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Meeting Report

Back to Stockholm for 'metabolism, epigenetics and cell death'

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19th ECDO Conference on Apoptosis, 14–17 September 2011, Stockholm, Sweden

The last ECDO Conference organized in Sweden (the 6th Euroconference on Apoptosis), an exciting meeting attended by around 150 participants, was in 1998 at Saltsjöbaden in the Stockholm archipelago. Thirteen years later the ECDO Conference is back in the heart of Stockholm City and is the biggest (300 participants) and the most important cell death research meeting hitherto organized in Sweden. Despite the nice weather, the lectures were well attended, thanks to the interesting program and the outstanding speakers.

For the past 9 years, an important event preceding the ECDO meetings is the training course on 'concepts and methods in programmed cell death'. During this course, experts in the field share their knowledge with young fellows. Such an event ensures the continuation of the ECDO traditions as well as the shaping of the next generation of cell death research leaders. To facilitate the participation at these meetings by as many young fellows as possible, several travel fellowships, sponsored by ECDO, were provided. The 9th training course was comprehensive and various methodological problems in cell death research were discussed. Thus, Josef Penninger (Institute of Molecular Biotechnology, Austria) focused on the efforts of his group to use genetic mouse models, in order to understand the impact of the mitochondrial OXPHOS changes and autophagy on the pathogenesis of lung cancer. They were able to identify a novel tumor suppressor gene implicated in multiple cancers that might be involved in the regulation of cell fate and the molecular switch of cells towards necroptosis. Detailed analysis of the cellular response to bioenergetics stress was done by Jochen Prehn (Royal College of Surgeons, Ireland). Importantly, he described various techniques that enable the detection of alterations in cellular bioenergetics both on a population and a single-cell level, and how bioenergetic stress activates complex cell responses, such as the activation of apoptosis or macroauthophagy. A very systematic explanation of the molecular mechanisms regulating autophagy and its assessment in mammalian cells was made by Hans-Uwe Simon (University of Bern, Switzerland). He also clarified how dysregulation of autophagy leads to various diseases and focused on the role of high autophagic activity in adult stem cells. Interestingly, blockage of autophagy in these cells increases their susceptibility towards cytotoxic therapy. Jean-Claude Martinou (University of Geneva, Switzerland) summarized recent progress made on the role of Bcl-2 family members in healthy cells and those undergoing apoptosis. He showed that activation of both Bax and Bak is essential for the fision/fusion of mitochondria. Moreover, the timing between Bax oligomerization at the contact sites of mitochondria and the drop in mitochondria membrane potential does not exceed 10–20s, suggesting an important link between these two processes. Finally, an excellent methodological presentation on how to use high accuracy mass spectrometry-based quantitative proteomics for the large-scale analysis of site-specific protein phosphorylation and acetylation dynamics in autophagy was done by Jens Andersen (University of Southern Denmark, Denmark). All presentations were much appreciated by young fellows who addressed many questions to get more detailed practical information essential for their research. They also asked to keep this tradition to run the courses before the ECDO Conference in the future.

It has become a tradition for the Euroconferences to focus each meeting on specific aspects of apoptosis. This time to celebrate the return to Sweden, we chose 'metabolism and epigenetics' as the theme of the meeting, two fields currently in the cutting edge of cell death research.

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Metabolic Control of Cell Death

The ECDO keynote lecture on metabolism was presented by Sir Salvador Moncada (University College London, UK) who provided fundamental insights on the links between the cell cycle and the metabolic substrates essential for its progression. He presented that the ubiquitin ligase anaphase-promoting complex/cyclosome (APC/C)-Cdh1, controls G1- to S-phase transition by targeting degradation motifs present on proteins promoting glycolysis (e.g. 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase isoform 3) and on proteins regulating glutaminolysis (e.g. glutaminase 1).

Evidence that metabolic adaptations synergize with the tumor microenvironment to select cancer cells for their ability to survive under the harsh conditions often found in solid tumors was presented by Tak Mak (Princess Margaret Hospital, Canada). In this context, carnitine palmitoyltransferase 1C may participate in metabolic transformation by upregulating fatty acid oxidation and ATP production, thereby, becoming resistant to glucose deprivation or hypoxia. He also showed how high DJ1 expression may promote tumorigenesis by reducing the oxidative stress and thereby preventing ROS-induced cell death. Gerry Melino (University of Rome 'Tor Vergata', Italy) described the involvement of p73 in senescence and metabolism. Mice deficient for the TAp73 isoforms showed a significant premature spontaneous aging phenotype. These mice were characterized by unbalanced redox defenses. TAp73 was able to drive the expression of glutaminase type 2, and TAp73-null cells showed clear metabolic defects in the glutamine pathway affecting GSH and redox balance. Eyal Gottlieb (Beatson Institute for Cancer Research, UK) described newly developed approaches to study metabolic transformation of cancer. Utilizing analytical chemistry (metabolomics) and computational approaches, the metabolic adaptation of cells that are deficient for the mitochondrial tumor suppressor fumarate hydratase was studied. In a more generic model of cancer metabolism, links between lipid metabolism (biosynthesis and modification), cell growth (biomass increase) and cell survival was demonstrated. Matthew Vander Heiden (Massachusetts Institute of Technology, USA) showed that there is strong selection for use of the M2 isoform of pyruvate kinase (PK-M2) to metabolize glucose in cancer cells. Together, their findings argue that distinct metabolic phenotypes exist among proliferating cells, and both environmental and genetic factors influence how metabolism is regulated to support tumor formation and growth. Steve Elledge (Harvard University Medical School, USA) showed his group's recent findings on the modular Cullin Ring Ligases (CLR) and the sets of proteins whose stabilities or ubiquitination status is regulated by the CLR family. One such substrate is NUSAP1, a microtubule binding, cell-cycle-regulated protein that is required for resistance to taxol. Nika Danial (Dana-Farber Cancer Institute, USA) reported their results from the analysis of gene expression signatures in a large number of newly diagnosed diffuse large B-cell lymphomas (DLBCL). Two distinct molecular subsets, the 'oxidative phosphorylation' subtype and the 'B-cell receptor/proliferation' subtype could be identified. An integrative approach using proteomics and metabolomics uncovered the first evidence of distinct quantitative differences in the metabolic profiles of DLBCL subtypes and these differences manifest at the level of mitochondrial oxidative capacity, preferential channeling of glucose- and fatty acid-derived carbons to biosynthetic pathways, ROS handling and select toxicity profiles to metabolic perturbants.

Epigenetic Control of Cell Death

The ECDO Keynote Lecture on epigenetics was presented by Michael G Rosenfeld (University of California, USA) and focused on the discovery of two non-coding RNAs, TUG1 and NEAT2, located in Polycomb bodies and interchromatin granules, respectively. Both ncRNAs control the relocation of growth control genes between these two subnuclear structures, depending on the methylation status of Polycomb 2 protein in response to growth signals. These ncRNAs direct the assembly of multiple transcriptional complexes and, thereby, specify growth control gene expression and cell proliferation in a spatially organized manner. Rosenfeld further presented data indicating that nuclear receptor-induced chromosomal proximity and DNA breaks underlie specific chromosomal translocations in cancer.

The results of a genome-wide loss-of-function screen that reveals an important role for the proteasome in HDAC inhibitor-induced cell death were reported by Nicholas La Thangue (University of Oxford, UK). His group also discovered that in cutaneous T-cell lymphoma where HDAC inhibitors have clinical utility, HR23B, which shuttles ubiquitinated cargo proteins to the proteasome and influences autophagy, acts as a biomarker for tumor sensitivity to HDAC inhibitor-based therapy. As such, HR23B represents a striking example of 'bench to clinic' translation. Cancer stem cells are thought to be responsible for tumor initiation, maintenance and spreading. These cells display selective resistance towards conventional therapies. Jan Paul Medema (University of Amsterdam, The Netherlands) described a novel approach to define colon cancer stem cells using a Wnt-reporter construct and observed that stemness is related to chemotherapy resistance and metastasis. Selcuk Colak (Academic Medical Center, The Netherlands) showed that HDAC inhibitors can break cancer stem cell resistance. In colon cancer induction of differentiation of stem cells sensitizes them to drug-induced apoptosis. Treatment with HDAC inhibitor is followed by downregulation of anti-apoptotic Bcl-2 family members and upregulation of pro-apoptotic BH3-only proteins. The ubiquitin ligase RNF8 regulates DNA damage signaling through the ubiquitination of histone H2A. Nico P Dantuma (Karolinska Institutet, Sweden) showed that the chromodomain helicase DNA-binding protein CHD4 is essential for RNF8-mediated chromatin unfolding. This finding suggested a new mechanism of chromatin remodeling-assisted ubiquitination, which involves the cooperation between CHD4 and RNF8 creating a local chromatin environment that is permissive to the assembly of checkpoint and repair machineries at DNA lesions. Jesus Gil (Imperial College London, UK) presented data on the epigenetic control of the *INK4/ARF* tumor suppressor locus, a key executor of cellular senescence. This locus appears to be regulated by the balance between polycomb repressive complexes and the

histone demethylase JMJD3. In addition, Gil reported that ANRIL, a ncRNA also transcribed from this locus, contributes to the epigenetic transcriptional repression of the *INK4/ARF* locus.

Experimental Models to Investigate Cell Death

Three presentations were focused on the description of different experimental models for investigation of cell death mechanisms. Using the worm *C. elegans*, Nektarios Tavernarakis (FORTH, Greece) investigated heat stroke-induced cell death mechanism, which is a highly conserved gene expression program. The removal of heat shock factor-1 (HSF-1), the transcription regulator of the heat shock response, abolished the protective effect of heat preconditioning. By contrast, overexpression of HSF-1 suppressed necrotic cell death triggered by various insults. The small heat shock protein HSP-16.1 was found to be both essential and sufficient for protection against necrosis. Its protective effect is exerted by modulating calcium release from the Golgi apparatus and involves the Golgi-specific Ca^{2+} pump pmr-1. Loss of pmr-1 function abolishes the capacity of hsp-16.1 overexpression to protect against necrosis. Altogether these data suggest that intervention strategies based on selective manipulation of the heat shock response may effectively counteract necrotic cell death. For the first time at the ECDO Conferences the evolution of programmed cell death from plants to man was presented (Peter Bozhkov, Swedish University of Agricultural Sciences, Sweden). Although plant genomes do not encode direct homologs of core apoptotic regulators found in animals, the presence of rigid cell walls in plants restricts the range of cell death pathways (e.g. breakdown of cells into membrane-surrounded bodies and engulfment of dead cells). On the other hand, the lack of an inflammatory response provides plants a possibility of utilizing cell corpses to grow their bodies, as well as to store and transport nutrients. Morphological changes in dying plant cells are classified into two broad classes: vacuolar cell death and necrosis. Molecular components of plant cell death regulation are still not well known and recently were shown to include metacaspases (proteases ancestral to metazoan caspases) and autophagy proteins. Herman Steller (The Rockefeller University, USA) investigated the role of IAPs in the balance between apoptosis and tumor suppression. The detailed analysis revealed apoptosis-related protein in the TGF-β signaling pathway (ARTS) as a pro-apoptotic IAP-antagonist encoded by the Sept4 gene. Expression of ARTS, but not the other non-apoptotic Sept4 isoforms, is frequently lost in a variety of human cancers and its silencing involves DNA hypermethylation of the ARTS-specific promoter and changes in the pattern of histone H3 methylation. Loss of ARTS function in the mouse accelerates the development of tumors, defining ARTS as a crucial tumor suppressor and uncovers a causal link between epigenetic inactivation of ARTS and oncogenesis. ARTS was shown by Sarita Larisch (University of Haifa, Israel) to be frequently lost in human cancers. Analysis of molecular mechanisms of ARTS-induced cell death revealed that it not only acts as an inhibitor of XIAP but also is involved in the initiation of a mitochondria-mediated apoptotic pathway.

Regulation of Cell Death

Guido Kroemer (Institut Gustave Roussy, France) presented recent findings on the usage of cardiac glycosides (e.g. digoxin) to induce the exposure of calreticulin on the cell surface, which, when combined with non-immunogenic anti-cancer agents (e.g. mitoxantrone), led to an immunogenic cancer cell death. To confer better chemotherapeutic efficacy, Andreas Strasser (Walter and Eliza Hall Institute, Australia) examined anti-apoptotic Bcl-2 family proteins and concluded that homozygous or even heterozygous deletion of *Mcl-1* (and not of *Bcl-x* or *Bcl-2*) led to regression of lymphomas and prolonged survival of tumor burdened mice, thereby, identifying Mcl-1 as the most suitable target for therapeutic intervention. Another potent cancer-specific cytotoxicity can be induced by chemical inhibition of sphingomyelinase (ASM) by siramesine. Marja Jäättelä (Danish Cancer Society, Denmark) showed that this compound reverts the MDR phenotype of prostate cancer cells both *in vitro* and *in vivo*. Mauro Piacentini (University of Rome 'Tor Vergata', Italy) showed that Ambra1, in addition to regulating the Beclin 1/class III PI3-kinase Vps34 complex activity, has a role in the late stages of autophagy by interacting and regulating the activity of the lysosomal protein Spinster-1. Ambra1 regulates both Spinster-1 protein stability by modulating its ubiquitination state through its interaction with specific ubiquitin E3 ligases and deubiquitinating enzymes. Paolo Bonaldo (University of Padova, Italy) presented *in vivo* evidence that defective activation of the autophagic machinery has a key pathogenic role in congenital muscular dystrophies linked to collagen VI deficiency. Indeed, skeletal muscles of collagen VI-null mice displayed impaired autophagic flux. New insights into how HIV hijacked dendritic cells (DCs) promote viral dissemination were demonstrated by Marie-Lise Gougeon (Institute Pasteur, France). HMGB1 was shown to be involved in the resistance of HIV-infected DCs to NK-dependent apoptosis. HMGB1 induced the upregulation of the two apoptosis inhibitors c-FLIP and c-IAP2 in the infected DCs. Eleonora Ottina (Innsbruck Medical University, Austria) generated an inducible as well as a tissue-specific transgenic mouse model to knockdown the anti-apoptotic protein A1/Bfl-1. First results suggest that A1 may be important for thymocyte survival during positive selection, for B cell maturation and for the differentiation potential into the granulocytic lineage. The different functions and regulatory mechanisms for caspases were discussed. Inna Lavrik (DKFZ, Germany) reported that stimulation of CD95 can result in the induction of apoptotic and non-apoptotic signaling pathways. c-FLIP, a known regulator of caspase-8 activation, can promote both pro- and anti-apoptotic pathways depending upon its concentration at the DISC. Jose L Venero (University of Seville, Spain) described how the orderly activation of caspase-8 and caspase-3/-7 are involved in microglia activation and how this signaling pathway potentially contributes to neurotoxicity in Parkinson's and Alzheimer's diseases. Rinke Stienstra (Radboud University Nijmegen Medical Centre, The Netherlands) reported that caspase-1 contributes to the development of obesity and insulin resistance. *In vivo,* the absence of caspase-1 protected against both phenomena, suggesting that inhibition of

caspase-1 may represent a useful therapeutic strategy for the treatment of obesity. Daniela De Zio (University of Rome 'Tor Vergata', Italy) suggested that Ku70 is a negative modulator of Apaf1 expression and, thereby, of caspase-9 activation, because of its ability to bind Apaf1 promoter upon DNA damage.

Clinical Implications of Cell Death

Technological advances in isolating and expanding *in vitro* competent cancer stem cells from several solid tumors were presented by Ruggero De Maria (Istituto Superiore di Sanità, Italy). Using a platform that quantifies relevant proteins and post-translational modifications of signaling proteins involved in cell survival, coupled with a functional screening of chemical inhibitors, several pathways active in cancer stem cells that can be targeted for effective therapies were mapped. Walter Malorni (Istituto Superiore di Sanità, Italy) reported that the pathogenesis of immune-mediated vascular diseases is associated with gender. Vascular cells from females showed an estrogen-dependent susceptibility to the disturbance of intracellular redox balance in comparison with cells from males because of the formation of autoimmune antibodies against the Ral binding protein1, a cell surface protein that catalyzes the extrusion from the cell of reduced glutathione conjugates.

ECDO Honorary Lecture

In every ECDO Conference, an outstanding European researcher is presented with the ECDO award for contributions to the field of cell death. This year the ECDO award was given to Professor Klaus-Michael Debatin (University of Ulm, Germany), and in his honorary lecture he focused on the translation and clinical perspectives of cell death research. Many years ago Klaus-Michael Debatin, together with Peter Krammer, discovered a key apoptosis signaling pathway, APO-1/Fas, CD95, followed by the first description of CD95-mediated apoptosis in human leukemia cells. Following this discovery, he has developed strategies using apoptosis modifiers to sensitize resistant tumor cells for cell death induction either by conventional chemotherapy or by novel apoptosis-inducing ligands, such as TRAIL. His group provided evidence for the impact of intact apoptosis signaling by showing that simultaneous analysis of cytochrome c and caspase-3 activation in individual leukemia cells *ex vivo* predicts treatment response and outcome. He presented the identification of specific survival pathways in a NOD/SCID/hu-ALL model that characterize high-risk leukemia with early relapse and fatal outcome. On the basis of his work and that of many groups, molecular insights into apoptosis regulation led to a better understanding of therapy response in conventional treatment, directed molecule-based rational treatment strategies and provided novel targets for therapeutic intervention.

Jürg Tschopp Memorial Lecture

In the beginning of 2011, the cell death community was shocked by the sudden death of Jürg Tschopp, the scientist, whose fundamental research brought striking and almost immediate benefits to patients suffering from several autoimmune disorders. Fabio Martinon (University of Lausanne, Switzerland) presented a Jürg Tschopp Memorial Lecture, describing perspectives on Jürg Tschopp, reminding us of the strong curiosity of Jürg and his determination to change the life of people by understanding the molecular mechanisms of diseases. Dr. Tschopp had an infectious enthusiasm both for small daily discoveries in the laboratory, and for major advances of science. It is likely that his legacy will continue to influence and inspire scientists all over the world for many more years to come.

Next year the ECDO will celebrate the 20th (Jubilee) Euroconference, which will take place in Rome (Italy) on 14–17 September 2012 and we look forward to meet many of you again.

Conflict of Interest

The authors declare no conflict of interest.

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