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COMMENTARY

From molecules and cells to diseases: West meets East for medical research in Tianjin

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The second Australia-China Biomedical Research Conference was held at Nankai University in China's north from 24-27 April, 2009. Students from a number of China's famous universities turned out to welcome almost 100 Australians and more than 200 Chinese scientists, researchers, university leaders and policy makers. The research findings presented at the conference, which included 16 plenary lectures and 8 symposia, covered basic to clinical investigations in the areas of stem cell biology, mechanisms of cancer, immunity and viral infection, membrane trafficking and neuroscience, cardiovascular and metabolic disorders, protein structures and microRNAs, intracellular signalling, biotechnology and drug development.

Infection and immunity

With the emergence of the swine flu threat at about the time of the conference, a presentation on how the immune system has evolved to deal with viral infection was very timely. Nobel Laureate Professor Peter Doherty, University of Melbourne, told the conference that the concept of immunological memory had been known since the time of the great plague in Europe, when it was noted that only those who had recovered from infection could safely tend new victims. He went on to describe the immunology for which he was awarded the Nobel Prize, namely describing the role of MHC molecules and how they evolved to present peptides to the immune system, in particular viral peptides to CD8 T cells. He reviewed his own work on immunity, which supported the conclusion that protection against viral infections is mediated primarily by circulating antibodies [1].

Metabolism and management of hormones

Medical researchers today are familiar with concept of translational research, given the interest in this area from both industry and funding agencies. However, as the conference heard, this view is somewhat restricted. Professor John Funder, Director of Research Strategies at Southern Health of Victoria, spoke of translational research, noting that while the bench-to-beside picture is true, it is too narrow. Translational research encompasses not only basic science, clinical care, but also health promotion, disease prevention and health policy. Professor Funder also expressed the view that insights from population studies and patient care should feed back to the lab, to inform the questions asked by scientists [2]. He presented three examples of clinical studies in the areas of hormones, high blood pressure and heart failure that have prompted a radical re-evaluation of how we think about a particular area of cardiovascular endocrinology.

First, a mutation in the mineralocorticoid receptor (MR) provided the basis for reclassification of the order in which the steroid hormone receptors branched off the ancestral receptor protein. Second, MRs are normally activated by aldosterone, but not the glucocorticoid cortisol. In clinical trials in progressive heart failure, pharmacological blocking of MRs with low doses of spironolactone in addition to standard therapy produced a remarkable 30% improvement in survival. In damaged tissue such as the failing heart, raised cortisol levels activate MR, explaining why spironolactone was effective. Professor Funder and his team have shown that MR inhibitors only need occupy a small proportion of available MR to produce their protective effect, suggesting that the effect is due to induction of protective genes and/or repression of harmful genes. So, clinical studies have led to a re-evaluation of the evolution of MRs, the role of aldosterone in heart failure, the action of cortisol on MR in damaged tissues and finally how an MR antagonist protects cardiac tissue [3].

Stem cells but not so stemness when effective in brain repair

Neuroscientists and stem cell researchers have grappled with longstand-

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ing questions surrounding the possible existence of brain stem cells and the cell populations that might function in this role. Professor Perry Bartlett, Director of the Queensland Brain Institute told the conference that his group has identified the first true hippocampal stem cell population. The cells are activated to divide and produce neurons only after stimulation by neural activity *in vitro* and *in vivo*. Although a number of latent progenitors are activated by prolonged neural excitation, as few as eight precursors per hippocampus possess the cardinal properties that define stem cells [4]. Transgenic animals in which these neurogenic precursors are eliminated before the learning of spatial navigation tasks lose the ability to learn such tasks.

It seems that there are limited numbers of residential stem cells in deep brain and that some peripheral stem cells may have a brain-homing capacity under particular conditions. The involvement of the blood-brain barrier in brain stem cell biology is supported by the presence of a stem cell niche in the subventricular zone and neural stem cell interaction with cells of the blood-brain barrier [5, 6]. Like foetal stem cells, mesenchymal stem cells (MSCs) appear to have the capacity to home to the maternal brain [7]. Professor Hsiao Chang Chan, Director of the Epithelial Cell Biology Research Center at the Chinese University of Hong Kong, said that the lack of significant immunogenicity of MSCs and their freedom from ethical controversies made them attractive potential sources of stem cells [8]. She described her findings of increased cell survival rates and efficiency of neuronal differentiation using dedifferentiated MSC-derived neuron progenitor cells.

Surprises from particular genes in brain development and malfunction

Evidence is accruing concerning the regulation of gene expression that directs cell development in the brain. Professor Yi-Zheng Wang, Head of the Laboratory of Neuronal Signal Transduction at the Institute of Neurosciences, Chinese Academy of Sciences, Shanghai, presented his discoveries that the transient receptor potential canonical channel TRPC6 is necessary to promote synapse formation. The TRPCs are Ca^{2+}-permeable, non-selective cation channels localized to the excitatory synapses of neurons. Professor Wang reported that the mechanism by which TRPC6 promotes excitatory synapse formation involves the signalling pathway from the calcium- and calmodulin-dependent protein kinase IV to the transcription factor CREB signalling pathway. Mice overexpressing TRPC6 have both enhanced spine formation, and spatial learning and memory [9].

Continuing the theme of specific genes and their association with neural diseases, Professor Peter Schofield, Director of the Prince of Wales Medical Research Institute reported a significant link of chromosome locus 15q25-26 and susceptibility to bipolar disorder in 35 Australian multi-generational families [10]. He and his team have so far genotyped 376 single nucleotide polymorphisms at 15-kb resolution across the high-priority region and 20-kb resolution across the remaining 6.2-Mb interval in an Australian case-control cohort. The gene ST8SIA2, previously associated with schizophrenia in Japanese and Chinese patients, was identified as a putative bipolar susceptibility gene within the 15q25-26 linkage region [10].

Conquering cancer

Reporting on oesophageal squamous cell carcinoma (ESCC), Professor Ming-Rong Wang, Vice-President of Cancer Institute (Hospital), Peking Union Medical College, Chinese Academy of Medical Sciences told the Conference that ESCC, an aggressive malignancy with poor prognosis, harbours frequent amplification and overexpression of the CTTN, PLK1 and PRKCI genes, with PLK1 overexpression being an independent poor prognostic factor. Professor Wang presented evidence that PLK1 inhibits the mitochondrial apoptosis pathway through a mechanism involving survivin, whereas conformal radiotherapy regulates the transcription of CTTN through STAT3, providing molecular targets in oesophageal cancer [11].

The mutant genes behind the development and progression of colon cancer appear to be relatively few. Professor Tony Burgess, Director of the Ludwig Institute for Cancer Research in Melbourne showed that a handful of mutations dominate the landscape of oncogenic lesions in the colon: apc, β-catenin, ras, PI3 kinase, B-Raf, TGFR and MLH1, however, signalling from the EGFR, FGFR, TGFR systems, several G-protein coupled receptors, notch and even cytokine receptors also appeared to influence the behaviour of colon cancer cells [12].

ATM, the product of the defective gene that causes ataxia-telangiectasia, plays a central role in cancer-related DNA damage [13]. Professor Martin Lavin provided new insights into the mechanism of ATM action. ATM binds DNA double-strand breaks (DSB) through the Mre11/Rad50/Nbs1 complex that senses the break in DNA. Once ATM is recruited and activated by autophosphorylation on DSB, it phosphorylates a number of substrates including Nbs1 and Rad50. Professor Lavin presented evidence that mutation of the phosphorylated site S635 on Rad50 allowed ATM activation but disrupted downstream signalling. He presented evidence that ATM-dependent phosphorylation of Rad50 plays a role in minimizing the risk of genome instability and, as a consequence, cancer and other pathologies.

In the future, therapeutics based on DNA may provide an alternative

to traditional chemotherapy, immune and surgical treatments for melanoma and non-melanoma skin cancers, the most common types of cancer among Caucasians [14]. Professor Levon Khachigian, Director of the University of NSW Centre for Vascular Research, presented evidence that the DNAzyme Dz13, which targets the bZIP transcription factor c-Jun mRNA, inhibits tumour growth in animal models. He demonstrated the capacity of Dz13 in a clinically suitable liposomal formulation to cause regression of melanoma growth in mouse models. Growth suppression by Dz13 was mediated through inhibition of c-Jun and numerous c-Jun-dependent pro-angiogenic and tumourigenic genes.

Based on studies on the BH3 mimetic ABT-737, targeting pro-survival Bcl-2 proteins has potential for the treatment of incurable lymphoid tumours [15]. Associate Professor Andrew Roberts of the Walter & Eliza Hall Institute of Medical Research presented his recent team's work, which showed that ABT-737 was active in cells that overexpressed Bcl-2 and/or Bcl-x_L, but ineffective as a single agent if the same cells also overexpressed Mcl-1, the antiapoptotic member of the BCL-2 family. Professor Roberts concluded that ABT-737 is highly active *in vivo* against murine c-Myc-driven lymphomas that overexpress Bcl-2. ABT-737 when combined with standard cytotoxics overcomes chemoresistance and is curative [16].

T cells matter

T lymphocytes, or T cells, have a function to temper initial innate immune response for protection, according to Prof Hong Tang, Head of the Center for Infection and Immunity, Chinese Academy of Sciences. The discovery was made from the analysis of cellular interactions between innate and adaptive immunities. Prof Tang reported that T cells are both necessary and sufficient to temper the initial innate immune response. While immunocompromised hosts often die from acute infection, presumably due to the lack of pathogen clearance by the adaptive immune response, the innate immune response can be a direct cause of death in the absence of residential T cells. The mechanism of the T cell effect involves not only natural regulatory T cells but also resting $CD4^+CD25^-Foxp3^-$ or $CD8^+$ T cells. Professor Hong Tang discussed that T cells play a critical role in protecting neonates from lethal over-active innate responses [17].

Th17 cells are a subset of $CD4^+$ T helper cells that produce interleukin 17 and induce tissue inflammation and autoimmunity. Prof Gang Pei, President of Shanghai Tongji University presented new findings concerning the role of Th17 cells in multiple sclerosis (MS). Working on the β-arrestin gene, which is important in G-protein-coupled receptor trafficking and signalling in immune cells [18], Professor Gang Pei and his team unveiled a surprising mechanism whereby the non-coding sequence of the β-arrestin gene gives rise to microRNA that regulates a set of transcription factors and cytokines in the regulation of Th17 differentiation. Whereas β-arrestin 1 is involved in regulating T helper cell function and autoimmunity through epigenetic mechanisms, the Th17 cell-associated intronic microRNA (miR-X) is expressed at high levels in MS patients and mice with experimental autoimmune encephalomyelitis. Various lines of evidence indicate that Th17 cells are key players in MS pathogenesis, which involves a regulatory mechanism by the microRNA from the β-arrestin gene.

γδ T cells differ from the bulk of T cells in their T cell receptor. Found primarily in the gut mucosa, the role of γδ T cells is poorly understood. Professor Zhinan Yin, Dean of Nankai University College of Life Sciences presented his studies that revealed unique features and functions of γδ T cells in regulating tumour immune surveillance. Professor Yin and his team found that reconstitution of $TCR\beta^{-/-}$ mice with Vγ4, but not Vγ1, γδ T cells restored the anti-tumor response in which IFN-γ and perforin were the critical elements. Mice enriched in the Vγ4 subset of γδ T cells through deletion of Vγ1 γδ T cells had increased resistance to tumor growth.

Continuing on the theme of T cells, Professor Ban Hock Toh of Monash University presented evidence that natural killer T (NKT) cells, particularly $CD4^+$ and double-negative NKT cells, have pro-atherogenic activity in the $ApoE^{-/-}$ mouse model of human atherosclerosis. Professor Toh and his team found that removal of NKT cells by thymectomy at neonatal day 3 in $ApoE^{-/-}$ mice resulted in markedly reduced atherosclerotic lesions when the mice were fed a high-fat diet. The effect was reversed by adoptive transfer of total NKT cells or by the transfer of $CD4^+$ (but not double negative) NKT cells [19].

Cell death – good, bad and the mechanisms

A number of presentations provided evidence that apoptosis is a predominant mechanism for the elimination of cancer cells. However, apoptosis and cell survival are also important processes in both healthy and arrhythmic cardiac functions in the hearts of humans and mice. Professor Baofeng Yang, President of Harbin Medical University, provided evidence that miR-1 and miR-133 are involved in regulating the fate of cardiac cells. Increased miR-1 and/or decreased miR-133 levels favour apoptosis. Conversely, decreased miR-1 and/or increased miR-133 levels favour survival of cardiomyocytes.

MicroRNA miR-1 reduced the levels of HSP60 and HSP70 proteins without changing the levels of their transcripts, whereas miR-133 repressed caspase-9 expression at both the protein and mRNA levels. Professor Yang provided evidence that miR-1 is overexpressed in patients with coronary artery dis-

ease, and that overexpression of miR-1 in normal or infarcted rat hearts was found to exacerbate arrhythmogenesis. His data supported the suggestion that miRNAs are a potential anti-arrhythmic target [20].

Dr David Huang discussed how the interactions between the three fractions of the Bcl-2 protein family determine whether a cell lives or dies. The pro-survival family members Bcl-2, Bcl-x_L, Bcl-w, Mcl-1 and A1 maintain cell survival, in large part by keeping the pro-apoptotic cell death mediators Bax and Bak in check. Unrestrained, Bax and/or Bak mediate organellar damage, principally disrupting the outer mitochondrial membrane to destroy mitochondrial function and trigger the release of pro-apoptotic factors such as cytochrome c into the cytosol. Cell stress or damage signals activate the BH3-only proteins, such as Bim, Bid and Bad, the other pro-apoptotic Bcl 2-related sub-family of proteins. They trigger apoptosis by inactivating the pro-survival proteins, and perhaps also directly activating Bax and Bak, resulting in apoptosis.

By investigating the molecular interactions between the three subfamilies, Dr Huang was able to show that pro-survival Bcl-x_L is the limiting factor for the survival of platelets, through its action in restraining the pro-apoptotic Bak. He proposed that the Bcl-x_L:Bak interaction is critical for determining platelet lifespan. Because Bak has a longer half-life than Bcl-x_L, Dr Huang suggested that investigation of the stability of Bcl-x_L, particularly the unstructured loop region between the first and second α-helices of Bcl-x_L, may be important for understanding how this pro-survival protein regulates platelet lifespan [21].

Mitochondria are dynamic organelles, undergoing fusion, fission and branching. The fusion process is mediated by the mitofusin family. Professor Quan Chen of Nankai University presented his novel findings on two members of this family, Mfn1 and Mfn2. Both proteins are normally polyubiquitinated and degraded by proteasomes. Treatment of cells with the proteasome inhibitor MG132 was found to increase Mfn1 and enhance mitochondrial fusion at the microtubule-organizing centre. Professor Chen and his team have identified the membrane-anchored ubiquitin E3 ligase Ring Finger Protein 5 (RNF5) for which Mfn1 is the substrate. Knockdown of RNF5 caused upregulation of Mfn1 and induced mitochondrial fusion, while overexpression of wild-type RNF5 caused downregulation of Mfn1, associated with enhanced ubiquitination and mitochondrial fragmentation. Professor Chen concluded that Mfn1 protein levels were finely tuned by an RNF5/polyubiquitination-dependent proteasomal pathway that regulates mitochondrial dynamics, and thereby cell survival and death.

The author was regretful to the speakers whose work was presented at 2nd Australia-China Biomedical Research Conference but not pertinently discussed in this commentary.

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