B2B-2K5: The new buzz in translational research*

Translational research is simply research that results in the development of new and improved medical treatments. The theme that discoveries made at the laboratory bench can lead to the delivery of more effective care at the hospital bedside, was captured in the catchy and alliterative subtitle 'B2B-2K5' during the recently held conference. This report is a brief overview of the 30 talks delivered at this conference by some of the leading clinicians and scientists from India and the United States.

Imaging technologies leave magical impressions, as anybody who has had an X-ray, ultrasound, CT or PET scan can attest. Jason A. Koutcher (Memorial Sloan-Kettering Cancer Center, New York City) described the recent progress made in applications of magnetic resonance imaging (MRI) and magnetic resonance spectroscopic imaging (MRSI). MRI can now be used to scan the whole mouse and detect the presence of hepatic tumours. Three-dimensional ^{31}P-MRSI can be used to detect the levels of individual metabolites (e.g. nucleotide triphosphates, phosphotidyl ethanolamine) in individual 'voxels' (volume pixels) of the liver. This allows one to determine when normal metabolism is restored following surgical resection of liver cancers. This is important for the administration of adjuvant chemotherapy. If chemotherapy is given too early it can lead to killing of the regenerating liver cells, but waiting too long post-surgery can allow the tumour to re-grow and the therapy would no longer be effective. MRSI can also be used to stage prostate cancers based on the levels of citrate and choline. Citrate levels are relatively high in the normal prostate, whereas high choline is an indication of rapidly proliferating tissues such as tumours. By measuring the levels of choline and citrate in individual voxels, one can localize where a tumour might be located in the prostate. This would allow biopsy to be directed to that location, rather than taking a random biopsy sample. About 130 patients examined in this way, who subsequently underwent radical prostatomy, revealed that MRSI had a sensitivity of 85–90% for high-grade tumours. The sensitivity was not as good for lower grade tumours. The approach is not without pitfalls. For example, spectral degeneration is seen in the region of haemorrhage and conditions like prostitis can cause false positives.

Irradiated tumours can serve as a source of tumour-associated antigens. Alan Alfieri (Albert Einstein College of Medicine, New York City) described the exciting possibilities of radiation-induced autologous tumour vaccination. Local tumour radiotherapy (60 Gy) was combined with injection of the cytokine Flt3-ligand (Flt3L) in a murine model of Lewis lung carcinoma. Dendritic cells (DCs) are known to present antigen from apoptotic cells, and Flt3L is known to expand DCs *in vivo* and thus was expected to improve antigen presentation from dying, irradiated tumour cells. Alfieri *et al.*, found that radiotherapy + Flt3L reduced pulmonary metastases and significantly improved survival in C57Bl/6 mice with established footpad tumours. Mice treated with Flt3L alone showed delayed tumour growth, but eventually succumbed to tumour progression. The combination therapy of radiotherapy + Flt3L failed to work in immunodeficient athymic (nude) mice, implicating the role of T-cells in prolonging survival. Thus sequential radiation and immunotherapy with Flt3L to enhance tumour antigen presentation may produce therapeutic responses against disseminated cancer and improvement in survival. Immunotherapy was also the theme of the talk by Ashok Khar (Centre for Cellular and Molecular Biology (CCMB), Hyderabad). He described studies on the spontaneous regression of a rat histiocytoma transplanted subcutaneously in a syngenic host. The major effector of this regression was the NK cell, which induced tumour antibody-dependent tumour cell death via necrosis and apoptosis. His studies showed that immune cells (NK cells, dendritic cells and macrophages) influx into the peritoneum. The NK cells get activated and then migrate back to the tumour to cause cell death.

Radiation also holds promise for facilitating recovery from spinal cord injury in a rat model. Kalderon and Alfieri had previously demonstrated the beneficial effects of X-rays after transection injury to the nervous tissue. They had found that radiation focused on the injury site prevented the degenerative process called cavitation and thus preserved structural integrity and electrophysiological function. Chitti R. Moorthy *et al.* (New York Medical College, Valhalla) did similar investigations, but with a contusion (crush) injury to the rat spinal cord and found

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that they could achieve improved locomotor function if the radiation was delivered early enough. Moorthy also gave two other talks; one, a historical perspective on cancer research and another where he overviewed recent developments in radiation therapy. Among them are the use of a robot in brachytherapy, which would spare medical personnel from accidental exposure. Another was the 4–6 fold enhancement of chemosensitivity of cancer cells when heated to 43°C. Richard J. Zeman (New York Medical College) described other approaches to enhance the recovery of locomotor function in the rat model of spinal cord injury. Ordinarily, injury causes inflammation, which increases the reactive oxygen species (ROS) and results in increased cell death, tissue loss and formation of a glial scar and, ultimately paralysis. Scavenging the ROS using either Tempol, a free-radical scavenger and antioxidant that is already used as a radioprotective agent to prevent radiation-induced hair loss, or Clenbuterol, a beta 2-agonist and anti-inflammatory agent resulted in more of the spinal tissue to be spared and in substantial recovery of locomotor function at the most severe levels of injury. These drugs were effective in a relatively prolonged therapeutic window. Similar results were seen in rats exposed to hyperbaric oxygen.

Moving up from the spinal cord, two talks described investigations with astrocytomas and gliomas. Malignant astrocytomas are among the most resistant tumours to curative treatments. Mean survival without treatment is measured in weeks, and even with treatment, the mean survival is <1 year. Astrocytomas also show extreme heterogeneity within any given tumour. Several oncogenes (growth factors, growth factor receptors, kinases, transcription factors, etc.) and tumour suppressor genes have been identified as important in astrocytoma initiation and progression. Interestingly, protein kinase C (PKC) appears to be over-expressed in the early stages of astocytomas and then the tumour cells lose PKC activity as they progress to higher grades. Deborah L. Benzil (New York Medical College) described studies to determine whether the inhibition of PKC by Tamoxifen (an anti-estrogen at low doses, but a PKC inhibitor at a high doses), retarded progression of low-grade astrocytomas to a higher grade. Encouragingly, 30% of the patients showed good response to this treatment. She also reported studies investigating the effects of anti-angiogenesis agents, including thalidomide. Transferrin is being used for targetted chemotherapy (transmid). Gliomas also are difficult to access, display heterogeneity, respond poorly to radio- and chemotherapy and have variable prognosis. Ravi Sirdeshmukh *et al.* (CCMB) analysed more than 3000 proteins from 30 tumours using a 2DE/MS proteomics approach, to identify about 850 proteins that appeared to be differentially expressed between the tumour and control tissues. They found the molecular chaperone HSP 70 to be down-regulated in many tumours. He suggested that this may correlate with destabilization of the cytoskeleton. Consistent with this idea, they found that glial fibrillary acidic protein (GFAP), an intermediate filament protein, showed aberrant phosphorylation at threonine and enhanced proteolysis in malignant cells. They observed distinct proteolytic forms of GFAP, which suggested that the protein underwent a regulated proteolysis. Thus GFAP proteolysis may serve to grade astrocytomas. Other differentially expressed proteins include the tumour suppressor protein prohibitin, and Rho-GDP dissociation inhibitor (Rho-GDI), a regulator of Rho proteins involved in cell growth regulation. It would be of interest to test whether the different molecular patterns observed in different tumours correlate with their clinicopathological differences.

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide. HCC risk is increased 100-fold in people with Hepatitis B viral infection and up to 900-fold in cirrhosis patients. The talk by Marcelo Facciuto (Westchester Medical Center and New York Medical College) described the criteria for eligibility for an orthologous liver transplant as well as several ways to control tumour growth during the wait for suitable donor tissue. These include ethanol injection and chemo-embolization of the arteries and living donor liver transplant. In living donor transplantation, it becomes imperative to optimize graft/host sizes to avoid 'small for size' syndrome (if the graft is too small) and graft versus host disease (if the graft is too large). Hepatocyte transplantation can ameliorate radiation-induced liver damage. Alfieri described studies in which rats were subjected to heavy irradiation of the liver, after which tagged autologous hepatocytes were introduced and were found to be capable of restoring full liver functionality. This work suggests that autologous transplantation of tagged hepatocytes sorted by magnetic cell separation or flow cytometry may be useful for patients with diffuse liver disease. In yet another talk, Alfieri described more applications of flow cytometry and laser scanning cytometry in cancer research. In particular, evaluating the extent of apoptosis on the basis of peaks indicating sub-G1 DNA content and dUTP-FITC incorporation to directly view DNA breaks. These tools have been used to monitor the effectiveness of chemotherapy in leukaemia.

Many cancers result from the formation of tumour-specific chromosomal changes, specifically translocations. Moreover the translocation breakpoints often correlate with the location of oncogenes. Suresh Jhanwar (Memorial Sloan-Kettering Cancer Center) gave several examples of cancer-associated chromosome aberrations that were instrumental in the identification of cancer genes. For example, chronic myelogenous leukaemia (CML) is caused by a translocation that leads to the formation of a consitutively active gene for a tyrosine kinase fusion protein called Bcr-Abl, which affects the cell signalling pathway. Acute promyelocytic leukaemia is due to translocation of the retinoic acid receptor with a novel gene *PML*, resulting in the inhibition of differentiation at the promyelocytic stage. Somatic mutations in many of the cancer genes identified in this way, are found to be responsible for sporadic cases of these cancers. For example, familial renal cell carcinoma (RCC) is seen in families segregating the translocation T(3; 8) (p14; q24). It was found that all the cancer cells had lost the derived chromosome 8 (der 8), which suggested that the cancer was caused by loss of heterozygosity for a locus on chromosome 3, in the region distal to the translocation breakpoint. When normal cells from patients with familial RCC were transfected with Ha-ras, cells showing the transformed phenotype had lost the gene in chromosome 3 that was later identified to be *VHL*, the gene for von Hippel–Lindau syndrome. The *VHL* gene is mutated in many sporadic cases of RCC. Levy Kopelovich (National Cancer Institute, Bethesda) described studies by his group on clinically normal cells from persons with conditions that impart a dominantly heritable risk for cancer (e.g. mutant tumour suppressor gene). They detected 'one-hit' RNA expression profiles, including aberrations of the clinically normal cells in a gene-specific manner,

in heterozygous individuals. Examples are microarray cluster analysis of different forms of renal cancer carcinoma caused by mutations in TSC2 (tuberous sclerosis 2) versus VHL (von Hippel–Lindau syndrome), and TP53 (Li-Fraumeni syndrome). This has significance to the discovery of cancer preventive agents. They also looked at highly malignant cells in fluctuation studies to isolate clones with different phenotypes. The clones are being studied for their response to chemopreventive agents. Stably normal clones are tagged with GFP/RFP, orthotopically implanted in recipient mice and their progression in the absence and presence of chemopreventive agents is monitored in real time. This may result in the discovery of chemopreventive agents.

E. Premkumar Reddy (Temple University School of Medicine) delivered the Gold Medal Address. He described recent studies on PLK-1 (polo-like kinase 1), a serine/threonine protein kinase that plays a regulatory role in microtubule assembly. During mitotic spindle assembly PLK-1 associates with the centrosomes, probably affecting tubulin polymerization, and is found in the mid-zone during telophase. Mutations in the *Drosophila* PLK-1 homologue showed blockage at different steps in mitosis. Overexpression of PLK-1 in NIH 3T3 cells was shown to result in a transformed phenotype and PLK-1 was also found to be over-expressed in many human tumours. Esophageal cancer patients with high levels of PLK-1 expression had a significantly poorer prognosis than patients with low expression. Antisense oligonucleotides and siRNA directed against the PLK-1 mRNA induced mitotic defects which led to apoptosis. All these results suggested that PLK-1 acted as an oncogene. Knockdown of PLK-1 results in mitotic arrest, spindle abnormalities and apoptosis. ON 01910 is a small molecule kinase inhibitor with potent activity against PLK-1 in assays that used CDC25c or casein as substrate. ON 01910 was found to compete for the substrate-binding pocket of PLK-1 and the inhibition was irreversible. Normal cells treated with ON 01910 did not undergo apoptosis, presumably because the presence of a checkpoint arrested them in G1. Treatment of a broad spectrum of human tumour cells, including those that are multi-drug resistant, with ON 01910 causes spindle and centrosome abnormalities that led to apoptosis. Inhibition and regression of tumour growth by ON 01910 was also seen in xenograft models of human cancers such as hepatic, pancreatic, colon and breast tumours and in lymphoma. ON 01910 caused complete remission when used in combination therapy with adriamycin. Indeed, it appeared to counter the weight loss seen with the use of adriamycin. This suggests that drugs that promote G1 arrest will overcome the toxic side effects of chemotherapy. ON 01910 also blocked other kinases such as Flt-1 and Met, and thus could be effective against tumour invasiveness and angiogenesis. It has been approved by the US FDA in phase-I clinical trials.

Geeta K. Vemuganti *et al.* (L. V. Prasad Eye Institute, Hyderabad) are developing cell-therapy approaches for the treatment of corneal diseases. She described the reconstitution of the corneal epithelium on an amniotic membrane support by co-culturing segments excised from the limbal region of the cornea of an unaffected eye together with conjunctival cells, using a small ring to separate the two cell types. The resulting cell mono-layer could then be used for transplantation and reconstruction of the ocular surface. Among 250 patients treated in this way, the success rate was 70%. Vemuganti *et al.* are also exploring the trans-differentiation of adult bone marrow stromal cells into retinal cells. Vijay Anand P. Reddy (Apollo Cancer Hospital, Hyderabad) described the treatment of introcular tumours (retinoblastoma and melanoma). His group uses ruthenium (Ru-109) radioactive plaques to deliver a high dose of radiation to a tumour and a negligible dose to the surrounding tissue. Brachytherapy is the procedure in which the radiation source is implanted onto the tumour. The success rate for his group was 16/17 eyes.

Clinical trials are essential to address questions of efficacy and toxicity of cancer therapies. D. Raghunadharao (Nizam's Institute of Medical Sciences, Hyderabad) discussed the conduct of clinical trials as attempts to answer scientific questions, while adhering to the principles of law, morals and ethics to protect the participants of such trials. He highlighted the problems arising from the general lack of awareness of both the scientific and ethical principles in India. S. H. Advani (Raheja Hospital, Mumbai) described the impressive gains in survival achieved by moving anti-cancer therapy from targetting DNA replication in dividing cells to interfering with a specific molecular target, usually a protein, that has a critical role in tumour growth or progression. Imatinib mesylate (phosphorylation blocker from Novartis) which targets tyrosine kinase and is used to treat CML and metastatic gastrointestinal stromal tumours, was a prime example. HER2/neu inhibitors like geftinib and trastuzumab are used to target tyrosine kinase receptors like EGFR and used in the treatment of breast cancers. Thalidomide blocks the interaction of plasma cells with bone marrow stromal cells, which causes bone resorption in multiple myeloma. Angiogenesis inhibitors like bevacizumab target VEGF and are used in metastatic colon cancer, and monoclonal antibodies like rituximab, an anti-CD20 antibody, are approved for B-cell non-Hodgkin's lymphoma. Thus by understanding the molecular changes that underlie cancer development, the malfunctioning molecules and pathways can be targetted to achieve more effective and rational therapy. Purvish M. Parikh (Tata Memorial Hospital, Mumbai) discussed the clinical use of imatinib to neutralize the tyrosine kinase activity, of the bcr-abl protein in CML, which heralded the era of targetted therapy. However, this drug caused dermatological side effects, and also resulted in myelosuppression, and imatinib resistance correlated with specific mutations in tumour cells. Real time PCR revealed that residual molecular disease persisted in patients treated with imatinib alone. Another example of dosage and schedule modification studies was gemcitabine, a pro-drug that needs to be activated in the body by deoxycytidine kinase. This enzyme gets saturated in 30 min. Therefore, the effect of prolonging the infusion time rather than increasing the dose of the drug was examined. This also results in the saving of as much as 66% of the cost of treatment.

Several talks described studies of a relatively more basic kind. Meena Jhanwar-Uniyal (New York Medical College) described a microarray-based study to unravel the genetic basis of cancer metastasis. In various malignancies, metastasis is inversely related to the abundance of nm23 protein expression. Jhanwar-Uniyal *et al.* used cDNA microarrays to identify novel genes and functional pathways in nm23-mediated spontaneous breast metastasis. The experiments were performed in a pair of cell lines derived from human mammary carcinoma cell line MDA-MB-435, namely C-100 (only vector transfected; highly metastatic) and H1-177 (nm23 transfected; low metastatic). They

found significant and consistent alterations in the expression (up- and down-regulation) of 2158 genes in a total of almost 20,000 genes between the high and low metastatic cells. The results suggest that the mechanism of action of metastasis suppression may involve nm23-induced down-regulation of genes associated with cell adhesion, motility (e.g. integrins alpha2, -8, -9, -L and -V, collagen type VIII alpha1, fibronectin 1, catenin, TGF-beta2, FGF7, MMP14 and 16, ErbB2) and certain tumour/metastasis suppressors (two members of SWI/SNF-related matrix-associated proteins 2 and 5 and PTEN). They also found that metastatic cells showed a greater expression of Rho, which would predict that inhibition of Rho, say by NFkB, might reduce the metastatic potential of cancer cells. Moni Abraham Kuriakose (Amrita Institute of Medical Science, Kochi) described a microarray-based profiling of tumour-specific gene expression that was done to recognize genes that might identify novel therapeutic targets in head and neck squamous cell carcinomas (HNSCCA) and oral cancers. HNSCCA tumour tissue and normal mucosal tissue were harvested at the time of surgery from patients with histologically confirmed HNSCCAs and cDNA libraries were constructed from the paired surgical specimens. Biotinylated RNA was transcribed from the cDNA library and hybridized to high-density microarrays containing approximately 12,000 human genes. Altered gene expression of HNSCCAs was identified by comparison to corresponding normal mucosal tissues and subjected to hierarchical clustering of data. Analysis revealed that the gene expression profiles could distinguish the tumours from non-malignant tissues. Reproducible gene expression changes were observed in 227 genes representing previously identified chemokines, tumour suppressors, differentiation markers, matrix molecules, membrane receptors, and transcription factors that correlated with neoplasia, including 46 previously uncharacterized genes. Significant expression of a collagen gene and a novel gene was reproducibly observed in the tumours, whereas these genes were virtually undetectable in their corresponding adjacent nonmalignant tissues. Kuriakose *et al.* now aim at combining gene chip technology with bioinformatics to develop a database of molecular taxonomy of the tumour. This could identify sensitive molecular markers for early tumour detection, prognosis, and novel targets for interceptive therapeutics.

Gopal C. Kundu (National Centre for Cell Science, Pune) spoke on Osteopontin (OPN), a chemokine-like extracellular matrix (ECM) protein that appears to induce degradation of the ECM in breast cancer and melanoma models by regulating cell motility, invasiveness and tumour growth. OPN was shown to regulate NFkB-mediated activation of pro-MMP-2, a promatrix metalloproteinase. OPN does this by stimulating IkB phosphorylation and degradation and this step can be inhibited by curcumin and might account for curcumin-induced regression of a transplanted melanoma. OPN also enhances the levels of nuclear factor inducing kinase (NIK) that results in the increased activity of urokinase-type plasminogen activator, a widely acting serine protease that degrades the ECM. In breast cancer cells, OPN was shown to activate PI-3 kinase and MAPK signalling pathways. These effects of OPN underlie its regulation of cell motility and invasiveness. According to Kumaravel Somasundaram (Indian Institute of Science, Bangalore), many cancers, in particular ~75% of invasive breast cancers, use epigenetic means (e.g. cytosine methylation) to turn-off expression of the transcription factor activator protein-2 alpha (AP-2a), which functions as a tumour suppressing pro-apoptotic factor. This causes cancerous cells to become insensitive to chemotherapeutic drugs that act via the induction of apoptosis. Treatment of such cancer cells with 5AzaC turns on AP-2a and restores the cancer cells to chemosensitivity.

Other highlights of the meeting were an inaugural session in which N. K. Ganguly (Indian Council of Medical Research, New Delhi) spoke on some of the challenges that confront medical research in India. For example, India has 600 districts but only about 20 field epidemiologists. Diagnostic kits developed by Indian scientists are not being used for any of the following reasons; they are not cost-effective, or they are not heat-stable, or they cannot be handled in the rural areas. Although in principle, the incidence of cervical cancer can be brought down by therapeutic vaccination against human papilloma virus or by on-site colposcopy and cryosurgery, sociological factors make it difficult to do so in a village. We are also weak in clinical trials and have never done trials on new entities. We need to have a research/policy interface. We lack the infrastructure to say, test GM foods, or answer questions such as 'what is the burden of cardiovascular disease in Karnataka?'. On the other hand, Lalji Singh (CCMB) pointed out the opportunities offered to basic and clinical scientists by the vast and diverse Indian population. Singh also touched upon the revolution in genetics that is going to transform clinical practice, particularly for diseases that run in families, but do not follow Mendelian rules.

This meeting was attended by about 400 participants, 57 of whom presented their work as posters. For more information on this and future meetings, contact ushaks@ccmb.res.in, vradha@ccmb.res.in or moorthycr@yahoo.com

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