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Caspases: more than just killers?

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apoptosis. Several recent observations suggest that caspases and apoptosis-

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including the control of T-cell proliferation and cell-cycle progression. Here, Los

and colleagues propose a model that directly connects cell suicide mechanisms to

the regulation of cell-cycle progression.

Since the discovery that the Caenorhabditis elegans

caspase-1, proteolytic activation of the caspase

cascade has emerged as the most central step of apoptosis. To date, 14 mammalian caspases have

been identified and implicated in different aspects of cell death, although the exact function of each

individual caspase is still largely unknown. Caspases cleave precursors to produce mature cytokines

(caspase-1 and -11), initiate the propagation of apoptotic signals (caspase-8 and -9) and execute the

apoptotic program through cleavage of several vital proteins (caspase-3, -6 and -7). Surprisingly, growing evidence now indicates a participation of caspases and other apoptosis regulators not only in cell death, but also in the control of the cell cycle.

Apoptosis and cell proliferation

Although the processes of cell proliferation and cell death appear to be opposing and mutually

contradictory, some evidence suggests that the two events are linked. The maintenance of genomic

stability is essential for the survival of organisms. To ensure this stability, checkpoints exist to interrupt cell-cycle progression when damage to the genome is detected. In multicellular organisms, a further

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Whether this reduced size is an indirect result of disturbed development and differentiation patterns, or is mediated by reduced cell proliferation or apoptosis, is unknown. Gene targeting of the death-receptor-associated caspase-8 and its adaptor molecule, the Fas-associated death domain protein (FADD), has provided even more striking data. Caspase-8-/- and FADD-/- mice exhibit impaired heart muscle development with thin trabeculae and ventricular musculature. In the immune system, hematopoietic precursor cells from these knockout mice reveal a strongly impaired colony-forming activity and a defect in maintaining sufficient numbers of T-cell progenitors entering thymic development.

**Proliferation of primary T cells is inhibited by cell-permeable caspase inhibitors**

In FADD-/- mice or animals expressing a dominant-negative FADD protein, activation-induced proliferation is impaired in T cells despite normal IL-2 production. T cells lacking FADD function arrest at the G0/G1 transition of the cell cycle. The role of FADD in proliferation is supported by data obtained from chimeric mice that are transgenic for dominant-negative FADD and are deficient for the recombinase-activating gene RAG-1. Pro-T cells from these animals fail to proliferate in response to CD3 epsilon ligation. Concomitant signaling through the pre-T-cell receptor (TCR) and death receptors appears to trigger cell survival, proliferation and differentiation, whereas death-receptor signaling in thymocytes that lack a pre-TCR induces apoptosis. Interestingly, FADD has been recently shown to become phosphorylated by an unknown serine protein kinase during the G2/M phase transition, whereas it is not phosphorylated in cells arrested in G1/S of the cell cycle. Together, these events could link FADD and possibly caspase-8 to the cell cycle. Indeed, it has been observed that caspase-8 is cleaved in non-apoptotic cells after TCR stimulation. Other supportive, yet indirect, evidence for a role of the FADD/caspase-8 pathway in cell growth is the observation that proliferation of primary T cells is inhibited by cell-permeable caspase inhibitors. However, it should also be noted that the ability of FADD to activate cell proliferation may be mediated by interaction with a signal transducer other than caspase-8. In this respect, it has been shown that expression of CrmA, a caspase-8 inhibitor, in T cells completely blocks CD95-mediated apoptosis, whereas T-cell proliferation is not affected. Nevertheless, there is considerable evidence involving FADD in T-cell proliferation. Whether the proliferative effect of FADD requires caspase-8 activation or unrelated signaling events is not fully established.
The role of caspases
Why should caspases be required for cell-cycle progression? One attractive hypothesis is that caspases could confer additional checkpoints, assuring that only healthy cells will complete the cell cycle. During apoptosis, FADD together with caspase-8 forms the death-inducing signaling complex (DISC). During mitosis, FADD could also associate with caspase-8 or with another hypothetical ‘mitotic DISC-forming protein’ (MDP), an event that might be facilitated by the phosphorylation of FADD at the G2/M transition. In the proposed model (Fig. 1), caspase activation could then be necessary either to protelytically remove a block of entry into mitosis, such as a hypothetical ‘mitosis entry blocker’ (MEB), or to activate other cell-cycle-regulatory proteins. The MEB function could be fulfilled by proteins such as topoisomerase-1 or nuclear replication factor MCM3, which are indeed targeted by caspases. Their elimination might be necessary to secure cell-cycle progression. Other proteins cleaved by caspases include several negative regulators such as Wee1, an inhibitor of the cell-cycle-regulatory kinases CDK2 and CDC2, and CDC27, which is a component of the anaphase-promoting complex. Wee1 is a critical factor of the G2/M cell-cycle checkpoint machinery and mediates cell-cycle arrest by phosphorylation of CDC2. Therefore, cleavage of Wee1 in proliferating lymphocytes could lead to its inactivation, thus allowing cell-cycle progression. Of note, Wee1 processing by caspases during apoptosis in Jurkat cells is correlated with a 20-fold decrease in Wee1 activity and an increase in CDC2 activity. Moreover, the cyclin inhibitors p21Waf1 and p27Kip1 are targeted by caspases, resulting in increased CDK2 activity that could allow cell-cycle progression. When caspases are activated during mitosis, a critical question is how caspase cleavage could be restricted to just these cell-cycle regulators, while leaving other vital proteins intact. The answer could lie in a specific subcellular compartmentalization of caspases, the existence of scaffold proteins, or a different accessibility of cleavable substrates. Some caspases are translocated to a certain organelle during activation and, in some cell types, certain caspases have been localized in the nucleus. Interestingly, it has been found that, although caspases were activated and Wee1 was cleaved after TCR triggering, neither DNA replication factor RFC140 nor DFF45, the inhibitor of caspase-activated DNase, were cleaved in proliferating T cells. Cleavage of RFC140 and DFF45 would lead to inhibition of DNA replication and fragmentation of genomic DNA, events that are not compatible with T-cell proliferation. Thus, selective substrate processing in non-apoptotic cells could explain why T cells survive and proliferate despite caspases being activated.

Apoptosis inhibitors and cell-cycle regulation
To protect cell-cycle-regulatory and other vital structures, the activity of caspases must be tightly regulated. This could be achieved by various apoptosis inhibitors. It has been observed that Bcl-2 (which, like FADD, is phosphorylated at the G2/M transition) delays the re-entry of resting NIH-3T3 cells into the cell cycle. Moreover, Bcl-2 transgenic mice have delayed T-cell proliferation, whereas transgenic overexpression of Bax accelerates cell-cycle progression and apoptosis. Cells overexpressing Bcl-2 also contain decreased levels of phosphorylated retinoblastoma protein, which is a key regulator of cell-cycle progression. Finally, downregulation of Bcl-2 by antisense approaches enhances proliferation of acute myeloid leukemic cells. Overexpression of the Bcl-2-related protein Mcl-1 has also recently been shown to inhibit cell-cycle progression through the G2/M phase, but no other Bcl-2 member, associates with the cell-cycle regulator PCNA (proliferating cell nuclear antigen). Interestingly, an Mcl-1 mutant that lacks the anti-apoptotic function of Bcl-2 can be genetically separated from its inhibitory effect on cell-cycle entry. However, as all mutations that suppress the anti-apoptotic activity of Bcl-2 also abolish the inhibitory effect on cell-cycle transition, these two activities of Bcl-2 may not be entirely independent. Thus, these findings would lend support to the idea that inactivation of pathways leading to caspase activity results in reduced cell-cycle progression.

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Much attention is being paid to the role of the inhibitor of apoptosis proteins (IAPs), in particular survivin, in the control of apoptosis and the cell cycle. Survivin is specifically induced in the G2/M phase and associates with microtubules of the spindle apparatus at the beginning of mitosis. Earlier work had suggested that survivin directly binds to and inhibits caspase 3 (Ref. 23). This led to the initial idea that survivin might function both as a cell-cycle regulator and apoptosis suppressor. Because caspase 3 and the CDK inhibitor p21Waf1 colocalize with survivin at the centrosomes, it had been speculated that caspase activity and survivin could act together as a central part of the G2/M checkpoint. Indeed, inhibition of survivin function induced caspase 3 activity and apoptosis and produced a defect characterized by hyperploidy and supernumerary centrosomes. More recent experiments, however, raise serious doubts that survivin is implicated in apoptosis at all. Survivin homologs have been found in yeast, worm and Drosophila. In these organisms, survivin...
proteins also localize to spindle zones, where they are required for efficient mitotic cell division. Because yeast genomes, however, do not encode caspases, the survivin of lower organisms might have a primary role in cell division rather than in inhibition of cell death. Moreover, also in C. elegans, survivin is presumably not involved in apoptosis but required for chromosome separation and cytokinesis. Overexpression of the survivin homolog Bir-1 was unable to inhibit developmentally occurring cell death in C. elegans and inhibition of Bir-1 expression did not increase cell death. Instead, embryos lacking Bir-1 did not complete cytokinesis and became multinucleate. A similar phenotype has very recently also been found in survivin-deficient mice that showed disrupted microtubule formation and polyploidy, and failed to survive beyond 4.5 days post calumum. Thus, these experiments in knockout mice, yeast and knockout worms suggest that there are two classes of IAP: the ancient forms (including mammalian survivin and the worm and yeast homologs) that control mitotic death but have no obvious role in apoptosis, and the other forms (for example, X-IAP) that do control caspase activation and thereby influence apoptosis, but have no influence on cell-cycle control.

Concluding remarks
Clearly, much remains to be learned about a potential dual role of caspases in apoptosis and cell proliferation. Although the model presented in Fig. 1 is hypothetical, it may provide an explanation for the proliferative abnormalities and developmental defects that are observed in caspase-8- and FADD-deficient mice, as well as in animals overexpressing an inactive FADD mutant. Likewise, it could explain why caspases are generally not deleted or silenced in most tumors. In contrast to earlier models suggesting that apoptosis is simply an aberrant form of mitosis, we would rather propose an inverse scenario in which a subset of apoptotic molecules may play a role in mitosis.

Certainly, many questions pertaining to the exact contribution of caspases to proliferation remain unanswered: how are the dual functions of caspases regulated and what are their cell-cycle-relevant targets? Is activation of caspases a normal part of the movement through the cell cycle, and which checkpoints do they control? Furthermore, is the cell-cycle-specific phosphorylation of Bcl-2 and FADD important for the regulation of M-phase events? Finally, does survivin suppress a default apoptotic pathway that is required for a certain step of the cell cycle or spindle assembly in mammals? Answering these questions might provide new insights into lymphocyte proliferation and the biological roles of caspases. The generation of conditional and cell-type-specific caspase-knockout mice will certainly shed more light on this issue.

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