Tumor Cell Death in Vienna

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Apoptotic cell death remains a contentious subject, with many topics unresolved, including the definition of apoptosis itself! In the opening talk of the 9th Euroconference on Apoptosis held in Vienna, Austria, between October 13-16, 2001, S. Orrenius (Stockholm, Sweden) addressed this deficiency by offering his definition of apoptosis. Orrenius defined apoptosis as caspase-dependent cell death that induces typical morphology. "Typical morphology" is thought by many researchers to involve nuclear condensation and DNA fragmentation. This definition is a useful reference point, even as the availability of molecular markers is increasingly expanding to complement the classical methodologies of morphological detection.

An Inhibitor of Granzyme B

T lymphocytes kill target cells by two main pathways, i) simultaneous release of perforin plus granzymes A and B, and ii) expression of the death-inducing ligand CD178 (CD95-ligand/Fas-ligand). Thus, granzyme B is a primary mediator of T lymphocyte-induced killing of tumor cells. After decades of research on granzyme B, an inhibitor of its activity was identified, named serpin proteinase inhibitor 9 (PI9). Expression of PI9 in T lymphocytes is believed to protect these cells from their own production of granzyme B [1]. J. Kummer (Amsterdam, Netherlands) reported that PI9 can be expressed by T-cell non-Hodgkin's lymphomas, B-cell non-Hodgkin's lymphomas, and Hodgkin's lymphomas, and PI9 expression was related to poor prognosis. Not suprisingly, expression of PI9 is hypothesized to offer these tumors protection from cytolytic T lymphocytes.

The Return of p53

Within the conference program, the return of presentations concerning the p53 tumor suppressor protein was notable. p53 was considered a "hot topic" at apoptosis conferences 5-10 years ago, but the focus subsequently intensified on the mitochondrial apoptotic pathway and death receptor pathways. The renewed interest in p53 reflects the recent identification and characterization of i) compelling experimental models illuminating the role of p53 in cancer development and response to treatment, ii) newly described targets of p53, and iii) novel p53-binding proteins, as described in the following paragraphs. S. Lowe and colleagues (Cold Spring Harbor, NY, USA) presented provocative data in a model of Myc-overexpressing $p53^{+/-}$ heterozygous lymphoma stem cells. After injection of these stem cells into mice, the arising tumors invariably have lost the wild-type *p53* allele. Interestingly, when apoptosis was "knocked-out" from the stem cells by overexpressing either a *bcl-2* gene or by introducing a dominant negative form of the *caspase-9* gene, the arising tumors retained their wild-type *p53* gene. These data were interpreted to indicate that disruption of apoptosis is sufficient to explain the selective pressure to mutate *p53* during lymphoma development. Thus, in this model, the remaining *p53* gene appears to be selectively lost solely for the survival advantage obtained by disrupting apoptosis, while defective cell cycle checkpoints (and the increased probablilty of aneuploidy) are byproducts of *p53* loss and are not critical events in lymphomagenesis. However, this does not argue that the p53 protein plays no role in the response to anti-cancer therapy. Indeed, it seems that p53 retarded tumor growth during therapy. After stem cell injection, therapeutically treated mice carrying $p53^{+/-}$ tumors (expressing the *bcl-2* or dominant negative *caspase-9* transgene) survived longer than apoptosis-competant mice whose tumors had lost the wild-type *p53* allele. Thus, although *p53* may be mutated during lymphomagenesis solely to disrupt apoptosis, these latter results have led Lowe to conclude that complete disruption of p53 activity can contribute to treatment failure by preventing both apoptosis *and* cell cycle arrest.

The scope of the activities of p53 is wide and varied. K. Vousden (Frederick, MD, USA) highlighted the known mechanisms of p53-dependent apoptosis. Pro-apoptotic transcriptional targets of p53 include genes for Noxa, PIDD, p53AIP1, PIG-3, and death receptors TRAIL-R2/DR5 and CD95/Fas. An additional p53-regulated pro-apoptotic protein named PUMA has been discovered and characterized by Vousden's lab. Inhibition of PUMA can reduce p53-dependent apoptosis *in vitro*. In addition to transcription-dependent regulation, some models indicate that p53 can regulate apoptosis in a transcription-independent manner, perhaps involving the release of previously translated death receptors from intracellular stores in the Golgi, and possibly other mechanisms. Towards explaining the transcription-independent activity, Vousden described the efficient induction of death in cell cultures by a 37 amino acid peptide derived from the DNA-binding portion of p53.

Being a potent inducer of apoptosis, p53 must be tightly regulated (probably by multiple mechanisms) in healthy cells. A novel family of proteins with members known as ASPP1 (apoptosis-stimulating protein of p53-1), ASPP2, and 53BP2 (iASPP) interact with p53 *in vivo*, as described by X. Lu (London, U.K.). ASPP1 and ASPP2 stimulate the transactivation function of p53 specifically on the promoters of apoptosis-related genes such as bax and *pig-3,* while leaving the promoters of other p53 targets such as *mdm-2, waf1,* and *cyclin g* unactivated. Underscoring the difficulties associated with research, the discovery of full-length ASPP2 was long postponed during ongoing studies of its fragmented form, 53BP2 [2, 3]. 53BP2 is an inhibitor of ASPP1 and ASPP2, and suppresses apoptosis induced by p53 in response to DNA damage. Lu reported that ASPP1 and ASPP2 are frequently (60%) down-regulated in human breast carcinomas, while 53BP2 is frequently up-regulated in tumors expressing normal levels of ASPP1 and ASPP2.

Further regulation of p53 expression and function is achieved by a newly characterized protein named Gas2, as descibed by R. Benetti (Trieste, Italy). It is well known that the cellular level of p53 expression is controlled by its enforced degradation in the ubiquitin:proteosome pathway. Benetti explained that, like HDM2 (human MDM-2), m-calpain is involved in enhancing p53 degradation. He presented evidence that Gas2 physically interacts with m-calpain, thereby promoting p53 stability (independently of HDM-2) and activity. Gas2 is a component of the microfilament system that is cleaved by caspase-3. Benetti additionally asserted that negative regulation of p53 can be achieved by another novel protein named hGTSE-1 (G2 and S phase-expressed protein-1). Evidence was provided that hGTSE-1 physically interacts with p53 *in vivo*, blocking its transactivation function and promoting p53 degradation.

The complexity of the p53 family recently became more apparent with the identification of splicing variants of family members p63 and p73. V. de Laurenzi (Rome, Italy) explained that the ΔN splicing form of p73 acts as a dominant negative regulator of p73 and p53 by competing with DNA binding sites on target genes. Interestingly, p53 and p73 can induce ΔNp73 expression, potentially providing a negative feedback loop. Currently, the mechanism(s) regulating the p73/ΔNp73 ratio is unknown.

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References

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