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Meeting Report

Second Cell Death Network symposium: the vital cell death

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Second Cell Death Network mini-symposium at Nobel Forum, Karolinska Institutet, Sweden, 24th April 2009.

The Network on the Mechanisms and Biomedical Implications of Cell Death or simply the 'Cell Death Network' (www.celldeathnetwork.com) was proud to organize its second symposium, on 24 April 24 2009, in the prestigious Nobel Forum lecture hall at Karolinska Institutet, Stockholm. Cell death is a broad notion, a process involved in a wide variety of cellular states. Cell death, in the form of apoptosis, necrosis or autophagy, is involved not only in the normal process of tissue renewal and development**,** but also in diseases such as cancer, diabetes, and immunological and neurodegenerative diseases. Thus, the aim of this meeting was to bring together researchers from different fields to discuss different angles of cell death. Local and international invited speakers covered cell death related topics within immunology, endocrinology, oncology as well as molecular signaling.

In his introductory remarks, Sten Orrenius acknowledged that, since the first Cell Death Network mini-symposium was organized at Nobel Forum 1 year ago, research activity in the cell death field continues to be very high and that considerable knowledge of the mechanisms involved has been gained. However, important gaps in our understanding of key events in the pathways triggering cell death still remain. For example, although we all agree that the cytosolic Bcl-2 family member, Bax, is critically involved in mitochondrial outer membrane permeabilization and in the release of cytochrome *c* and other proapoptotic proteins from the intermembrane space, the detailed mechanisms leading to Bax activation, translocation to the mitochondria and insertion into the outer membrane are still unclear. Also, the question of presence of mitochondrial receptor(s) for activated Bax has not been answered yet, although some recent work has suggested that member(s) of the TOM (translocase of the outer mitochondrial membrane) complex are involved in targeting activated Bax to the mitochondria. However, this is still controversial, and may serve as one example of key events in cell death signaling that have still not been resolved. Hence, it is clear that further research is required to characterize the cell death signaling pathways in detail as well as their biomedical implications.

Physiological cell death, which occurs as a continuous byproduct of cellular turnover, is nonimmunogenic or even tolerogenic, thereby avoiding autoimmunity. By contrast, cancer cell death elicited by radiotherapy and some chemotherapeutic agents, such as anthracyclines, is immunogenic. Recent data suggest that innate and cognate immune responses elicited by such anticancer agents are required for an optimal therapeutic outcome, underscoring the clinical relevance of immunogenic cell death. Guido Kroemer presented the 'key–lock' concept for an immunogenic cancer cell death to occur, demonstrating that changes in the composition of the cell surface, as well as the release of soluble immunogenic signals that occur in a defined temporal sequence represent the 'key' required to reveal the immunogenic properties of cancer cell death. This 'key' then operates on a series of receptors expressed by dendritic cells (DC, the 'lock'), which present tumor antigens to T cells and initiate a productive immune response. Immunogenic cell death is characterized by the early cell surface exposure of chaperones including calreticulin and/or heat shock proteins, which determine the uptake of tumor antigens and/or affect DC maturation. Moreover, the late release of High mobility group box 1 (HMGB1), which acts on toll-like receptor 4 (TLR4), is required for optimal presentation of antigens from dying tumor cells.

Further evidences for the immunogenicity of cell death were presented by Anna-Lena Spetz and coworkers. They showed in a mouse model that activated apoptotic splenocytes infected with a MuLV/HIV-1 pseudovirus are immunogenic *in vivo*. Immunization with Con A-activated apoptotic cells infected with MuLV/HIV-1 led to induction of Nef- and p24-specific CD4 + and CD8 + T cells as well as B-cell responses that could neutralize HIV-1. The mice were protected from infection after challenge with MuLV/HIV-1 virus. In addition, they showed that activated but not resting apoptotic PBMCs induce human dendritic cell maturation supporting T-cell proliferation and production of interferon-γ. A recent paper showed that LPS-activated apoptotic B cells induce Th17 cell

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differentiation, whereas resting apoptotic cells induce T regulatory cells (T regs are involved in control of autoimmunity), further supporting a role for certain activated apoptotic cells to promote immunity, whereas resting apoptotic cells instead evoke regulatory mechanisms. Apoptosis is a hallmark of HIV-1 pathogenesis inducing death of both infected and noninfected cells, which may result in a fine balancing act between induction of Th17 *versus* T reg cells after uptake of apoptotic cells during HIV-1 infection.

Embryonal neural tumors in the peripheral sympathetic nervous system and the posterior fossa of the central nervous system, neuroblastoma and medulloblastoma, are the most common malignant solid tumors of childhood. The team lead by Per Kogner has shown that these tumors show deficiencies in the apoptotic machinery adopted from their respective progenitor cells. He reported that the poor outcome is related to metastatic spread and resistance to current chemotherapeutic drugs. P Kogner also presented promising preclinical results on novel targeted therapies developed by his team.

Compounds that induce apoptosis of carcinoma cells can be identified by cell-based screening. Stig Linder's group has developed a screening assay that facilitates the identification of compounds triggering apoptosis of carcinoma cells grown in 3D culture (spheroids). It is hoped that hit compounds from this assay will be effective in the treatment of solid tumors. A number of interesting compounds have already been identified, including various inhibitors of the proteasome system and microtubuli. These compounds are evaluated in combination with cisplatin, a DNA damaging agent that the group has demonstrated to induce premature cell senescence at IC50 concentrations (and not apoptosis). To push forward apoptosis-inducing drugs to animal models and clinical studies the Linder lab has in collaboration with Peviva AB (Bromma) developed M30-Apoptosense, a serum biomarker for apoptosis. The analyte measured in the M30-Apoptosense apoptosis assay is caspase-cleaved cytokeratin 18. A second assay (M65ELISA) can be used to monitor total epithelial cell death. M30/M65 serum biomarkers have been used by a number of laboratories to study drug-induced cell death in xenograft models and in clinical studies.

Adi Kimchi's laboratory has been investigating the importance of autophagy in cancer development and in the therapeutic efficacy outcome of anticancer agents. Autophagy is an evolutionarily conserved process, with both cytoprotective and programmed cell death mechanisms. Beclin 1, an essential autophagic protein, was recently identified as a BH3-domain-only protein that binds to Bcl-2 antiapoptotic family members. The dissociation of beclin 1 from its Bcl-2 inhibitors is essential for its autophagic activity, and is therefore tightly controlled. Kimchi has showed that the death-associated protein kinase (DAPK) is one important regulator of this process. The activated form of DAPK triggers autophagy in a beclin-1-dependent manner. DAPK phosphorylates beclin 1 on Thr 119 located at a crucial position within its BH3 domain, and thus promotes the dissociation of beclin 1 from Bcl-XL and the induction of autophagy. These results reveal a substrate for DAPK that acts as one of the core proteins of the autophagic machinery, and they provide a new phosphorylation-based mechanism that reduces the interaction of beclin 1 with its inhibitors to activate the autophagic machinery rather than apoptosis.

The lab of Lars Holmgren has shown that tumor cells can recycle and reuse tumor DNA from cells that have died off through apoptosis. This recycling, called horizontal gene transfer, occurs when genetic material from a donor cell transfers to and propagates in a recipient cell. This process may allow the tumor cells to accumulate genetic alterations that promote malignant transformation or resistance to therapy. Targeting the tumor vasculature and primarily the endothelial cells have been promising approaches to new cancer therapies. One of the possible advantages is that the target cell is considered genomically stable. However, recent evidences argue otherwise, first, tumor-specific translocations have been detected in the tumor endothelium in patients, and second, endothelium harvested from experimental tumors has been show to be cytogenetically abnormal. The Holmgren lab showed that tumor DNA is also transferred from tumor cells to endothelial cells *in vivo*. Transfer of tumor DNA containing oncogenes as well as genes that dominantly inactivate the p53 pathway in the recipient cell allowed for propagation of the tumor DNA in endothelial cells. FISH analysis showed that these cells contained DNA of both tumor and endothelial origin. Interestingly they maintained their endothelial phenotype and could still form functional vessels when reinjected into mice. The presented data indicate that functional gene transfer between tumor cells and endothelium may be a novel mechanism by which tumors manipulate their microenvironment to support their growth.

Per-Olof Berggren discussed signal transduction in relation to β-cell dysfunction and death in diabetes. In this context he noted that the insulin secretory process is regulated by a sophisticated interplay between glucose and a plethora of additional factors. The function of insulin producing β-cells is modulated by the action of other nutrients, incretin factors, innervation and systemic growth factors, also, autocrine and paracrine regulatory loops within the islet of Langerhans. Although this modulatory role is well appreciated, the underlying molecular mechanisms involved remain poorly understood. Exposure of the pancreatic β-cell to stimulatory glucose concentrations leads to an increase in cytoplasmic free Ca^{2+} concentration, $[Ca^{2+}]_i$, which promotes insulin secretion. P-O Berggren discussed their own findings that serum from patients with type 1 diabetes increases L-type voltage-gated Ca^{2+} channel activity in insulin-producing cells. The subsequent unphysiological increase in cytoplasmic free Ca^{2+} concentration, $[Ca^{2+}]_i$, is associated with DNA fragmentation typical of programmed cell death or apoptosis. Verapamil, a blocker of voltage-gated L-type Ca^{2+} channels prevented these effects. A serum-mediated increase in Ca^{2+}-influx may thus work in concert with the autoimmune reaction associated with type 1 diabetes and contribute to the destruction of β-cells *in vivo*, and thereby aggravate the disease progression. In this context P-O Berggren's group has shown that serum from type 1 diabetic patients contains increased concentrations of apoliprotein CIII (apoCIII). This factor increases $[Ca^{2+}]_i$ and promotes β-cell death. The effects of type 1 diabetic serum and apoCIII on $[Ca^{2+}]_i$ and β-cell death are abolished when β-cells are coincubated with antisera against apoCIII. Finally, P-O Berggren also discussed a novel systems biology

approach that he has developed, enabling studies of pancreatic β-cell function and survival noninvasively, longitudinally at single-cell resolution.

Reconstitution of p53 functions might open new therapeutic avenues against cancer. Galina Selivanova's group has previously identified the p53-reactivating compound RITA (reactivated p53 is required for efficient apoptosis) in a cell-based screen. They undertook an unbiased examination of the molecular mechanism of the activity of RITA by analyzing the gene expression profiles induced by RITA using DNA microarrays. They detected major changes in gene expression in wild-type p53-expressing cells on RITA treatment including the differential regulation of known p53 target genes. In contrast, no changes in gene expression were detected in p53-negative cells were detected. Pathway analysis revealed preferential induction of p53-mediated apoptosis. These results are in line with what was observed in various cell lines following RITA treatment. In many tumor cells, p53 is inactivated by binding to another molecule, MDM-2. G Selivanova reported that RITA blocks the p53/MDM-2 interaction, resulting in MDM2-dependent downregulation of p21 and hnRNPK, providing a switch between apoptosis and growth arrest. G Selivanova showed data, demonstrating that reactivation of p53 by RITA leads to potent inhibition of several oncogenes, for example, antiapoptotic proteins, Akt pathway, c-Myc. The inhibition of oncogenes by p53 reduces the cell ability to buffer proapoptotic signals and elicits robust apoptosis. She concluded her talk by emphasizing the utility of targeting wild-type p53 protein itself as a promising approach for anticancer therapy.

Elias Arnér discussed the several levels of impact on cellular growth, cell death and cancer progression engender by the trace element selenium. He discussed the complexity of timing and concentration of selenium metabolites in relation to the role of selenoproteins, most notably glutathione peroxidase and thioredoxin reductase, and the consequences on cellular growth and tumor development. Knowing that the selenocysteine residue of thioredoxin reductase is a target of several electrophilic anticancer compounds, selenoprotein has been suggested to serve as a molecular target for anticancer therapy. E Arnér showed data, however, demonstrating that a vast complexity of selenium-dependent systems result in drug-dependent effects of thioredoxin reductase targeting, whereby certain drugs were more cytotoxic on reactions with the selenoprotein whereas others became less toxic due to cellular effects exerted by the same selenoprotein. Finally knockdown of the enzyme by more than 90% apparently had little effect on cell viability. E Arnér concluded his talk by stating that 'certain selenoproteins, such as thioredoxin reductase, are clearly important mediators of cell viability or induction of cell death in response to cellular stressors, but the underlying mechanisms are highly complex'.

Concluding remarks

This meeting gave a succinct update on the wide field of cell death and highlighted the importance of this process in biology and medicine. Despite thousands of articles published on the topic, many questions still remain to be answered and many aspects remain to be investigated. Besides presenting promising development in the use and understanding of cell death to cure diseases, this meeting showed the importance of crosstalk between different research fields, studying mechanisms from different angles, cell death signaling cascades. As organizers, we are very pleased with the second Cell Death Network mini-symposium, at Nobel Forum, and especially for the discussion it generated between juniors and more seniors participants, the establishment of collaborative work or more simplistically the exchange of research methods to study cell death. We are looking forward to the next year's symposium and a third round.

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