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Previews

Necrosis and the Serpin Under't

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Caspase proteases play essential roles in apoptotic cell death, while other proteases are active in necrotic cell death. In a recent paper in *Cell*, Luke et al. (2007) present findings demonstrating that a gene believed to be a natural protease inhibitor may have a role in preventing necrosis.

Cell death can be classified by molecules, morphology, and modus operandi. The best understood cell death is apoptosis, characterized by caspase protease activation and by changes to cellular architecture, including chromatin compaction. Apoptosis occurs as a natural part of development and as a consequence of cellular malfunction. Genes regulating apoptosis are conserved among metazoans and control the reproducible elimination of cells during development (Horvitz, 2003). Several of these genes, including some caspase and *bcl-2* family members, seem to have evolved principally to either annihilate or protect cells, since animals containing mutations in these genes generally exhibit only cell-death-related defects. Another form of developmental cell death, recently characterized in *C. elegans*, does not require genes mediating apoptosis and involves distinct cellular changes that are morphologically conserved with a form of vertebrate developmental cell death termed type III (Abraham et al., 2007). The molecular program governing this cell death process is not yet known.

Necrosis, yet another type of cell death, is less well defined, but is usually accompanied by activation of calpain and/or cathepsin proteases, by changes in calcium compartmentalization, and by cellular swelling (Hall et al., 1997; Xu et al., 2001; Syntichaki et al., 2002). Necrosis is generally associated with cellular trauma, and, unlike apoptosis, is thought of as an anarchic form of cell death, less efficient in enabling controlled demolition of cells; however, studies in *C. elegans* suggest that necrotic cells may be engulfed and degraded just like apoptotic cells (Chung et al., 2000).

Developmental roles for necrosis have not been identified. An important unanswered question, therefore, is whether necrosis is programmed cell death, involving dedicated effector proteins. A well-characterized genetic model of necrosis is the neurodegeneration of *C. elegans* mechanosensory neurons expressing a mutant MEC-4 degenerin-family ENaC ion channel (Driscoll and Chalfie, 1991). This gain-of–function protein contains an amino-acid substitution likely to cause increased ion flow across the plasma membrane, as shown for its human counterpart (Waldmann et al., 1996). Tavernarakis, Driscoll, and colleagues (Syntichaki et al., 2002) identified genetic suppressors of *mec-4*-induced neuronal death, demonstrating that aspartyl and calpain protease genes are required for the necrotic phenotype. These results suggest that this type of necrotic death involves proteases, but it remains unclear whether these enzymes primarily evolved to promote insult-induced necrosis, or whether their activities during necrosis represent an unselected coincidence.

A paper by Luke et al. (2007) in a recent issue of *Cell* investigates a new model of necrosis, pointing to a role for serine protease inhibitors in this cell death paradigm. Serpins are a large conserved family of serine protease inhibitors, extensively studied in the blood-clotting cascade, that irreversibly bind to target proteases. Most serpins are extracellular, apart from clade B serpins, of which *C. elegans* has nine members (Luke et al., 2006).

While investigating intracellular serpins in *C. elegans*, Luke et al. (2007) found that animals homozygous for a deletion in the *srp-6* intracellular serpin-related gene died rapidly when placed in water, a phenotype they called hypo-*o*smotic *s*tress *l*ethal (Osl). Examination of dying animals revealed that cells within them exhibited necrotic features. Cellular disintegration did not appear to be due to defects in the ability of animals to control fluid balance, since the kidney-like cells comprising the osmoregulation system of the animal formed normally, and mutant animals exhibited no defects under isotonic conditions. Furthermore, *srp-6* mutant cells underwent the normal volume and calcium changes associated with hypotonic shock, suggesting that these cells were not impaired in sensing osmolarity perturbations, but were likely defective in the response to such changes. Interestingly, other stress stimuli, including heat shock and hypoxia, also rendered *srp-6* mutants susceptible to induction of cellular necrosis.

The cell death ensuing after the exposure of *srp-6* mutants to hypotonic shock was not mediated by apoptotic genes and did not require the autophagy gene *bec-1*. However, some of the genes mediating *mec-4*-induced necrosis were required for the necrotic demise of *srp-6* mutant cells. Furthermore, overexpression of *srp-6* could prevent necrosis of cells expressing a degeneration-inducing MEC-4 channel, suggesting this degeneration and the Osl response are mechanistically linked.

Transmission electron microscopy and other imaging studies indicated that *srp-6* hypotonic cell death is characterized by lysosomal rupture, a result supported by genetic studies consistent with the notion that rupture of the lysosome-like granules of the intestine was responsible for organismal death.

These results suggest that *srp-6* may function to regulate lysosome integrity under stress. How might it function? Although *srp-6* is related to serpins by sequence, the authors were unable to inhibit serine peptidases with SRP-6 protein in vitro but were able to block cysteine peptidases, including a calpain, suggesting that cysteine proteases may initiate the catastrophic events leading to cell death. However, whether these proteases are lysosomal or not and whether lysosomal rupture is secondary to another event is not yet clear.

The role of *srp-6* in blocking necrosis induced by multiple cellular stressors led the authors to propose that *srp-6* acts as a buffer to pro-necrotic stimuli. Implied in this is the idea that a central function of this serpin-related gene is to prevent stress-induced necrosis. Thus, although it remains unclear whether necrosis occurs by an evolutionarily selected cellular demise process, the results presented here suggest the possibility that *srp-6* is an evolutionarily selected anti-necrosis factor. The expression pattern of *srp-6* lends some support to this idea. The gene is expressed mainly in the intestine, which, in animals placed in hypotonic media, is probably one of the most vulnerable surfaces, since it is not protected by a solute/solvent-impermeable cuticle. However, although *srp-6* may represent part of an anti-necrosis program, it is still too early to tell whether this is indeed the case. The results presented in the paper are also consistent with *srp-6* regulating a structural aspect of the lysosome or controlling lysosome biogenesis, among other possible models. Thus, the key role of this gene may not be to protect cells against necrosis, but to allow lysosomes to work or form properly. If this were the case, *srp-6* would likely function redundantly with other genes, since *srp-6* mutants only display defects when stressed.

Whether or not a dedicated anti-necrosis module exists in *C. elegans*, the results in this paper (Luke et al., 2007) do suggest that SRP-6 and related human proteins may be useful for modeling therapeutics for human disease states involving necrosis, such as stroke or acute pancreatitis.

A number of important questions regarding SRP-6 function remain to be addressed. Is expression or function of the protein regulated by chronic or sudden exposure to stress? Do other serpins exhibit similar activities, and, if so, do they share structural properties with SRP-6? Finally, identification of the main target of SRP-6 action would be key to elucidating the mechanisms by which SRP-6 inhibits necrosis.

The paper by Luke et al. (2007) introduces a new player upon the stage of necrotic death. Further studies on the way to dusty death should light tomorrow, and to-morrow, and to-morrow.

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