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Meeting Report

Long live the cell death!

A Mastino*[,1,2], S Basu[3], T Brunner[4], JE Ricci[5] and M Diederich[6]

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Apoptosis World 2008—From Mechanisms to Applications: Kirchberg, Luxembourg, 23–26 January 2008.

It is not yet time to move to a more exciting and fertile field of research! For those who attended the 'Apoptosis World 2008—From Mechanisms to Applications' meeting at the European Congress Center in Kirchberg, in the neighbourhood of Luxembourg, this is the reassuring message they can bring home. There is still a lot of work to be done for researchers interested in cell death! A number of 'black holes' in the basic knowledge of molecular mechanisms governing apoptosis and sister forms of cell death, such as autophagy and anoikis, still remain, as numerous speakers highlighted during their talks. Among these, insufficient understanding of 'who controls whom' in the network of signalling that positively and negatively regulate cell death or inadequate knowledge of the evolutionary meaning of proteins implicated in the apoptosis and autophagy main pathways. Are these proteins just moonlighters of death whose death-unrelated daily function allowed them to be phylogenetically conserved, as suggested during the keynote session and by various data presented during the meeting? Actually, this could explain why caspase 8 plays a role in the differentiation of haematopoietic cells without killing them or why tumour cells maintain a potential apoptotic machinery, as described during the meeting. Moreover, there is at least another good reason why this is not a time to shrink from apoptosis. The past decade was marked by terrific progress in deciphering the main pathways involved in apoptosis, but frustrating attempts to transfer basic knowledge into real benefits for human health. It seems that we are now entering in a new phase where translational research on apoptosis will be more remunerative. This was the 'air people breathed' while listening, for example, to the recent progress in preclinical studies on the Bcl-2 antagonist ABT-737 in the superb 'Hemicycle Room', where all the talks have taken place, or browsing among the posters in the lobby and the corridors of the European Congress Center.

Going into some details of this well-organized and well-attended conference, it is worthy to remind ourselves that it covered the whole gamut of apoptosis research, from mechanisms to applications, as advertised. It was gratifying to see high-quality talks examining the roles of signalling pathways and proteins in the regulation of apoptotic machinery and in their possible therapeutic use. As a consequence, rather than report an unavoidably defective summary of the talks presented by the numerous speakers, here we prefer to discuss some of the major points emerged during the meeting, without mentioning each one of the speakers.

On the mitochondria front, many interesting talks with important ramifications in terms of cancer therapy have been presented. As mitochondria play a crucial role in the regulation and induction of apoptosis because of their ability to release several pro-apoptotic proteins, the Bcl-2 family of proteins, and also the importance of the mitochondrial dynamic (fusion–fission) in cytochrome c release, was largely discussed during this meeting. In a nutshell it seemed clear that fragmentation of the mitochondrial network was probably not required for cytochrome c release. This conclusion came from the fact that Bcl-xL was able to antagonize Bax- and Bak-induced cytochrome c release but not mitochondrial fragmentation. In the same line, the fact that individual mitochondria could undergo permeabilization without fission was monitored. Nevertheless, it was also suggested that DRP1 (dynamin-related GTPases required for mitochondrial division) inhibition can block cytochrome c release. It seems that mitochondrial fission may not always be necessary for the release, but it may be possible that mitochondrial division proteins can be involved in the regulation of this event. The importance of mitochondrial fusion was also addressed. In response to diverse stimuli, mitochondria were found to be hyperfused. This SIMH (stress-induced mitochondrial hyperfusion) was involved in delaying death. This SIMH could represent a way used by the cell to survive transient stresses.

A burning question was addressed later: Why do cancer cells not lose their core apoptotic machinery? The primary answer was that several members of this machinery (mainly AIF, multidomain protein of the Bcl-2 family and Apaf-1) were also involved in response to fundamental cellular stresses (respectively, redox, nutriment and genotoxic stresses).

[1]Department of Life Sciences, University of Messina, Messina 98166, Italy; [2]IRCCS Centro Neurolesi 'Bonino-Pulejo', Messina, Italy; [3]Cell Survival Signalling Laboratory, Centre for Molecular Oncology, Institute of Cancer, Barts and The London School of Medicine and Dentistry, John Vane Science Centre, Charterhouse Square, London, UK; [4]Division of Immunopathology, Institute of Pathology, University of Bern, Bern, Switzerland; [5]INSERM, U895, Centre Méditerranéen de Médecine Moléculaire (C3M), Cell Death and Survival AVENIR team, Nice, France and [6]Laboratoire de Biologie Moleculaire et Cellulaire du Cancer, Luxembourg, Luxembourg
*Corresponding author: A Mastino, Department of Life Sciences, Section of Microbiology, Genetics and Molecular Biology, University of Messina, Salita Sperone 31, Messina 98166, Italy. Tel: +39 090 6765193; Fax: +39 090 392733; E-mail: antonio.mastino@unime.it

Of course, Bcl-2 members probably have a night job, but their day job is to be involved in the control of the MOMP (mitochondrial outer membrane permeabilization).

The specificity of the BH3-only protein depending on the anticancer treatment used was addressed. DNA damaging drugs involve Puma but it was described that a loss of Bim could further impair drug response, in particular in the absence of Noxa.

We got deeper into BH3-only protein mechanisms. Would replacing the BH3 domain of Bim with that of Bad, Noxa or Puma modify the biological activity of Bim? It appeared that even if those modifications could efficiently change the specificity of interaction among different Bcl-2 members, the activity of BH3-substituted Bim was not equivalent to that of the native protein. This work indicates that binding of the pro-survival member of the Bcl-2 family is probably not the only function of Bim.

As many tumours overexpress a Bcl-2 homologue, interest is growing in the potential of anticancer drugs that, like the BH3 domain, bind one or more Bcl-2 homologues and trigger apoptosis. A structure-based approach led to the identification of small organic BH3 mimics such as ABT-737. This compound binds strongly to Bcl-2, Bcl-xL and Bcl-w but not to Mcl-1 or A1. One intriguing talk reported that in primary chronic lymphocytic leukaemia cells, ABT-737 induced classical features of apoptosis and also mitochondrial outer and inner membrane permeabilization. This observation seemed to be specific for primary cell lines, as it was not observed in several culture cells. Of course, the impact of ABT-737 in nonmalignant primary cells will be of great interest to better appreciate this mechanism, which could be more general than expected as most of our techniques would not be able to discriminate between MOMP and permeabilization of both mitochondrial membranes.

Another issue that they dealt with at the meeting was the interplay between the c-Jun N-terminal protein kinase (JNK) and the Bcl-2 families of proteins. In fact, the role of JNKs in apoptosis, although well recognized, is still controversial in terms of apparent stimuli-specific and tissue-specific differences. In elucidating mechanisms that may underlie this, talks focused on which are the most relevant targets of JNK phosphorylation. Integration of stimulation of the BH3-only pro-apoptotic family members and inhibition of the anti-apoptotic Bcl-2 family members in the eventual activation of BAX was proposed to be central for JNK regulation of the mitochondrial apoptotic pathway. However, issues of which members are direct targets of the JNKs *in vivo* still need to be resolved. Specificity of action of the many JNK isoforms was proposed to be regulated by differences in their subcellular localization. One talk showed that in the adult rat brain, upon middle cerebral artery occlusion, JNK3 increased at the mitochondria (whereas JNKs 1 and 2 decreased) and accounted for the majority of JNK activity. It will be interesting to see if reported differences in activity among the isoforms in other tissues and stimuli contexts are also underpinned by differences in subcellular localization.

It was refreshing that in a general apoptosis meeting there was also an emphasis on the *other* p53 family members, i.e. p63 and p73. We say 'other' though, as many of the speakers reminded us, p63 and p73 are evolutionarily older! The differential role of the isoforms of p63 was highlighted in a talk that demonstrated that whereas the ΔNp63 isoforms are more widely expressed, the TAp63 isoform, the archetypal member of the 'p53' family, is necessary for protecting the female germ line from DNA damage and chromosomal abnormalities. Therefore, although p53 may be known as the 'guardian of the genome', TA p63 is the 'guardian of the germ line.' Keeping to the evolutionary theme, an NMR-based structure–model analysis of the invertebrate homologue of this family was reported, which shed new light on the importance of the different domains in conferring oligomerization specificity to the isoforms.

Building on recent studies demonstrating the role of the E3 ligase, ITCH, in degrading p63 and p73, the development of a small molecule inhibitor to ITCH was reported. It is hoped that this could be used to synergize with chemotherapy. Another related subject that was discussed is the role of the Yes-associated protein (YAP) in signalling DNA damage. It was shown that this occurs in part by competing with ITCH for binding and stabilizing p73, and that YAP functions as a tumour suppressor in breast. Vitamin D analogues are used in combination with DNA damaging agents in chemotherapy and it was reported that p73 and p63 but not p53 regulate the induction of the vitamin D receptor.

However, p53 itself was not forgotten in this meeting and exciting developments in the microRNA world were discussed, where upon p53 activation, several miRNAs are upregulated, many of which have putative tumour suppressive roles. It was also reported that the kinase DYRK2 phosphorylates p53 on Ser-46 and that this is important for the pro-apoptotic role of p53, showing us again that there is still much to be learned about the regulation of the much studied p53, let alone its more recently discovered, though evolutionarily older siblings.

Obviously, much discussion at this meeting was also on how and when death receptors induce apoptosis. Of particular interest in this regard is the role of TRAIL in the induction of apoptosis in tumour cells versus primary tissue cells. Several TRAIL-based clinical trials are currently ongoing, suggesting that primary cells tolerate TRAIL quite well. However, the role of TRAIL in primary cells, in particular in hepatocytes, is far from being well understood. Also why TRAIL induces apoptosis in only 50% of tumour cell lines (NCI panel) and not in the other 50% is the subject of intense research. Proteasome inhibition has a sensitizing effect on TRAIL-induced apoptosis in many different tumour cell lines suggesting (a) common resistance mechanism(s). Interestingly, this sensitizing effect appears to be distinct from the inhibition of NF-κB activation, although NF-κB regulates various anti-apoptotic genes. The identification of specific resistance genes confirming resistance to TRAIL-induced apoptosis is thus a clear focus of applied cancer research. Modulating these gene products may, however, also sensitize primary cells to TRAIL-induced apoptosis. Controversial results were presented at this meeting regarding the apoptosis-inducing potential of TRAIL in primary hepatocytes. Although TRAIL alone was found to be an inefficient trigger of cell death in primary murine and human hepatocytes, data were presented that chemotherapeutic agents can not only sensitize tumour cells, but also primary hepatocytes to

TRAIL-induced apoptosis, indicating extensive interaction between the extrinsic and intrinsic apoptosis pathways in given cell types. Several studies discussed the role of JNK, ERK and the BH3-only protein Bim in this crosstalk between the death receptor and mitochondrial pathway. Differential phosphorylation of Bim appears to define its fate and activity. Whereas growth factor-induced ERK activation and ERK-mediated Bim phosphorylation promote its ubiquitination and degradation by the proteasome, and subsequently survival of the cell, JNK-mediated phosphorylation of Bim was found to activate its pro-apoptotic activity and to accelerate the mitochondrial pathway. Proteasomal degradation of Bim may thus represent a mechanism by which proteasome inhibitors, such as bortezomib, sensitize tumour cells to TRAIL-induced apoptosis.

A number of talks and posters also focused on apoptosis and diseases, mainly neurodegenerative disorders and infections, demonstrating the growing link between basic and applied research on apoptosis.

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