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Editorial

Novel job opportunities in cell death!☆

Almost 40 years have passed from the historical discovery that cell death in the majority of the cases is not an accidental event, but corresponds to an extremely conscious and altruistic, thus highly regulated, process. Since the first observation in 1972 marked the "birth" of apoptosis, many advances have been made in the comprehension of the implicated mechanisms. Now, we are evolving to a new phase, in which we are learning that apoptosis is not the only paradigmatic form of active cell death. A cell, indeed, depending on its origin and/or the nature of the stimulus, may actively die by executing programs alternative to apoptosis. Autophagy and anoikis are only two of the most cited examples of these sister programs. At the same time, after several years of deep investigations and findings, a critical re-reading of the discoveries related to the apoptosis world has initiated. This is leading to re-evaluate the roles played by apparently well-known actors of the apoptotic process, thus depicturing novel and non-canonical functions for old players and thus contributing to delineate unexpected connections and interactions among different intracellular events.

The meeting "Apoptosis World 2008—From mechanisms to applications" held last January in Luxembourg, gathered more then 750 participants and is the largest international cell death forum covering both fundamental as well as translational research in this field.

This years gathering reflected the current spirit to read novel meanings behind the apparently consolidated notion of apoptosis and its main actors, as well as focusing at the same time to alternative forms of cell death. In this view, the intriguing hypothesis that typical apoptotic regulators/executors may have unrelated functions with respect to their well-known "day job" might contribute to depicture novel roles of apoptotic markers in other intracellular processes, like cell growth, migration and differentiation. Likewise, we are so delineating novel non-apoptotic functions of caspases, particularly of caspase-8, in orchestrating important steps of hematopoietic differentiation or in mediating pro-inflammatory response. During the conference, indeed, it was shown that caspase-8 is recruited at a very early step of differentiation in the formation of a multi-molecular complex required for monocytic/macrophagic differentiation. This occurs without the involvement of any currently known death receptor, thus reinforcing the hypothesis of a non-canonical activation, in addition to a non-apoptotic role for caspase-8 in this process. Similarly, a non-apoptotic involvement of caspase-8 was shown to be required in the intracellular signaling downstream to the activation of specific classes of immune response receptors, as Toll-like receptors family, in order to ensure a correct pro-inflammatory response against pathogens.

Moreover, other non-conventional roles were depictured for typical downstream anti-apoptotic regulators in processes like cell migration and proliferation. A good candidate in the control of cell migration could be XIAP, the best-known member of anti-apoptotic IAPs family. Indeed, it has been shown that XIAP controls upstream the stability of kinases involved in the activation of MAP kinases cascade, in tumor, as well as in primary healthy cells in consequence of cell growth factors stimulation. In this case, XIAP seems to prevent cell spreading and migrations, since an inadequate XIAP expression strongly to exacerbates these phenomena.

Besides not conventional jobs, the meeting was a good opportunity to discuss the non-canonical apoptotic programs that, depending on different cellular and tissue models, cells may undergo. It is emerging that in cells/tissues defective in caspase expression, thus unable to die following caspase-dependent pathways, other proteins may lead to the same pattern of apoptotic features. Intriguing is the case of proteins, such as the serine protease inhibitor LEI. This protein normally plays an anti-protease, thus anti-apoptotic, role in the cells expressing caspases. During the conference it was shown that LEI is able to undergo conformational change, thus a functional conversion, leading to exert an endonuclease activity. This occurs in cells/tissues with caspase-defective pathways. Thus, proteins like LEI may play opposite roles, pro-apoptotic *vs.* anti-apoptotic, by changing completely the nature of the reaction that they control.

Concerning the alternative, non-apoptotic forms of cell death, a growing attention has been focused on autophagy. The mechanisms at the base of this phenomenon are still difficult to interpret. One fact is that a mutually negative interaction exists among the members of autophagic and apoptotic machinery. This leads to a complex interference

☆ Apoptosis World 2008—From mechanisms to applications, Kirchberg, Luxembourg, January 23rd to 26th, 2008.

between autophagic and apoptotic signaling, that makes very difficult to disentangle who controls whom. An interesting approach is to identify novel simple biological models in which one of the two cell death machineries is lacking. As shown during the conference, the protist organism *Dictyostelium discoideum* may represent a valid tool for this aim. Its genome, indeed, typically does not encode executors or crucial modulators of apoptosis, such as caspases and Bcl-2 family members. First studies permitted to define important players in autophagy. It is conceivable to think that the use of these simple biological models might help also to discriminate what is behind the mutual inhibitory phenomenon existing between autophagy and apoptosis.

Regarding canonical apoptotic pathways, it is not surprising that the hottest debates dealt with newly presented data on functionality, activity and specificity of the proteins involved in the apoptotic intrinsic pathway. The mitochondrial front appeared one more time the most promising target for new drugs and therapies. Moreover, BH3-only proteins, the upstream mediator of the mitochondrial pathway was at the center of investigations of a number of talks and posters, that helped to clarify how these proteins are regulated and activated in extremely selective manner from different drugs used to trigger apoptosis.

Reconstituted liposomal systems showed how Bim or t-Bid are the main players of alternative strategies to ensure in any case the mitochondrial outer membrane permeabilization (MOMP). Indeed, Bim mediates MOMP only when high levels of pro-survival proteins are present, whereas in their absence, t-Bid achieves the same goal, thus suggesting that the intracellular protein environment plays a crucial role in determining the alternative involvement of Bim or t-Bid. Moreover, since the substitution of the BH3-domain of Puma, Noxa and Bad with BH3-domain of Bim could change the specificity of interaction, but does not reproduce the same behavior of the native protein in the chimeric constructs, a further function, not yet elucidated, was hypothesized for Bim during the congress. The growing interest of Bim is reflecting the converging opinion that Bim might be the actual earliest upstream mediator of mitochondrial pathway.

Downstream to BH3-only proteins, convincing data were also presented in order to propose a new model for another still unknown mechanism related to Bak oligomerisation. Indeed, as already described for the binding of BH3-only proteins to pro-survival members of Bcl-2 family, the formation of a disulfide-bond between the BH3 domain of one and the groove of another Bak molecule was proposed. What was hypothesized for Bak in Apoptosis 2008 reminds what was described in the previous meeting Apoptosis 2003, also held in Luxembourg, for Bax dimers activation, thus implying that all the members of Bcl-2 family might be able to activate themselves by exploiting the same strategy based on cysteines reactivity.

On one hand, once activated, Bax and Bak promote apoptosis by perturbing the permeability of mitochondria membrane and facilitating the release of cytochrome c; on the other hand, it is also known that Bax and Bak may promote fragmentation of the mitochondrial network. Thus, it has been suggested in the latest years that Bax/Bak-induced mitochondrial fission might be required for cytochrome c release. Surprisingly, during the congress amazing pictures demolished this axiom of apoptosis literature; indeed, it was shown that, even if mitochondrial fission accompanies apoptosis, an enforced mitochondrial fusion or inhibition of fission does not block cytochrome *c* release or other apoptotic downstream parameters. Moreover, it was shown that, even if Bax and Bak activation promotes mitochondrial fission, this phenomenon can be uncoupled from cytochrome c release by pro-survival Bcl-2 family proteins. These data suggest also that the anti-apoptotic protein Bcl-2, independently of the role in cell death control, might be involved in the mitochondrial fission-fusion behaviors, since a perturbation of the Bcl-2 network alters the mitochondrial dynamics. Again, another strong evidence of the existence of alternative non-canonical ''jobs''!

Altogether, this deep knowledge acquired along these latest two decades on apoptosis starts to be exploited for applicative and more general therapeutical aims. Thus, during the conference, a panel of investigations was presented concerning the modulation of apoptosis by natural compounds with novel therapeutic aims. Today, about 25% of the drugs prescribed worldwide come from plants, 121 such active compounds being in current use. During the meeting several new natural compounds have been shown to have pro-apoptotic activities. The use of natural products with therapeutic properties is as ancient as human civilization and, for a long time, mineral, plant and animal products were the main sources of drugs. Among them, (−)-epigallocatechin gallate, the major constituent of green tea, causes induction of apoptosis and cell cycle arrest in many types of cancer cells without affecting normal cells as well as pectic acid which derives from apple, causes apoptosis and necrosis in distinct tumoral cells. Similarly, sanguinarine, an alkaloid present in different plant extracts obtained from *Sanguinaria canadensis* and other plants from the Papaveraceae family, causes apoptosis in melanoma cells by a mechanism involving nuclear interaction and p53 expression. Moreover, a drug isolated from Chinese herb has been shown to strongly inhibit the proliferation and induce apoptosis in leukemia cells, encouraging potential future use in clinics. Furthermore, anthocyanins, a class of flavonoids, were shown to exhibit antioxidant, anti-inflammatory and chemotherapeutic properties. Indeed, they show anti-proliferative, anti-invasive effects and thus may induce apoptosis through inhibition of Bcl-2- and Bcl-xL-sensitive pathways.

Besides this, various novel clinical applications for drugs, which were already used for various diseases, have been presented revealing previously unknown pro-apoptotic properties. Thus, Metformin, an oral anti-diabetic drug originating from French lilac (*Galega officinalis*), leads to a significant reduction of tumor growth in mice. Moreover, the main component of ginseng, Ginsenosides, used in treating anemia for thousands of years, and the antimalaric artemisinin have been shown to inhibit proliferation and induce apoptosis.

By converting the fundamental knowledge to direct applications, another approach of the congress was focused on applied medicine. Some posters focused on the development of apoptosis-related markers to be used in the diagnostics of different diseases. Concerning liver disease, the related common cell death of hepatocytes, which is considered as a characteristic feature of liver damage,

contributed to an interesting discussion on serological caspases activity as an early and reliable diagnostic biomarker, especially for undiagnosed liver disease. Likewise, the caspase-cleaved serum marker cytokeratin-18 is a promising marker in clinics to evaluate epithelial apoptosis in liver and intestinal graft-versus-host disease. The same biomarkers can be used to evaluate treatment responses to anti-cancer therapies that do not lead to significant tumor shrinkage and therefore are difficult to monitor by imaging techniques (e.g. stable disease). Regarding the use of apoptotic biomarkers in the prediction of treatment outcome, the expression of distinct apoptosis-related genes apparently permits to evaluate the risk of treatment failure and relapse in diseases like Hodgkin's lymphoma and leukemia.

Interestingly, techniques commonly used until now for basic research only, are finding a therapeutical application. Among them, the use of small interfering RNAs can be considered as potent agents for gene silencing in order to overcome multidrug resistance of cancer cells. Thus, the use of siRNAs against multidrug resistance-related or Bcl-2 genes in combination with conventional chemotherapy was shown to sensitize tumor cells to enter apoptosis. Adenoviral vector encoding proapoptotic genes represents another gene therapy approach successfully used in the treatment of melanoma cells to selectively induce apoptosis in these malignant cells.

Overcoming apoptosis resistance seems to be a more and more challenging task. After the Gleevec period and its implied resistance, researchers are now focusing on the apoptotic effects of oxidative stress induction in CML. In multidrug resistant T-ALL, perifosine, a novel Akt inhibitor and promising therapeutic agent, was shown to induce apoptosis by a JNK dependent mechanism. Similar results were obtained in different leukemia cell lines using the flavonoid quercetin. As shown during the conference, besides its proapoptotic properties, quercetin is able to overcome resistance to death receptor- and drug-induced apoptosis in leukemia cells. Moreover, the development of carbon nanotubes (CNT) as vehicle to ensure the delivery of therapeutical drugs was presented as future potential anti-cancer therapy to overcome chemoresistance.

Finally, another major part of this congress was dedicated to apoptosis and neurodegenerative diseases. In this context, p53 was suggested as a main apoptotic mediator in the etiology of Parkinson's disease. In addition, processing of the amyloid precursor protein seems to be prevalent during apoptosis in Alzheimer's disease. The importance of the pro-apoptotic protein Bim was highlighted by the observation that Bim −/− mice are resistant to a number of cell death stimuli, thus emphasizing its possible role in neuronal degeneration.

Mark your calendar: Inflammation 2010, Inflammatory cell signaling mechanisms as therapeutic targets, January 27–30, 2010.

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