NECROTIC NEURODEGENERATION IN CAENORHABDITIS ELEGANS

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Necrosis plays a central role in neuronal injury associated with stroke and ischemia. Unlike apoptotic cell death, little is understood of molecular mechanisms of necrosis. The two distinct forms of cell death, necrosis and apoptosis, are easily distinguishable by morphological characteristics in the nematode Caenorhabditis elegans. At the level of light microscopy, necrotic cells appear as swollen vacuoles several times larger than normal cells; apoptotic cells become compacted and raised. Under the electron microscope the first visible step in necrotic-like death is the involution of the plasma membrane, followed by the appearance of membrane whorls [1]. Later, the electron density of the cytoplasm is reduced, the cell swells and cytoplasmic vacuoles arise. The chromatin forms aggregates and becomes fragmented. Organelles swell and finally lyse. The dead cell is removed by phagocytosis [2]. The key characterized initiators and regulators of apoptotic cell death do not influence the initiation or progression of necrotic cell death [2].

A variety of different insults can initiate necrotic-like neurodegeneration in C. elegans and lead to similar morphological changes in the affected cells. Necrosis-inducing genes include dominant alleles of the mec-4 and deg-1 degenerins which encode ion-channel subunits with similarity to mammalian epithelial Na⁺-ion channels (ENaC) [3, 4, 5], a dominant allele of the deg-3 acetylcholine receptor α-subunit [6], and a transgenically expressed constitutively activated mutant of the G-protein subunit GαS [7]. That channel hyperactivating mutations induce necrosis is reminiscent of the initiation of excitotoxic cell death in humans.

In our lab we study necrotic neurodegeneration with a focus on channel-hyperactivating mutations in the mec-4 gene as the death-initiating event. The MEC-4 protein is expressed in the six touch neurons in C. elegans, where it participates in the formation of mechanically gated ion channels and is involved in the signal transduction of mechanical stimuli. The dominant mec-4(d) allele encodes a hyperactive channel that leads to necrotic demise of the touch receptor neurons. We established a behavioral assay that allowed us to isolate suppressor mutations that block mec-4(d)-induced neurodegeneration. Ectopic expression of mec-4(d) in the ventral nerve cord leads to the appearance of a number of swelling and degenerating cells visible as vacuoles during the larval stage L1. As a consequence of this cell death, worms are severely paralyzed. We mutagenized 45,000 haploid genomes and isolated 24 worms with reconstituted locomotion. Such strains harbor candidate suppressors of mec-4(d)-induced neurodegeneration. Ten of the suppressors proved to be alleles of mec-6, which is a known suppressor of ectopically expressed mec-4(d). The other loci define several previously unknown genes involved in mec-4(d)-induced cell death.
We have identified one strong suppressor locus as calreticulin [8]. Calreticulin is a Ca\(^{2+}\) binding protein of the ER that plays critical roles in chaperone function and in maintenance of Ca\(^{2+}\) homeostasis. We have also shown that mutations in Ca\(^{2+}\) release channels \(itr-1\) (IP3 receptor) and \(unc-68\) (ryanodine receptor) also significantly suppress \(mec-4(d)\)-induced neurodegeneration. Our data suggest release of ER Ca\(^{2+}\) stores is a critical step in necrotic cell death in \(C. elegans\). Since ER Ca\(^{2+}\) release has been implicated in excitotoxic cell death, our data also supports that necrotic death mechanisms may be conserved from nematodes to humans.

We are currently investigating the function and identity of the remaining death suppressor alleles to decipher molecular mechanisms of necrotic-like cell death in the nematode.

**REFERENCES.**
