: Synergistic effect of ATO and Bo in acute promyelocytic leukemia

in a pre-clinical model. This combination is also effective in ATO resistant cell lines with high levels of synergism.

PL 2 Immunotoxin therapy for cancer

Raffit Hassan, M.D.

Center for Cancer Research, National Cancer Institute (NCI), Bethesda, USA.

Immunotoxins are recombinant proteins that consist of a bacterial or plant toxin linked to an antibody to direct them to antigens that are highly expressed on tumor cells. These are extremely potent molecules that kill tumor cells by inhibiting protein synthesis. Our group has been working on making immunotoxins, using *Pseudomonas* exotoxin A, targeting the tumor differentiation antigen mesothelin that is highly expressed in many cancers including malignant mesothelioma, lung, pancreatic and ovarian adenocarcinoma. The first generation anti-mesothelin immunotoxin, SS1P has produced major cancer regressions in patients with treatment refractory mesothelioma and is now being evaluated in patients with pancreatic and lung cancer. Since immunotoxins are immunogenic proteins that limits re-treatment of patients, our group has made second generation immunotoxins that are inherently less immunogenic by modifying the B and T cell epitopes of *Pseudomonas* exotoxin A. These molecules retain their anti-tumor activity and are less immunogenic in preclinical studies. One of these molecules, RG7787 is now being tested in the clinic in patients with advanced solid tumors expressing mesothelin.

IS 8 A novel self-lipid antigen targets human T cells against CD1c$^+$ leukemias

Gennaro De Libero

SIgN, A-STAR, Singapore and Department of Biomedicine, University of Basel, Switzerland

T-cells that recognize tumor antigens represent an optimal source of TCR genes to use in T cell immunotherapy of tumors. An ideal tumor-specific TCR should recognize a conserved tumor-associated antigen presented by a non-polymorphic antigen-presenting molecule. We have identified self-reactive T-cells restricted to CD1c, a non-polymorphic antigen-presenting molecule expressed by a variety of leukemic cells. CD1c-reactive T-cells recognize a novel class of self-lipid antigens, that we identified as methyl-lysophosphatidic acids. These unique lipids are expressed in primary leukemia blasts and cell lines and are almost absent in non-transformed cells, suggesting tumor-dependent changes in lipid metabolism. CD1 molecules were found expressed in both primary leukemic blasts in both acute myeloid leukemia and B-cell acute lymphoblastic leukemia. In addition, CD1c self-reactive T-cells efficiently recognized CD1c$^+$ leukemia cells whereas were poorly responsive to non-transformed CD1c-expressing cells. In a leukemia xenograft model, methyl-lysophosphatidic acid-specific T-cells protected NOD/scid mice against grafted CD1c$^+$ human leukemia cells *in vivo*. The identification of a novel self-lipid antigens expressed in leukemia cells and the observed control of malignant cell growth by lipid-specific T-cells *in vivo* provide a new conceptual framework for leukemia immune surveillance and immunotherapy.

IS 9 Targeting stathmin: discovery of the Next Generation broad-spectrum anticancer drugs

Lijun Sun

Harvard Medical School, Department of Surgery, Center for Drug Discovery and Translational Research, Beth Israel Deaconess Medical Center, Boston, MA, USA.

Stathmin (STMN1) is a microtubule destabilizing protein that plays an important role in mitotic spindle dynamics. STMN1 is a major regulator of cell proliferation and migration/invasion, as well as responses to well-known anti-microtubule drugs such as paclitaxel. Cancer cells overexpress STMN1 in order to sustain their rapid divisions and proliferations. STMN1 is also a prognostic factor in predicting disease free survival rates for prostate, bladder, pancreatic, breast, endometrial cancer, esophageal adenocarcinoma, and leukemia. A unique property of STMN1 is that both positive and negative deviation from homeostasis states of its expression or phopshorylation results in blockage of cell cycle, yet the underlying mechanism is different. We hypothesize that disruption of the heightened STMN1 homeostasis in tumors leads to inhibition of growth and metastasis, thus representing a novel and attractive strategy for therapeutic intervention. We have generated a series of novel compounds that induced rapid mitotic catastrophe in prostate cancer cells but did not affect the morphology of normal epithelial cells. We further identified that the profound anticancer activity of these novel compounds was due to, at least in part, their selective modulation of STMN1. In this presentation we will provide an update on our on-going efforts aimed at characterizing the anticancer activities of the novel small molecules, as well as our plan to develop similar compounds as antitumor therapies.

IS 10 Targeting aberrant cellular bioenergetics in glioblastoma: implications in therapy

Deobrat Dixit, Ruchi Ghildiyal, Fahim Ahmad and Ellora Sen

National Brain Research Centre, Manesar 122051, Haryana

Tumor cells undergo extensive metabolic rewiring to maintain their heightened energy demands, arising from increased proliferative potential. This altered metabolic reprogramming involving a Warburg-like switch to aerobic glycolysis, alteration in the pentose phosphate pathway and lipid metabolism; offers a survival advantage to cancer cells. Aberrant metabolic state is associated with the poor prognosis of Glioblastoma multiforme (GBM) - the most malignant of brain tumors. Interestingly, de-differentiated glioma stem-like cells endowed with heightened tumorigenic potential and chemoresistance, possess a metabolic status distinct from that of differentiated glioma cells. Also, aberrant metabolic profile of glioma cells is concomitant with changes in biochemical/ signaling pathways and transcriptional networks. Our studies are focused on investigating how such aberrant circuitries regulate cellular bioenergetic homeostasis to affect glioma (i) cell survival and differentiation potential and (ii) resistance to apoptosis. Moreover, as targeting these aberrant signalling/ transcriptional circuitries could result in the development of new therapeutic modalities, efforts are also directed towards identifying potential anti-glioma targets with ability to modulate aberrant cellular bioenergetics.

IS 11 Biomarkers in gliomas

Dr. Chitra Sarkar

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

Diffuse gliomas are a heterogenous group of tumors and thus far, they have been classified based on their morphologic resemblance to normal glial cells, such as astrocytes, oligodendrocytes, etc., and on pathological grade. However, integration of genomic, transcriptomic, and morphologic data from numerous high throughput studies in recent years has shown that there is substantial diversity in biological behaviour within each of these morphologically defined entities, and identification of behaviourally distinct molecular subgroups has diagnostic, prognostic as well as therapeutic connotations. Most recently, the International Society of Neuropathology (ISN) has set down guidelines for incorporation of molecular biomarkers into the classification of CNS tumors for providing an integrated diagnosis. Narrowing down definitions of diagnostic entities in this manner also aims at reducing inter-observer reproducibility and ensuring uniformity in clinical trials. The most important molecular markers in the classification of gliomas include p53, EGFR, IDH1, ATRX, 1p/19q and MGMT. Mutation of the tumour suppressor gene TP53 in astrocytomas has been known for decades while mutations in the IDH1/2 and ATRX genes have been identified more recently. These alterations are seen in majority of Grade II and III astrocytic tumors, as well as in secondary GBM, while primary GBM are devoid of them. Primary GBM, on the other hand, demonstrate alterations in the EGFR oncogene, either as amplification or as EGFR VIII mutation. Mutations in TP53, IDH and ATRX genes can be identified

by sequencing. In addition, immunohistochemistry with p53, ATRX and mutation specific IDH1 antibodies can be used as a surrogate technique, which is inexpensive and pathologist friendly. Oligodendroglial tumors are characterized by a signature set of genetic alterations viz. 1p/19q co-deletion, IDH1/2 mutation and wild-type ATRX. 1p/19q deletion is the hallmark of these tumors and is of diagnostic, prognostic and predictive value. It can be detected easily by fluorescence in situ hybridization. MGMT promoter methylation is an important epigenetic modification, relevant from a therapeutic point of view. GBMs harbouring MGMT promoter methylation are associated with a better clinical response to alkylating agents as well as better clinical outcome. Recently, mutations of the TERT gene have been reported in gliomas associated with poor prognosis. All this molecular data has served to enhance our knowledge of brain tumor development and to challenge our grading systems, underscoring the importance of developing subclassifications with prognostic and potentially therapeutic implications. A classification scheme incorporating clinical, pathologic, and molecular information may facilitate improved prognostication, development of more effective clinical trials, and rational testing of targeted therapeutics.

IS 12 Identification of aberrantly activated pathways in cancers

Harsha Gowda, Ph.D.

Wellcome Trust/ DBT India Alliance Early Career Research Fellow, Institute of Bioinformatics, Bangalore.

Several studies over the last decade have firmly established role of aberrantly activated kinase signaling pathways in driving cancers. Cancer genome sequencing efforts have also revealed several kinases that are frequently mutated. This has resulted in development of targeted therapies that are being extensively used to treat various malignancies. Mass spectrometry offers a unique opportunity to profile global phosphoproteomic changes that can serve as a surrogate to study signaling activity in cancers. We have used this approach to identify aberrantly activated signaling pathways in various malignancies. These approaches in conjunction with cancer genome sequencing would prove useful to find putative targets to treat cancers.

IS 13 Differential microRNA expression in molecular subgroups of medulloblastomas and its impact on medulloblastoma biology

Neelam Shirsat

Advanced Centre for Treatment, Research & Education in Cancer, Tata Memorial Centre, Navi Mumbai.

Medulloblastoma is a common primary malignant brain tumor in children. Genome wide expression profiling studies including our study has demonstrated that medulloblastoma is comprised of 4 core molecular subgroups *viz*. WNT, SHH, Group 3 and Group 4, that are not only distinct in their underlying biology but also vary in their clinical characteristics like age related incidence, presence of metastasis and survival rates. In addition to the clinical parameters, molecular classification of medulloblastomas is now necessary for better risk assessment and management of the disease. The four molecular subgroups also differ in their microRNA profiles. We have validated the differential miRNA expression in over 150 medulloblastoma. We have developed a miRNA based real time PCR assay for molecular classification, that is particularly useful for formalin fixed paraffin embedded tissues, as miRNAs are relatively resistant to fragmentation during formalin fixation. The assay has an accuracy of 97% and was validated on an independent set of FFPE tissues from DKFZ, Germany. Further, Non-WNT, non-SHH medulloblastomas over-expressing *miR-182* or under-expressing *miR-592* were found to have significantly inferior survival rates indicating utility of these two miRNAs as markers for risk stratification of medulloblastomas. The WNT subgroup medulloblastomas have the most distinctive miRNA profile. The WNT subgroup tumors overexpress a number of miRNAs like miR-193a, miR-224, miR-148a. Studies investigating the effect of expression of these miRNAs in medulloblastoma cell lines, suggest role for these miRNAs in better survival rates of the WNT subgroup medulloblastomas.

IS 14 Epigenetics and oral cancer: Role of histone chaperone NPM1 and acetylation

Tapas K. Kundu

JNCASR, India

Epigenetic alterations in gene expression are responsible for the development and progression of vast majority of cancers, mediated mainly by changes in histone modifications and DNA methylation. Oral squamous cell carcinoma (OSCC) is one of the most common and highly aggressive malignancies worldwide, especially in India. Many groups have been trying to identify the molecular markers in OSCC that would help in the understanding of the clinical outcome of oral cancers using approaches such as high-throughput genome sequencing. However, till date, the epigenetic changes in OSCC are not well understood and there is no reliable marker for prognosis of oral cancer. We have been investigating the epigenetic alterations in OSCC. Unlike genetic alterations, epigenetic alterations are potentially reversible using inhibitors designed to target the enzymes required for the post-translational modifications of histone and non-histone proteins. We found that oral tumors showed elevated levels of a non-histone protein Nucleophosmin (NPM1) which is also a histone chaperone. Interestingly, we found that NPM1 is acetylated by histone acetyltransferase p300 and the acetylated form is significantly higher in the oral tumor tissues as compared to the adjacent normal tissues. Further, we found that NPM1 could enhance the autoacetylation of p300 thereby increasing its catalytic activity. In line with this, we also found global histone acetylation marks such as H3K9ac, H3K14ac, H2AK5ac, H3K56ac to be elevated in oral tumor tissues. We designed a water soluble inhibitor that could target p300 catalytic activity and found that it could bring about a significant reduction in the acetylation levels in oral tumors generated in nude mice consequently bringing about tumor regression. Interestingly, NPM1 is a histone chaperone and activates Pol II driven transcription. Further, acetylation of NPM1 enhances its histone chaperoning and transcriptional activation ability. We are currently trying to address the transcriptional network controlled by NPM1/AcNPM1 during oral tumorigenesis.

IS 15 Homology and enzymatic requirements of alternate, microhomology dependent NHEJ

Sathees C. Raghavan

Department of Biochemistry, Indian Institute of Science, Bangalore - 560012

DNA double-strand breaks (DSBs) are prerequisites for the generation of chromosomal translocations in cancer. Our studies show that formation of altered DNA structures like G-quadruplexes, triplexes etc. leads to chromosomal fragility. The V(D)J recombinase, RAGs, can acts as a structure-specific nuclease, besides being sequence-specific. Further, a nonamer of V(D)J recombination enhances RAG cleavage on non-B DNA and the sequence of such a structure itself can direct RAG activity. Non-homologous DNA end joining (NHEJ) is one of the major DNA double-strand break (DSB) repair pathways in higher eukaryotes. Alternative NHEJ (A-NHEJ) has been proposed as a possible cause for the generation of chromosomal translocations in cancer. However, little is known whether alternative NHEJ can be operative when classical NHEJ machinery is intact. Besides, it appears that there is not much consensus over its presence and relevance in normal cells. Therefore, several aspects of the alternative NHEJ are still need to be resolved. For example, its precise mechanism and the microhomology length requirements are yet to be fully uncovered. Although, there are independent studies showing the involvement of multiple proteins using knock down or knock out strategies, involvement of suggested proteins needs to be confirmed in a biochemical system. In the present study, we have established a cell-free repair assay system to investigate alternative NHEJ using which we demonstrate that MMEJ is operative even in the presence of classical NHEJ machinery. More importantly, we provide the evidence

that MMEJ operates not only in cancer cells, but also in normal cells. A minimum of 5 nt microhomology is required for MMEJ to operate. In a direct demonstration for the first time, we show that MMEJ is dependent on MRE11, NBS1, LIGASE III, XRCC1, FEN1 and PARP1 both *in vitro* and inside cells, while C-NHEJ proteins like, DNA-PKcs, KU70, KU80, XRCC4 and LIGASE IV were dispensable. Thus, we define the enzymatic and homology requirements of microhomology mediated A-NHEJ in normal and cancer cells.

IS 16 Targeting the anchorage-independent growth of cancer cells: Links with stemness

Annapoorni Rangarajan

Associate Professor, Department of Molecular Reproduction, Development and Genetics, Indian Institute of Science, Bangalore 560012, India. Email: anu@mrdg.iisc.ernet.in

Normal epithelial cells require attachment for cell growth; matrix-detachment triggers a form of apoptosis termed as 'anoikis'. In contrast, acquisition of anoikis-resistance is a hallmark of solid tumors that facilitates survival of cancer cells in circulation, as well as growth at distant sites. Therefore, understanding the molecular mechanisms that contribute to anoikis-resistance is likely to identify novel therapeutic targets to curb cancer spread. Recent studies have, interestingly, uncovered that even within the normal epithelia, there exists a very small population of cells that can survive matrix-detachment and generate three-dimensional structures when cultured in suspension in vitro. Such floating spheroids generated by normal human mammary epithelial cells (HMECs) have been termed as "mammospheres" which are enriched in stem/progenitor cells. Furthermore, studies in the past decade have revealed that even within cancers, only the sub-population of *cancer stem cells* that are enriched in stemness properties appear to be capable of generating 3-dimensioanl spheroids in vitro and tumor initiation in vivo. Thus, anchorage-independent growth appears to be linked with stemness properties. Therefore, we exploited the 'mammosphere' system to try to decipher the molecular mechanisms that contribute to anoikis-resistant growth. Signaling mechanisms that enable cancer cell growth in 3-dimension, their links to stemness and drug resistance, as well as strategies to target them will be discussed.

PL 3 Cervical cancer screening in India

Surendra S. Shastri, MBBS; MD; DPh; DHA

Department of Preventive Oncology, Tata Memorial Hospital, TMC, Mumbai.

Despite a large burden of cervical cancer developing countries are not in a position to set up organized cytology-based cervical screening programmes due to lack of infrastructure and trained personnel, and issues related to quality assurance and costs. In the last one decade VIA and HPV testing have been tested in a number of developing countries. At least three randomized controlled trials have demonstrated a significant mortality benefit following cervical screening by either VIA or HPV testing. A single round of VIA screening performed by trained nurses in Dindigul district of Southern India resulted in a significant 35% reduction in mortality from cervical cancer. A single round of HPV testing performed by technicians in Osmanabad district in western India, showed a significant 48% mortality reduction. Another RCT from Mumbai district also in western India demonstrated a significant 31% cervical cancer mortality reduction after 4 rounds of VIA screening performed by trained PHWs at 24-month interval. These studies present 3 attractive options for cervical screening in developing countries. In situations where only a single round of screening is feasible, the best option would be HPV-DNA testing. In situations where adequate number of trained nurses could be deployed to 'screen and treat', VIA testing followed by colposcopy and cryotherapy should be the choice. The third and most cost-effective and widely implementable strategy for cervical cancer control in developing countries would be four rounds of biennial VIA screening by PHWs. The Indian States of Tamil Nadu and Sikkim have already started statewide organized cervical screening with VIA. Neighboring country Bangladesh is also piloting a VIA-based national cervical screening program.

IS 17 Clinical aspects and advances In the treatment of locally advanced head and neck squamous cell carcinoma

Victoria Villaflor

Department of Medicine, University of Chicago, USA

Head and neck squamous cell carcinoma (SCCHN) worldwide is the 6th leading cause of death. There are approximately, 550,000 new cases annually. In the USA there are approximately 55,000 new cases with approximately 12,000 deaths annually [1, 2]. Most of these patients will present with locally or locoregionally advanced disease. Many of these patients will be offered curative intent therapy using multidisciplinary approaches including surgery; radiotherapy and chemotherapy [3]. Sadly, cure rates do remain poor for locoregionally advanced SCCHN not related to Human Papillomavirus (HPV). Concurrent chemoradiotherapy has become a standard of care for patients with locoregionally advanced, non-metastatic disease. Concurrent chemoradiotherapy demonstrated an 8% absolute improvement in 5-year overall survival of concurrent chemoradiotherapy over radiotherapy alone [4]. Additionally, high objective response rates with neoadjuvant chemotherapy have been observed demonstrating chemotherapy-sensitivity in locoregionally advanced SCCHN. None-the-less, the same meta-analysis demonstrated on 2.4% improvement in 5-year overall survival not statistically significant [4]. Further studies evaluating neoadjuvant chemotherapy using a three-drug regimen of docetaxel, cisplatin, and 5-fluorouracil (TPF) compared to cisplatin and 5-fluorouracil have demonstrated an advantage to TPF [5, 6]. Further studies, including DECIDE and PARADIGM have failed to demonstrate a statistically significant benefit with neoadjuvant chemotherapy compared to no neoadjuvant chemotherapy but, are often criticized for early closure, and heterogeneity of patients [7, 8]. Hence, neoadjuvant chemotherapy remains controversial. More recently, there has appeared to be an increase in HPV-positive oropharyngeal cancers. HPV-positive patients who have a 10 pack year or less of tobacco use, have a greater than 80% 5 year overall survival [9]. These improved outcomes have suggested that toxic multimodality treatment regimens may be de-intensified in order to prolong survival while minimizing toxicity. Previous studies have suggested that response to induction chemotherapy is a reliable prognosticator for outcome following definitive treatment. ECOG 1308, a phase II study involved 90 patients with stage III/IV HPV-positive oropharyngeal squamous cell carcinoma was recently presented at ASCO 2014 meeting. The study stratified patients based on response to neoadjuvant chemotherapy to receive either 70 or 54 GY IMRT. At 2 years, overall survival favored the low-dose group over standard-dose (93% vs 87%) and progression–free survival (80% vs 65%) also favored low-dose group [10]. These results are provocative, and further study is ongoing evaluating the role of reduced therapy in this subset of HNSCC patients.

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IS 18 Can a single dose of HPV vaccination prevent cervical neoplasia?

R. Sankaranarayanan, M.D.

Special Advisor on Cancer Control (CCO); Head, Early Detection & Prevention Section (EDP) and Screening Group (SCR), World Health Organization - International Agency for Research on Cancer (WHO-IARC), Lyon, France.

World Health Organization recently recommended a two dose Human Papillomavirus (HPV) vaccine regime targeting 9-13 year old girls for implementation in national immunization programs (NIP). Currently 8 countries are implementing a two dose HPV vaccine schedule in their NIPs. There is currently a great interest if a single dose of HPV vaccine will be effective in preventing cervical neoplasia based on some findings in observational studies involving girls/ women who defaulted their prescribed dosage. It is an interesting proposition, given the potential cost savings, ease of administration/ delivery logistics and scaling-up of vaccination. Results from a Costa Rican study suggested that 1 or 2 doses of HPV vaccine are as protective as 3 doses against incident HPV-16 or -18 infections that persisted for 1 year. Early results from an Indian study are consistent with the Costa Rica results. In the Indian study, 20,000 girls aged 10-18 years were randomized to two groups to receive 3 or 2 doses of quadrivalent HPV vaccine. However, due to the unfortunate suspension of vaccination midway through recruitment because of events unrelated to this study, four groups evolved by default: 4,950 girls received one dose; 3,452 received 2 doses (days 1 and 60); 4,979 received 2 doses (days 1 and 180+) and 4,348 received 3 doses (days 1, 60 and 180+). These girls are being followed up for immune response, frequency of persistent HPV infection and cervical neoplasia. Although the immunogenicity of 1 dose was clearly inferior to that of 3 doses (ATP) at 12, 18, 24 and 36 months from the last dose, it was higher than following natural infection. The frequency of incident infection based on cervical cells from 1288 married women and persistent infection (based on cervical cell samples from 171 married women) for vaccine targeted HPV infections were similar across the different dose groups. No persistent HPV 16 or 18 infection was found in the 171 girls from whom cervical cells were available. Long-term follow-up of girls who received only one dose by default in national programs and in research settings will clarify the cervical cancer preventive prospects associated with a single dose of HPV vaccination.

IS 19 Explosive rise of mouth cancer in India

Prakash C. Gupta

Director, Healis - Sekhsaria Institute for Public Health, Navi Mumbai.

Oral cancer is a major of cancer in India as revealed by cancer registry data. The overall trend does not show any specific increase over time. Due to reports of an increasing prevalence of oral submucous fibrosis, a highly pprecancerous condition, an increase in the incidence or oral cancer has been predicted. Since the increase in oral submucous fibrosis in more among young individuals, it was postulated that increase in oral cancer would also occur first among young persons. A comparison of the age specific incidence rates of mouth cancer (ICD 143-5) during 1983-87 and 1995 in the city of Ahmedabad showed that the incidence had significantly increased in the younger population (< 50 yr). From recent data sets, age-specific incidence rates of mouth cancer (International Classification of Diseases [ICD]-9:143–5; ICD-10:C03–06) in five year age groups among men aged ≥15 years for the city of Ahmedabad for years 1985, 1995, 2007 and 2010 were extracted from published reports. For comparison, lung cancer (ICD-9:169; ICD-10:C33–C34) rates were also abstracted. A cohort approach was used for further analysis of mouth cancer incidence. Age adjusted incidence rates of mouth and lung cancer for men aged ≥15 years were calculated and compared. The age specific incidence rates of mouth cancer among men increased over the 25-year period while lung cancer rates showed a net decrease. Using a cohort approach for mouth cancer, a very rapid increase in younger age cohorts was found. Mouth cancer incidence increased markedly among men in urban Ahmedabad between 1985 and 2010 but not the lung cancer so this increase is not due to smoking. The reason for this unprecedented increase in oral submucous fibrosis and oral cancer is clearly due to increasing consumption of areca nut products especially gutka in Ahmedabad and all over India. Gutka has now been banned all over India, but a more vigorous implementation is necessary.