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Meeting Report

9th Euroconference on Apoptosis

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Apoptosis is implicated in a number of human and animal pathologies including infectious diseases, neurodegenerative diseases, cancer and veterinary diseases the prevention or treatment of which are of major concern for assuring and maintaining of quality of life. Therefore, apoptosis became a major biomedical and biotechnology issue as illustrated by publication statistics: of the total number of papers on apoptosis published over the past 30 years, 25% (13 040 publications) appeared in 2000. In the past decade, the Euroconferences turned out to represent a unique platform for communication between not only European apoptosis experts but also from scientists all over the world. At the 9th Euroconference on Apoptosis, 177 participants from EU-countries as well as Canada, Czech Republic, Hungary, Israrel, Poland, Republic of Korea, Russia, Slovenia, South Korea, Switzerland and USA assembled in Vienna. The specific topics that were addressed comprised cell organelles, DNA damage and cancer, neurodegeneration and preclinical models.

In his keynote address Sten Orrenius emphasized that it has become increasingly clear that apoptosis is an elaborate multilevel process that requires the coordinated involvement of most, if not all, cellular compartments. Depending on the type of injury, one or more cellular compartment, including the plasma membrane, endoplasmic reticulum, Golgi apparatus, lysosomes, cytoskeleton, mitochondrion, and nucleus, normally serve as the key death 'sensor' and trigger the onset of the apoptotic process by engaging a core apoptotic machinery which – according to his view – always involves caspase activation. There are significant interplays among the organelles during apoptosis as exemplified by the link between the death receptor pathway and mitochondria, caspase activation following damage of endoplasmic reticulum and targetting released nuclear proteins to the mitochondria following DNA damage. Mitochondria appear to be the central components in most of the inter-organelle cross-talks during apoptosis and particularly the space between their inner and outer membrane which may vary even in different mitochondrial morphology. He addressed two specific issues related to mitochondria: the significance of a two step process for the release of cytochrome *c* (one from its interaction with cardiolopin and then the release of its soluble forms) and the importance of ATP level in cells to determine exposure of PS on the surface (low level of ATP suppresses aminophospholipid translocase). Thus, different organelles play specific roles in mediating divers or relatively unspecific signals to the final coordinated collapse of cells – or, in view of the meeting site – very much like the highly coordinated, rhythmic movements of dancers performing the Viennese waltz. These aspects were extended in the following session on Cell Organelles.

Cell Organelles

Wilfried Bursch has demonstrated the wide occurrence of autophagic-lysosomal type of programmed cell death death and showed that proteasomes move into the nucleus, possibly providing a link betweeen cytoplasmic and nuclear events during this type of cell death. Using 4D-Nomarski time lapse video microscopy to follow in detail how cell death genes regulate the extent and kinetics of apoptotic cell death and removal, Michael Hengartner showed how engulfment genes cooperate with ced-3 to kill in the *C. elegans* system, especially when a cell is between 'life or death'. Thus engulfment genes not only mediate apoptotic corpse removal, but can also act as pro-apoptotic factors. A possibly similar phenomenon in mammals was reported by Zsuzsa Szondy: tissue transglutaminase knockout mice develop autoantibodies and have a defect in phagocytosis of apoptotic cells. This is because sufficient active TGFβ, which facilitates apoptosis and activates macrophages through an autocrine loop, is not formed by the macrophages. Guy Salvesen summarized the current understanding of caspase activation by lysosomes: it occurs through cleavage of bid by lysosomal proteases. In support of this view, Karin Roberg presented experiments showing that lysosomal proteases are translocated to the cytosol in apoptosis; microinjection of cathepsin D caused caspase activation and apoptosis. The group of Peter Vandenabeele found two new mitochondrial enzymes which are released and involved in the molecular events of apoptosis: endonuclease G mediates caspase-independent DNA fragmentation in the nucleus, the serine protease Omi interacts with XIAP and enhances caspase activity. According to Carmen Garrido apoptosis inducing factor (AIF) is the main player in the onset of caspase-independent apoptosis after heat shock, serum withdrawal, addition of staurosporin or vinblastin and HSP70 can neutralize the effect of AIF. Saskia Lippens showed that the expression of caspase 14 is limited to the ectodermal epithelia and may participate in the

elimination of cellular organelles during terminal differentiation of keratinocytes. In transgenic tobacco plants with BHRF1, a viral member of Bcl-2 family capable to influence mitochondria, spontaneous and pathogen-induced necrotic lesions developed and these plants were more sensitive to the induction of senescence as demonstrated by Artur Pfitzner.

Cancer and DNA Damage

Rolf Schulte-Hermann reviewed the extensive data of liver carcinogenesis pointing out the importance of the rate of apoptosis which is exceeded by proliferation but can be increased by promoter withdrawal, food restriction or TGF-β1 (at least in the early stage of tumor development). Using a *myc*-dependent model in which loss of p53+/− was part of the development of highly invasive lymphoma, Scott Lowe showed that disruption of apoptosis is sufficient to explain the selective pressure to mutate p53 during lymphoma development (Bcl-2 or dominant negative caspase 9 prevented p53 loss from lymphoma but not the development of highly invasive tumors), whereas aneuploidy and defective cell cycle checkpoints are simply byproducts of p53 loss; however, for treatment of tumors the importance of p53 functions other than apoptosis induction became apparent. Christos Paraskeva demonstrated how dietary and pharmacological interventions (butyrate, vitamin D analogs, cyclooxygenase(COX)-2 inhibitors) leading to apoptosis of colorectal cells may be critical in novel strategies for the prevention and treatment of cancer. The survival function and antiapoptotic effect of HGF/Met, including its signalling mechanism, was emphasized by Andrea Rasola. Christine Watson talked about transcriptional regulation of apoptosis in mammary gland *in vitro* and *in vivo*: conditional deletion of Stat3 results in diminished apoptosis and delayed postlactational involution whilst loss of transcription factor IRF-1, a downstream-target, accelerates the first phase of involution of mammary gland. In cultured epithelial cells Stat5 provides a signal for differentiation and survival. According to the results of Manuela Baccarini (based on gene ablation experiments) the main function of raf-1 is to prevent apoptosis and other members of the raf family are responsible for the regulation of cell proliferation. It was reported by Alain Kummer that the presence of the granzyme B inhibitor PI9 may be responsible for the escape of various lymphoma cells from their elimination by cytotoxic lymphocytes. Boris Zhivotovsky showed that non-small cell lung carcinoma cells are resistant to radiation but could be killed in a caspase-independent way by agents triggering mitochondrial dysfunction and the release of AIF. Elena Dudich suggested that alpha-fetoprotein may kill tumor cells via induction of cytochrome *c* release from mitochondria. According to Jiri Neuzil vitamine E succinate is a potent novel anti-neoplastic agent with tumor selectivity and cooperativity with TRAIL. Haya Lorberbaum-Galski and colleagues have successfuly developed chimeric proteins containing proapoptotic participants for killing unwanted cells in the body [Fc_ε-Bax, Fc_ε-Bak, GnRH-Bax, GnRH-Bik, GnRH-Bak, GNRH-DFF40, Il-2-Bax either targeting mast cells (Fc_ε), adenocarcinoma (GnRH), activated T-cells (Il-2)] for killing unwanted cells in the body. Karen Vousden reviewed the current status of how apoptosis is regulated by p53 pointing out p53 inducible genes (particularly the recently described PUMA), transcriptionally independent apoptotic functions (a 37 amino acid peptide derived from p53 efficiently induces apoptosis in their hands) and the potential benefit of developing inhibitors of MDM2 which ubiquitinates and thereby removes p53. Roberta Benedetti described the mechanism of action of two proteins which regulate susceptibility to p53-dependent apoptosis: (1) hGTSE-1 downregulates p53 thereby blocking its apoptosis-inducing and transcriptional ability, (2) Gas2 induces susceptibility to apoptosis in stressed cells by enhancing p53 stability through inhibiting its cleavage by calpain. According to Xin Lu the apoptotic function of p53 can be also regulated by a novel family of proteins termed ASPP (apoptosis stimulating proteins of p53); all members interact with p53, they target p53 to apoptosis gene sites such as Bax and PIG-3, their natural inhibitor iASPP inhibits this function and ASPP1 and 2 are frequently downregulated in human breast carcinoma. DNA repair deficient knockout mice reveal altered apoptosis as demonstrated by Tomas Lindahl: (1) deletion of DNA ligase IV leads to embryonic death with massive apoptosis (involving Atm and p53) of neurons as a result of unrepaired DNA strand breaks; (2) ablation of DNase III results in autoimmunity because of the failure of apoptosis of developing immunocytes. Non-repaired DNA alkylation damage triggers apoptosis and, as sugested by Bernd Kaina, processing of DNA alkylation lesions generate replication-dependent DNA double-strand breaks that act as critical ultimate apoptotic lesions activating the mitochondrial damage pathway but not involving p53. Vincenzo De Laurenzi reported that the expression of a shorter form of p73 negatively regulates the apoptosis-inducing ability of both p73 and p53.

Neurodegeneration

Mauro Piacentini presented results suggesting that that the ablation of tissue transglutaminase prevents cell death in a mouse model of Huntington disease; it looks that this enzyme may play a crucial role in an autophagic type of cell death but not in the formation on nuclear inclusion in Huntington brain tissues. Based on experiments in a toxic (dysplacement of dopamine to cytosol and generation of reactive oxygen species) and genetic model (modelling α-synuclein mutation), a significant role of dopamine in nigral cell loss was proposed by Julie Lotharius. Detailed investigations of the group of John Olney have revealed that the deleterious effect of ethanol (mimicking the effect of NMDA antagonists and GABA mimetic compounds) on the human fetal brain is related to massive apoptosis coinciding with time of synaptogenesis. *Ad libitum* diet seems to be also harmful as shown by Timothy Cowen: it increases vulnerability to free radical induced apoptotic neuronal death in ageing rodents via a mechanism that includes enhanced sensitivity to PI-kinase inhibition.

Preclinical models

The significance of caspase activation in a number of pathologic conditions has become apparent providing the basis of the clinical use of a broad selection of caspase inhibitors. Don Nicholson presented several model systems – including a septic model and Alzheimer disease – for the potential use of such inhibitors. He reported the discovery of a

new family of death domain containing proteins in neurons (examples are HIP-1 and Hippi) which are detached from huntingtin after its cleavage by caspases and synergise in cytotoxicity recruiting and activating procaspase 8. As detailed by Jörg Tschopp the Fas-FasL interaction (when both are in trimeric form) has been shown to play an important role in several diseases (e.g. alcohol-induced hepatitis, graft *versus* host disease, toxic epidermal necrolysis, multiple sclerosis) and the generation of novel inhibitors of Fas-mediated apoptosis has become a major challenge for the development of future therapeutic protocols. Soluble FasL released by metalloproteinases after interaction of FasL with Fas may have beneficial effects. This seems to be also the case when treatment of cerebellar neurons by Aβ peptide leads to FasL- dependent apoptosis: metalloproteinase inhibitors facilitate death as demonstrated by Doug Green. Since hybrids of TNF-deficient and gld mice were known to be resistant towards stroke-induced damage Anna Martin-Villalba and colleagues now used neutralizing antibodies to TNF and FasL and thereby, successfully decreased infarct size and mortality. The results of Ingrid Herr suggest that an autoamplification loop can mediate apoptosis by FasL, Trail and TNF-α involving the stress signalling pathway throgh JNK/SAPKs and c-Jun.

The presentation of the scientific data was supplemented by a session on the EU-Fith Framework Programme (FP5), Christian Krassnig, Mohamed Al-Rubeai and Laszlo Fesus presented ongoing apoptosis research projects and future strategic initiatives of the EU. Taken together, about 15 apoptosis-related projects are currently funded within the FP5 thematic programme 'Quality of Life and Management of Living Resources' mainly within the generic activity 7 'Chronic and degenerative diseases, cancer, diabetes, cardiovascular diseases and rare diseases'. Some links can also be found in the key action 6 Ageing (e.g. Age-related diseases). Furthermore, in the future post-genomic era the major diseases in Europe such as cancer, neurodegenerative diseases, age-related diseases infectious diseases will remain a very important field, good funding opportunities for apoptosis reasearch will be offered within the priority thematic area 'Genomics and biotechnology for health.'

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