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Meeting Report

A place to die for: apoptosis in cancer and infection, Capri 2002

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The Third International SASS/1st Cell Death and Differentiation Conference 'Apoptosis in Cancer and Infection', Capri, Naples, Italy, 6–10 October 2002. Melino G, Gupta S, and Green DR Chairs

Like real estate, the three most important considerations when organizing a conference are 'location, location, and location'. In this spirit, the fourth International SASS/1st Cell Death and Differentiation Conference, focusing on Apoptosis in Cancer and Infection, was held on the stunning Italian island of Capri from 6 to 10 October 2002. It is challenging to produce superlatives adequate for this setting; from the wild cliffs rising thousands of feet above the brilliant blue sea to the trendy designer shops and shockingly expensive cappuccinos of the piazza, Capri is a study in both harmony and contrast. The fourth most important consideration in conference organization is the speaker list and this conference measured up well, with many of the most recognized researchers in the field on hand to present their latest findings on a wide variety of topics related to cancer and apoptosis. The weather cooperated as well, with the bravest attendees indulging in late-season swimming. Spirits were kept high by superb local cuisine and minds were kept keen by a combination of late nights and strong coffee. Attendees were further galvanized midway through the conference by the news of the Nobel Prize awarded for work on *C. elegans* as a genetic system and, significantly, pioneering studies of apoptosis by Brenner, Sulston, and Horvitz. Conference cochair Gerry Melino shared tidbits of history and restaurant recommendations, and the piazza played host to constantly changing groups of meeting participants engaged in animated discussions of topics, scientific and otherwise. Of course, most important is the science itself and it was generally agreed that the quality was at least a match for the geographical surroundings. A partial list of conference highlights appears below.

Decisions and pathways to death

Doug Green presented provocative results that question the current thinking on the mechanism of mitochondrial premeabilization prior to caspase activation. Specifically, it appears that cytochrome c release precedes Bax translocation to the mitochondria. Using liposomes derived from mitochondrial membranes, it was found that cardiolipin is an important outer membrane component required for permeabilization. Interestingly, in this model, Bid formed oligomeric pore structures with Bax that allowed even very large proteins (up to 2000 kDa) to exit the mitochondria. Elegant results concerning the switch between apoptosis and necrosis triggered by the TNF receptor 1 (TNFR1) were presented by Peter Vandenabeele. Specifically, inhibition of HSP90 was shown to alter the TNFR1 death-inducing signaling complex leading to apoptotic death via RIP. In the absence of HSP90 inhibitors, necrotic death occurred through a FADD-dependent mechanism. It is remarkable that cells can be switched between apoptosis and necrosis by simply adding the appropriate factor to the same cell line. As a departure from the more dogmatic views of cell death, Gerry Melino presented findings on terminal differentiation in the skin. This process is associated with programmed cell death and the construction of a cornified cell envelope, a cross-linked protein structure that gives the epidermis its strength and resistance. The transglutaminase proteins are responsible for the crosslinking of this structure, and also mediate differentiation-related cell death in the skin. Melino challenged participants to consider alternative forms of genetic-regulated cell death (in addition to apoptosis), and this theme was repeated by several meeting participants.

Caspases, p53, and other death-effecting molecules

The function of caspase-12 in apoptosis has been a hot topic of late, and Don Nicholson first stoked the fire and then extinguished it by deconstructing the current thinking on the

role of this caspase in human cell death. It has been proposed that caspase-12 is involved in apoptosis in response to endoplasmic reticulum (ER) stress. However, an analysis of human cells shows that caspase-12 is not present in a functional form (except in about 1% of African individuals) as the gene is truncated. While the function of the truncated protein is not clear, it does not appear to be active in the ER stress response. This work clearly questions several previous studies of the relevance of caspase-12 in apoptosis, and Nicholson elaborated how the confusion in this area is confounded by the crossreactivity of anti-mouse caspase-12 antibodies with caspase-4 and caspase-5.

Continuing the theme of caspases, Seamus Martin and co-workers have used a combination of immunodepletion and analysis of caspase substrate cleavage, to determine the hierarchy of caspase activation in dying cells. Further, proteomic approaches were used to identify more than 200 possible caspase substrates. Martin was cautious to interpret these substrates as critical factors in the regulation of stereotypic hallmarks of apoptosis, as many of these proteins could simply be 'innocent bystanders'. He also emphasized that while caspase-3 and caspase-7 are redundant with respect to cleavage of PARP, caspase-7 cannot compensate for the absence of caspase-3.

One of the most intriguing new results in the caspase field was presented by David Wallach, who described a novel regulatory nuclear protein, CARY (caspase-8-binding protein with RNA-binding motifs), involved in death receptor signaling. CARY was shown to regulate the activation of procaspase-8 in the cytosol and thus sensitize cells to apoptosis. As CARY is itself a substrate for caspase-8, its effects are likely to be subject to a feedback inhibition loop. Wallach suggested that the regulation of active CARY, either by its release from the nucleus or by phosphorylation of its caspase-8 cleavage site, add further levels of control to caspase-8 activity.

PML plays an important role in both retinoic acid-induced differentiation and various forms of tumor suppression, and Pierpaolo Pandolfi shared recent data on the importance of this factor in apoptosis. PML forms novel structures in the nucleus, which appear to mediate its interactions with a number of apoptotic proteins, including p53. Recent results indicate that the sumoylation state of PML could be the key for its action. Specifically, sumoylated PML forms nuclear complexes, and upon irradiation-induced nuclearization of p53, PML is activated by desumoylation.

Continuing the theme of p53-binding proteins, Xin Lu presented findings on the growing ASPP family. ASPP 1 and 2 are recently described proteins that interact with p53, stimulating its apoptotic effect. Lu introduced us to a third member of the family, iASPP, which also binds but does not activate p53, thereby competing with ASPP 1 and 2 and exerting an antiapoptotic effect. iASPP is the most evolutionarily conserved of the ASPP proteins, is upregulated in human breast carcinomas and can also cooperate with Ras, E1A and E7.

Concluding the p53 session, Frank McKeon provided a different perspective by presenting his work on the possible role of p53 in the mitotic aneuploidy checkpoint. Bub-1 is a mitotic checkpoint protein that localizes to the kinetochores during mitosis, and has recently been shown to be important in triggering apoptosis in response to adaptation to the mitotic checkpoint and subsequent aneuploidy. Indeed, the polyploid phenotype of Bub-1 $-/-$ cells resembles that of p53 $-/-$, and p53 upregulation is markedly reduced in Bub-1 $-/-$ cells, possibly implying that defects in Bub-1 signaling to p53 result in tumor-related aneuploidies.

Apoptosis and cancer

As has become his hallmark, Gerard Evan gave an extremely provocative talk, challenging the idea that cancers arise through a complex series of diverse mutations. In his search for the minimum number of mutations required for neoplasia, Evan has constructed 'switchable' mice in which the c-Myc oncogene can be activated in specific tissues. In the absence of other genetic abnormalities, c-Myc-induced proliferation followed by apoptosis in pancreatic β cells; however, if apoptosis was suppressed by concurrent activation of antiapoptotic Bcl-X_L, or concurrent suppression of proapoptotic p53 or p19ARF, β-cell neoplasia (followed by angiogenesis and local invasion) followed. This work shows that as few as two genetic mutations are sufficient to give rise to a tumor, and suggests that one therapeutic approach to treating tumors with deregulated p53 might be to reactivate this protein. Expounding this theme, Karen Vousden presented the results of a high-throughput molecular screen for inhibitors of MDM2, a protein involved in p53 degradation. In normal cells undergoing stress-induced apoptosis, stable p53 transcriptionally activates PUMA, a BH3 protein that induces cell death by binding Bcl-2. This is mediated, in part, by inhibition of MDM2, but some tumor cells are defective for MDM2 regulation. A new class of MDM2-blocking molecules has been found to induce apoptosis in these tumors by restabilizing p53 function.

On the other hand, active p53 may not necessarily be good news for all cancer patients. Andre Gudkov described how pharmacological inhibition of p53 in patients undergoing chemotherapy for cancers in which p53 had already been lost could reduce the side effects caused by p53-dependent apoptosis. Furthermore, in some tumors in which p53 is partially active, p53-induced cell cycle arrest and subsequent DNA repair contributes to radiation resistance. p53 can therefore modulate the response to chemotherapeutic agents in previously unsuspected ways. Likewise, Klaus–Michael Debatin presented novel ideas on the therapeutic manipulation of cell death: In many tumors, inhibitors of apoptosis proteins (IAPs) are highly expressed. Perhaps surprisingly, SMAC can still induce apoptosis in these cells, but must first be released from the mitochondria. Positive results have been obtained by fusing SMAC peptides to HIVtat, leading to its transport across the cell membrane and into the cytosol. Combined therapy involving SMAC-HIVtat combined with the death receptor ligand, TRAIL, has successfully eliminated gliomas in a mouse model.

Hidenori Ichijo presented evidence for a critical role of ASK1 MAP kinase signaling in oxidative stress- and ER stress-induced apoptosis. Importantly, ASK1 knockout mice reveal that stress-induced activation of ASK1 in turn activates JNK/p38 to induce apoptosis.

Pathology and apoptosis

Peter Krammer discussed recent findings on apoptosis following the immune response of T cells; antibody binding leads to expression of CD95-L via IL-2. On the first day following immune activation, T cells show resistance to apoptosis, but become susceptible by day 6. Analysis of receptor-mediated T-cell death is a direct prognosticator of survival in sepsis patients. In a related presentation, Sudhir Gupta presented his work on molecular signaling of TNF-induced apoptosis to explain the attenuated immune response of aging individuals. Specifically, increased signaling via TNFR-1 may be mediated by an age-dependent accumulation of FADD, while reduced TNFR-2 signaling may result from decreased NF-κB activation, mediated by lower I-κB phosphorylation.

Continuing the theme of T-cell apoptosis, Jean-Claude Ameisen suggested an influential role for apoptosis in HIV infection. Apoptosis in uninfected CD4 T cells is a characteristic of AIDS pathogenesis. Ameisen's findings indicate that this may be a mechanism of early AIDS infection intended to condition the host's capacity to respond to viral infection. HIV viral particles were found to induce apoptosis in uninfected CD4 cells only if the particles were taken from dying, infected cells. This implies that cell death in infected cells creates an amplification loop that destroys uninfected cells, with serious pathogenic consequences. Finally, Chris Reutelingsperger presented recent developments in the emerging field of *in vivo* annexin V visualization of apoptosis. He reported initial successes in using real-time annexin V binding to predict tumor malignancy. This technology has shown promise, particularly in the study of intracardial tumors, of eliminating statistics as a predictor of clinical outcome, instead tailoring therapy and predicting treatment results on an individual basis in real time. Furthermore, while most investigators were using antibodies for pull-downs, Reutelingsperger challenged the stomachs of all present by including in his presentation a video of a novel 'pullout' technique, to remove cardiac tumors (specifics are left to the reader's imagination).

This summary gives only a taste of the scientific quality of the meeting and the authors apologize to those speakers not specifically mentioned; this was due only to space restrictions. In the final analysis, the conference was extremely successful. Many new ideas were presented and a variety of successful therapeutic applications of apoptosis were highlighted. Realistically, many presenters emphasized the need for perspective; while many of the key cell death pathways have been solved, there are still gaps between the specifics of the apoptotic program and current thinking on mitosis, necrosis, differentiation and cancer. Correlation and integration of current knowledge of apoptosis with that of other fields will lead both to a more holistic view of cell function and more effective cell death-related therapeutics.