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News and Commentary

Lysosomes and mitochondria in the commitment to apoptosis: a potential role for cathepsin D and AIF

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Apoptosis is frequently viewed as the result of an explosive activation of the 'caspase cascade'. In this version, the regulation of apoptosis functions as a multidimensional network of activatory chain reactions involving activators, inhibitors, and even inhibitors of inhibitors, much like the complement system. Nonetheless, it appears clear that apoptosis is not just synonymous of caspase activation. The idea that apoptosis can be initiated and sometimes executed by caspase-independent processes has been challenged by the difficulty of outlining complete pathways in which each of the molecular actors is defined. Although we are still far from a complete view of the caspase-independent death-initiating machinery, a recent paper by Anna Senik's group[1] sheds some light on a novel connection between lysosomes and mitochondria.

Lysosomes as Death Signal Integrators

In several paradigms of apoptosis, lysosomes may function as death signal integrators.[2–4] Lysosomal cathepsins including cathepsins B, D, and L translocate from the lysosomal lumen to the cytosol in response to a variety of signals such as TNF receptor ligation,[5,6] p53 activation,[7] oxidative stress,[8] and the lipid second messenger sphingosine.[9] Such a translocation can also be induced by lysosomotropic agents such as cyprofloxacin, norfloxacin, and hydroxychloroquine.[10,11] Anna Senik's group now describes yet another model in which lysosomal membrane permeabilization (LMP) and cathepsin release constitutes a crucial step of the death cascade.[1] Human circulating T cells activated *in vitro* with a combination of mitogenic CD2 antibodies and interleukin-2 can be driven into apoptosis upon stimulation with CD2 or staurosporine. This apoptotic process can be subdivided into at least two steps, a caspase-independent commitment step and a caspase-mediated degradation step.[12] The early phase of commitment to apoptosis is characterized by cell shrinkage, peripheral chromatin condensation, and the translocation of apoptosis-inducing factor (AIF) from mitochondria to the cytosol and to the nucleus. At this stage, no other mitochondrial intermembrane proteins (such as cytochrome *c*, endonuclease G, Smac/DIABLO, or Omi/HtrA2) are released to the same extent as AIF from mitochondria, although Bax is inserted in the outer mitochondrial membrane. Importantly, at this stage lysosomal cathepsins are found in a diffuse, cytosolic localization.[1] It should be noted that the interpretation of the immunofluorescence data demonstrating the early release of AIF is complicated by the fact that the intensity of the AIF staining increases considerably upon translocation to the cytosol, whereas the immunoblot analysis does not support the increase in the total amount of the protein. Similarly, cathepsins B, D, and L stain far more intensively when translocated into the cytosol following staurosporine treatment than when in the lysosomal compartment in untreated cells. Additional methods to show the release of AIF as well as cathepsins could therefore have considerably strengthened the paper.

A Lethal Cascade Linking Lysosomes to Mitochondria

Pepstatin A, a specific inhibitor of cathepsin D, as well as the knockdown of cathepsin D expression using small interfering RNA (siRNA) inhibited all signs of staurosporine-induced apoptotic commitment, including insertion of Bax into mitochondrial membranes, AIF release, cell volume reduction, and chromatin condensation. It should, however, be noted that even though pepstatin A delayed staurosporine-induced cell death, it did not increase a long-time survival of the cells, indicating that redundant cathepsin D-independent pathways can substitute the death pathway described in this paper. Importantly, pepstatin A did not inhibit the release of cathepsin D,[1] indicating that cathepsin D activity is not required for its translocation. siRNAs knocking down Bax or AIF had no effect on the redistribution of cathepsin D. However, Bax-specific siRNA clearly prevented the mitochondrial release of AIF. Moreover, siRNA knocking down AIF (which had no effect on the conformational change of Bax accompanying its mitochondrial insertion) delayed markedly cellular shrinkage, chromatin condensation, and cell death.[1] Altogether these data suggest a linear sequence of events in which cathepsin D translocation is required for the Bax-mediated release of AIF, which in turn causes chromatin remodeling and commitment to apoptosis (Figure 1). Similarly, it has been found that lysosomotropic toxins fail to kill cells when the Bax and/or Bak genes are removed from the system or when mitochondrial membranes are sealed by overexpression of Bcl-2 (or the strictly mitochondrion-specific vMIA protein from Cytomegalovirus).[10,11] In such a system, the absence of Bax and/or Bak or the presence of Bcl-2 or vMIA fails to affect the redistribution of cathepsins B and D, yet does block the release of mitochondrial death effectors, including cytochrome *c*.[10,11] These data point to an obligatory participation of mitochondria in the transmission of lethal signal initially perceived at the lysosomal level.

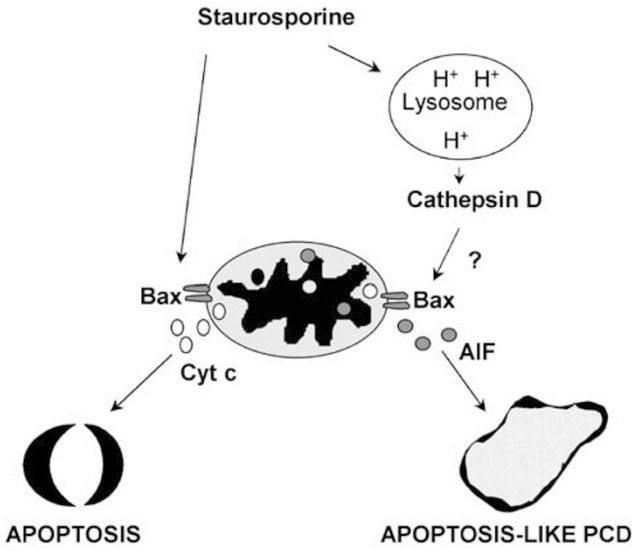

Figure 1 Early cathepsin D-dependent (right) and the later cathepsin D-independent death pathways triggered by staurosporine in T lymphocytes

Selective Permeabilization of Organelle Membranes during Apoptosis

During apoptosis, the strict compartmentalization of potentially lethal proteins such as cytochrome *c*, cathepsins, and AIF is selectively disrupted. As suggested by Anna Senik's group,[1] activated T cells treated for a short period with staurosporine exhibit a major change in lysosomal function. Lysosomes released cathepsins ($\sim$30 kDa) and FITC–dextran conjugates of up to 40 kDa but not β-hexosaminidase (250 kDa) or FITC–dextran molecules $\geqslant$70 kDa. Simultaneously, however, the cytosol–lysosome pH gradient was at least partially preserved, as indicated by labeling with an acidophilic fluorochrome[1]. This points to a highly selective membrane permeabilization process in which membrane pores selective for proteins of a limited size open, presumably in a transient fashion. Alternatively, a selective transport system could become activated during the commitment phase of apoptosis. In addition, mitochondria incorporating Bax were found to release AIF selectively.[1] This is at odds with reports suggesting that Bax and staurosporine trigger an early caspase-independent cytochrome *c* release and late caspase-dependent AIF release,[13] or that Bax suffices to release all mitochondrial intermembrane proteins (and FITC–dextran molecules of up to 2000 kDa),[14] yet in accord with previous observations indicating that AIF can be released from mitochondria in a selective, caspase-independent fashion.[15–17] Of note, reversible and partial membrane permeabilization has, however, been observed for the inner mitochondrial membrane during the prelude of apoptosis.[18,19] How exactly such a selective permeabilization is obtained remains an ongoing mystery and will certainly concentrate much of the efforts of the cell death research community during the forthcoming years.

Missing Links between Lysosomes and Mitochondria

It remains a conundrum how LMP leads to the mitochondrial membrane permeabilization (MMP). In preapoptotic-activated T cells, apparently the enzymatic activity of cathepsin D is required for Bax to become inserted into the mitochondrial membrane.[1] In several other models of LMP-mediated cell death, the inhibition of individual cathepsins (or their knockout) is not sufficient to block the activation of Bax.[10,11] Thus, several cathepsins (and perhaps even other lysosomal hydrolases) may be able to constitute the link between LMP and MMP. Indeed, cysteine cathepsins B, H, L, S, and K have recently been shown to cleave and activate the Bax-activating Bcl-2 family member, Bid, *in vitro* (Boris Turk, personal communication), and the TNF-induced MMP in hepatocytes depends on the activity of cathepsin B rather than cathepsin D.[5] Such variations in the requirement of individual cathepsins between the diverse apoptosis models may reflect differences in the expression levels of cathepsins themselves or their endogenous cathepsin inhibitors, which are highly cell type dependent. No signs of cathepsin D-mediated cleavage of either Bid or Bax were readily detectable in staurosporine-treated activated T cells.[1] Thus, the exact mechanism of cathepsin D-mediated activation of Bax in this model remains unclear. In addition to Bid and Bax, other mediators may modify the link between LMP and MMP. The activation of LMP and cathepsin activity has, for example, been linked to the phospholipase A2 activation[20] and the production of arachidonic acid,[21] a potential MMP inducer[22] that requires Bax to induce apoptosis.[23] Even though the recent data have clearly established the link between LMP and MMP and some of the molecular players have been revealed, the exact molecular cascade leading from LMP to MMP still remains to be established.

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