Meeting Report

The Controlling Cancer Summit, 17-19 May 2016, London, UK

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Abstract

The Controlling Cancer Summit is an intimate informal meeting that annually gathers international academic and clinical researchers to network and debate the current advancements and challenges of oncology research. This year it focused not only on diagnostic/prognostic biomarkers and genetic influences in cancer, but also novel and sometimes unconventional therapeutic interventions. This report will summarise the meeting highlights that contribute to our comprehension of cancer biology and new innovative ways to target this disease.

DNA damage and repair

TP53 is activated by DNA damage, and consequently initiates a cascade of events leading to DNA repair or programmed cell death. It is therefore crucial for

suppressing tumourigenesis, and several talks at the summit were dedicated to p53 function.

Dr. Leandro Castellano from Imperial College London presented findings describing the role of p53 in miRNA processing and function. Using isogenic HCT116 ($TP53^{+/+}$ and $TP53^{-/-}$) cell lines, the group identified that variable direct association of p53 with AGO2 (a catalytic component of the RNA-induced silencing complex) regulates its interaction with miRNA during DNA damage response, triggered by doxorubicin. Various oncogenic mutant forms of TP53 decreased miRNA binding to AGO2, compared to wild-type $TP53^{1}$, suggesting that p53 regulates miRNA-mediated post-transcriptional gene repression in cancer.

Platinum agents, such as cisplatin or dicycloplatin, are widely used in cancer therapy and also trigger DNA damage repair pathway. Dr. Jing Jie Yu of Mary Babb Randolph Cancer Centre demonstrated a direct relationship between p53 activation in response to platinum therapy and checkpoint kinase 2 (Chk2) phosphorylation. Using transient overexpression of *TP53* in ovarian cancer cell lines, the author showed doubling of Chk2 phosphorylation. Similarly, siRNA-mediated knockdown of p53 significantly reduced Chk2 phosphorylation. Importantly, inhibition of Chk2 with a small molecule inhibitor enhanced efficacy of cisplatin *in vitro*, suggesting a role for such compounds in targeting resistance to platinum agents.

Temozolomide is another DNA-damaging agent, widely used in treatment of brain cancer, but prone to swift development of resistance. Prof. Anthony Berdis of Cleveland State University shared his findings on tackling this issue using metal-containing nucleosides. Following analysis of several nucleoside analogues, one analogue demonstrated consistent insertion opposite cytidine and thymine; and a corresponding nucleoside revealed potent anti-proliferative effects in haemotological

and adherent cancer cell lines. These findings were validated *in vivo* using solid cancer subcutaneous xenografts[2]. Given the unique ability of this analogue to be incorporated in the lesions created by temozolomide, misreplication was inhibited, thereby enhancing temozolomide's therapeutic window.

Exosomes: broad applications in cancer

In our stride for personalised medicine, as a cancer research community, we have explored the power of biomarkers for targeted treatment, genetic testing and more recently, "liquid biopsies" to investigate the potential of circulating tumour DNA and exosomes in cancer. Exosomes are small membrane vesicles (30-100nm) hosting specific proteins and lipids, mRNA and miRNA, and can be released by any cell type[3]. Given numerous functional properties of exosomes, such as molecule transport and cell-cell communication, they received a lot of attention in cancer research as stimulators of immune response and vehicles for drug delivery. Both of these areas were discussed at the summit.

Prof. Susanne Gabrielsson from Karolinka Institute described the role of exosomes derived from dendritic cells (dexosomes) in stimulating the immune system. Dexosomes are capable of stimulating several types of immune response, and together with molecule-carrying ability, they comprise an appealing putative cancer treatment. Using various T and B cell knockout mouse models, Prof. Gabriellson's group showed that dexosomes loaded with MHC class I and II peptides were not sufficient to elicit CD4+ or CD8+ T cell responses and that immune stimulation depended on functional B cells[4]. *In vivo* CD8+ T cell activation was superior when dexosomes were loaded with a whole-molecule antigen, rather than a peptide, as dexosomes

containing whole ovalbumin (OVA) protein offered better protection against OVA-overexpressing tumour. This tumour-specific response was amplified upon co-loading of exosomes with alpha-galactosylceramide, a glycolipid that induces a rapid activation of invariant natural killer T cells[5]. Interestingly, Prof. Gabrielsson's work challenges the central role of MHC class I in exosome stimulation of T cell response. In a B16/OVA mouse melanoma model, allogeneic and syngeneic exosomes stimulated similar percentages of T cells infiltrating the tumour. The author hypothesised that dendritic cells most likely take up both types of exosomes, consequently inducing antigen-specific T and B cell response, thereby bypassing the requirement for patient's own exosomes[6].

Another function of exosomes discussed at the summit was its potential to deliver anti-cancer compounds. Brain cancers are among the deadliest cancers and are particularly hard to reach with a systemic therapy due to blood-brain barrier. Yet neural stem cells, such as human mesenchymal stromal stem cells (MSC), have been shown to infiltrate glioma cells in the brain. Prof. Chaya Brodie of Bar-Ilan University demonstrated the use of these stem cells in therapy against brain tumours. Expression of select miRNAs demonstrated therapeutic potential in many *in vitro* models, but this approach still lacks effective delivery *in vivo*. Specifically, miR-124 and miR-145 overexpression in glioma (stem) cells was reported to significantly decrease cell migration and self-renewal *in vitro,* respectively[7]. The author suggested that miR-124 regulates cell migration via laminin γ 1 and intergrin β 1, whereas differentiation was altered by its effect on CDK6. Inhibitory effects of miR-145 were attributed to its repression of Sox2 and Oct4. In glioma xenografts, successful delivery of these fluorescently labelled miRNAs (and also pre-miRNA) enclosed in exosomes was demonstrated to be gap junction-dependent and cell contact-

independent[8]. Altogether, these findings have a major implication in therapeutic miRNA delivery to the brain in cancer and also in neurodegenerative disorders.

Natural nanomedicines as cancer therapeutics

Of course, in addition to miRNA and siRNA, natural (and synthetic) molecules may also be entrapped in lipid vesicles. There were several natural anti-cancer compounds discussed at the summit and are summarised below.

Dr. Christine Dufès of University of Strathclyde spoke about the therapeutic potential of a green tea extract, epigallocatechin-3-gallate (EGCG). The author firstly demonstrated that 3-diaminobutyric polypropylenimine dendrimer (DAB) conjugation to transferrin resulted in highly selective tumour targeting. Intravenous administration of this dendriplex complexed with TNFa-encoding DNA resulted in sustained tumour regression *in vivo*, using A431 epidermoid carcinoma xenograft model, with 90% complete response and 10% partial regression over a course of one month of treatment[9]. Consequently, the authors encapsulated the natural EGCG in these transferrin-bearing vesicles and used it for treatment of A431 (epidermoid) and B16-F10 (melanoma) xenografts. Tumour suppression reached 40% in both models, with improved overall survival, compared to the control mice[10], thereby highlighting a role for this green tea extract as an anti-cancer therapy.

Anti-tumour activities of cardiac glycosides have been known for decades, yet their molecular mechanism of tumour suppression remained unclear. One such type of cardiac glycosides, named UNBS1450, is extracted from the plant *Calotropis procera*, and Prof. Marc Diederich described its anticancer properties at the summit. The group focused on the effects exerted by UNBS1450 on Bcl-2 anti-apoptotic

proteins. Using adherent and non-adherent cell lines of various cancer types they identified MCL-1 as a key mediator of anti-proliferative properties of UNBS1450. MCL-1 downregulation occurred at the protein level, via proteasomal degradation, and was an essential early step in cell death response mediated by UNBS1450[11]. Furthermore, UNBS1450 triggered accumulation of mitochondria in phagophores, consequently triggering apoptosis in N-type SH-SY5Y cells[12]. These studies provided a detailed mechanism of anti-proliferative action of select cardiac glycosides.

However perhaps the most controversial and widely debated natural anti-cancer compounds are cannabinoids. Over 60 phytocannabinoids have been identified, yet to date there have been only a few attempts at identifying specific cannabinoids harbouring therapeutic potential against cancer and their mechanism of action. Dr. David Meiri of Technion-Israel Institute of Technology presented some early findings using different cannabinoids as anti-proliferative agents in colorectal cancer cell line models. His group identified several cannabis extracts with potent anti-proliferative properties, exerted through G-coupled receptors, CB1 and CB2. Interestingly, Dr. Meiri showed that the delivery method (ingest/inhale) dramatically altered effects of individual cannabinoids in a cancer-dependent manner.

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