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Meeting Report

A Sino–British frontier workshop of cancer biology

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As part of the collaboration between the British Medical Research Council (MRC) and representatives of the Chinese scientific community, 12 scientists specifically interested in death/life decision-making met in Shanghai. The goal of this workshop, co-sponsored by the MRC, the State-key Lab of Oncogenes and Related Genes of Shanghai Cancer Institute and the journal 'Cell Death & Differentiation', was to present the latest developments in apoptosis research, with a focus on how these discoveries can improve our understanding of cancer pathogenesis and lead to new therapeutic approaches.

The workshop began with a talk by Karen H Vousden (Beatson Institute for Cancer Research, Glasgow, UK) that focused on the role of p53 in metabolism. The apoptosis field started to develop around the time when the p53 tumour suppressor gene was discovered, some 30 years ago. Not that p53 plays a mechanistic role in every cell death event, but rather that the loss of its function in malignant cells profoundly alters their propensity to undergo apoptosis. Recent discoveries from Karen Vousden's laboratory and others have revealed a new function for p53 in the regulation of glucose metabolism and oxidative stress, thus bridging metabolism and cell death regulation. These novel p53 activities appear to play a key role in tumour suppression, and shed new light on the pathways that underlie the metabolic changes in cancer cells. It appears that the differential regulation of p53 target genes determines the choice of response. For instance, under mild stress conditions, low levels of p53 are sufficient to induce the expression of several genes involved in cell cycle arrest, DNA repair and protection against oxidative stress, thus allowing cell survival. In contrast, after extended stress or irreparable DNA damage, elevated levels of p53 lead to induction of pro-oxidant genes and concomitant repression of several antioxidant genes, thus causing elevated intracellular ROS levels and cell death or senescence. Based on this model, loss of p53 function in normal cells increases intracellular levels of ROS, which damage DNA and increase the mutation rate. In this context, failure to induce p53-dependent cell death, cell cycle arrest or DNA repair leads to tumorigenesis. A p53-inducible gene named TIGAR (TP53-induced glycolysis and apoptosis regulator), has been shown to function to lower the intracellular levels of fructose-2,6-bisphosphate (Fru-2,6-P2), a substrate that promotes glycolysis by activation of 6-phospho-1-kinase, a key enzyme in the glycolytic pathway. Enhanced TIGAR expression lowers Fru-2,6-P2 levels, thereby slowing glycolysis and directing glucose to an alternative pathway, the pentose phosphate pathway (PPP). The activation of the PPP leads to the production of NADPH, which is needed for the removal of ROS by reduced glutathione. Thus, through TIGAR, p53 can lower cellular sensitivity to ROS-associated apoptosis, potentially contributing to the survival of cells undergoing mild, reversible stress. In addition, TIGAR expression can also play a role in modulating autophagy, with an increase in autophagy following inhibition of TIGAR expression in nutrient-starved cells. It seems possible that the ability of TIGAR to promote PPP, lower ROS levels and enhance survival might contribute to the genesis of cancer. In support of this proposal, Vousden's study has found overexpression of TIGAR in a number of cancer cell lines (regardless of p53 status) and in a significant proportion of primary malignant colon cancers.

Regulation of metabolism in cancer was also the main subject of the presentation given by Xin Lu (Ludwig Institute for Cancer Research, Oxford, UK). She discussed an apoptosis-stimulating protein of p53 (ASPP) family member ASPP2, which was discovered more than 10 years ago as a binding partner of p53. Its role as a positive regulator of p53-mediated apoptosis has been clearly established *in vitro* in previous studies from her laboratory, but its physiological importance *in vivo* has only just emerged through the generation and characterization of *ASPP2*-deficient mice. New data from Xin Lu's laboratory suggests that ASPP2 may connect autophagy to senescence and therefore be a novel target for autophagy-based therapy.

Of course, p53 no longer stands alone, and its cousins may have an important hand in regulating cell survival. Gerry Melino (MRC Toxicology Unit, Leicester, UK) presented new interesting data about the role of the p53 family member, p63. Phylogenetically, p53 is considered to be the latest addition to the gene family, explaining its primary involvement in tumour

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suppression whereas the oldest, p63, appears to be largely involved in development. In particular, p63 is involved in the conservation of stemness in epithelial tissues. Specifically, the micro RNA miR-203, through its ability to regulate ΔNp63 expression level, appears to be a key molecule in the control of p63-dependent proliferative potential in epithelial precursor cells both during keratinocyte differentiation and in epithelial development. The activity of p63 also depends on its steady state protein levels. In this respect, Melino's laboratory has identified the Hect-containing E3 (Nedd-4-like) ubiquitin-protein ligase Aip4/Itch as responsible for the proteosomal degradation of both p73 and p63. The PY motif-containing C-terminal region of p73/p63 binds to the WW domain of Itch, resulting in their ubiquitination and degradation. In addition, his group has also identified a regulator of Itch activity, NEDD4-binding protein 1 (N4BP1), which is therefore able to regulate the function of Itch's substrates.

A component of the p53 tumour suppressive network is the promyelocytic leukaemia protein PML. A novel role for PML in the nervous system was discussed by Paolo Salomoni (MRC Toxicology Unit, Leicester, UK) who found that in the developing neocortex the expression of PML is restricted to the level of neural progenitor/stem cells and is present in a complex with the tumour suppressor retinoblastoma protein (pRb) and the protein phosphatase 1 (PP1), and triggers pRb dephosphorylation. In this way, PML modulates cell fate during neocortical development by regulating cell cycle progression in concert with pRb. This opens up the possibility that alterations of PML function, as revealed in human brain tumours, may originate from the neuroepithelial compartment of the nervous system.

After lunch, the workshop continued on a different tone with a talk by Sir David Lane (IMCB, Singapore), co-discoverer of p53, who has developed a new model to study the p53 pathway in Zebrafish (*Danio rerio*). In a normal cell, levels of p53 increase rapidly following stresses such as UV treatment and ionizing radiation. However, there is little genetic evidence to show if p53 is necessary for organogenesis during embryogenesis. In Zebrafish, p53, as shown for p63 and p73, exists as multiple isoforms derived from either alternative splicing or the usage of an alternative promoter. One of the p53 isoforms, Δ133p53, is derived from an alternative promoter in intron 4 of p53 gene. However, little is known about how Δ133p53 expression is regulated and what kind of biological function it plays. He presented recent studies on the *def* (digestive-organ expansion factor) gene and the *def*^{hi429} mutant in zebrafish. The expression of *def* in these animals is enriched in the digestive organs at later stages of organogenesis. Histological analysis and *in situ* hybridization showed that the initiation and early development of digestive organs are not obviously altered in the *def*^{hi429} mutant. However, at a later stage, all digestive organs display hypoplasia. Studies using organ-specific markers showed that cell differentiation does occur but organ expansion and maturation are compromised in the mutant. Surprisingly, detailed studies showed that the expression of Δ113p53, a counterpart to the human isoform Δ133p53, is selectively upregulated in the def^{hi429} mutant. The increase in Δ113p53 expression, limited to the mutant digestive organs, selectively induced the expression of p53-responsive genes to trigger cell cycle arrest but not apoptosis, resulting in compromised organ growth in the mutant. Thus, while induction of p53 and/or its isoforms is crucial to suppress abnormal cell growth, Δ113p53 is tightly regulated by the organ/tissue-specific factor def, especially during organogenesis, to prevent adverse inhibition of organ/tissue growth.

Recently, RNA interference (RNAi) has emerged as a promising therapeutic weapon. The advantage of RNAi lies in its high specificity and potent gene silencing, coupled with the fact that it can potentially target every gene, and every cell has the necessary machinery. But, is siRNA therapy possible *in vivo*? a question addressed by Erwei Song (Sun-Yat-Sen University, Guangzhou, China) in his talk. The main obstacle to developing siRNA as a small-molecule drug is the *in vivo* delivery of RNAi oligonucleotides across the cell membrane to the cytoplasm where it can enter the RNAi pathway and guide sequence-specific mRNA degradation. Song's group designed a protamine-antibody fusion protein to deliver siRNA to HIV-infected or envelope-transfected cells. The fusion protein (F105-P) was designed with the protamine coding sequence linked to the C terminus of the heavy chain Fab fragment of an HIV-1 envelope antibody. siRNAs bound to F105-P induced silencing only in cells expressing HIV-1 envelope. In addition, siRNAs targeted against the HIV-1 capsid gene gag inhibited HIV replication in hard-to-transfect, HIV-infected primary T cells. Intratumoral or intravenous injection of F105-P-complexed siRNAs into mice targeted HIV envelope-expressing B16 melanoma cells, but not normal tissue or envelope-negative B16 cells; injection of F105-P with siRNAs targeting c-myc, MDM2 and VEGF inhibited envelope-expressing subcutaneous B16 tumours. Furthermore, an ErbB2 single-chain antibody fused with protamine delivered siRNAs specifically into ErbB2-expressing cancer cells. This study demonstrated the potential for systemic, cell type-specific, antibody-mediated siRNA delivery.

Members of the tumour necrosis factor receptor (TNFR) superfamily are important for cell growth and survival. In addition to providing costimulatory signals for cell proliferation, ligation of both TNFR1 and Fas results in apoptosis. Mian Wu's (University of Science and Technology of China, Hefei) presentation focused on CD27, a member of the TNFR family that binds to Siva, a proapoptotic protein, and induces apoptosis. Siva1 is able to interact with HDM2 and can therefore release p53 from negative control leading to effective stabilization of p53 and activation of the p53 pathway.

Jingde Zhu (Shanghai Cancer Institute, China) discussed use of DNA methylation for cancer detection, presenting his latest results on DNA methylation profiling of urine sediments for sensitive/specific detection of bladder cancer, DNA methylation profiling for the staging and classification of liver cancer, and the MBD affinity approach to genome-wide methylation profiling of human cancer cells.

The complexity of tumour angiogenesis has been discovered in the primary tumour of clear cell renal carcinomas (CCRCC), where two distinct types of microvessels have been identified: undifferentiated (CD31(+)/CD34(−)) and differentiated (CD34(+)) vessels. Chao-Nan Qian (Sun-Yat-Sen University, Guangzhou, China; Van Andel Research Institute, Grand Rapids, USA) showed that these

distinct types correlate with contrasting prognoses, and this may influence the therapeutic effects of anti-angiogenic therapy.

Xuebiao Yao (University of Science and Technology of China, Hefei), using a combination of mass spectrometry-based identification with siRNA-based target protein manipulation, discussed the molecular dissection of centromere plasticity in cell division.

Mesenchymal stem cells (MSC) are one of the most promising adult stem cell types due to their availability and the relatively simple requirements for *in vitro* expansion. They have the capacity to differentiate into several tissues, including bone, cartilage, tendon, muscle and fat, and produce growth factors and cytokines that promote hematopoietic cell expansion and differentiation. *In vivo*, MSCs are able to repair damaged tissue from kidney, heart, liver, pancreas and gastrointestinal tract. Furthermore, they also have anti-proliferative, immunomodulatory and anti-inflammatory effects, and this ability of MSCs is activated by proinflammatory cytokines. Yufang Shi (Institute of Health Science, Shanghai Institutes for Biological Sciences, China) discussed the mechanism underlying the immunosuppressive effects of MSCs. Interestingly, dendritic cells exposed to apoptotic cells are strongly immunosuppressive *in vitro* and *in vivo.* It was further discovered that the apoptotic cell-sensitized dendritic cells are highly sensitive to IFN-γ-induced production of NO, which plays a key role in apoptotic cell-mediated immunosuppression.

The workshop ended with a closing lecture by Doug Green (St Jude Children's Research Hospital, Memphis, USA). He was scheduled to speak on 'mitochondria and cell death regulation', however, he asked us in the audience to decide which among three different talks we wanted to hear. After a quick vote, one of the three talks was selected: 'the induction of immunological tolerance by apoptotic cells'. His work mainly focused on how caspases and mitochondria can regulate the immune response. In particular, his group has identified the 75 kDa subunit of respiratory complex I as a critical caspase substrate in the mitochondria. Its cleavage leads to disruption of electron transport, loss of mitochondrial transmembrane potential, decline in ATP levels, loss of mitochondrial structural integrity and production of reactive oxygen species (ROS), which are critical to tolerance induction by apoptotic cells. ROS oxidize the potential danger signal high-mobility group box-1 protein (HMGB1) released from dying cells and thereby neutralized its stimulatory activity. Apoptotic cells failed to induce tolerance and instead stimulated immune responses by scavenging or by mutating a mitochondrial caspase target protein when ROS activity was inhibited. Similarly, blocking sites of oxidation in HMGB1 prevented tolerance induction by apoptotic cells. These results suggest that caspase-orchestrated mitochondrial events determine the impact of apoptotic cells on the immune response.

This meeting gave a succinct update on mechanistic and translational aspects of cell death regulation in cancer covering topics within immunology, neurology and oncology as well as growth and aging. Although several mechanisms have been resolved, as numerous speakers highlighted during their talks, many questions still remain and more arise as new theories emerge.