Genes Required for Cell Shedding, a Caspase-Independent Mechanism of Programmed Cell Elimination

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Programmed cell death plays critical roles in metazoan development and in the removal of damaged, infected or cancerous cells. Although most developmental cell deaths in *C. elegans* require the CED-3 caspase, some cells die in mutants completely lacking *ced-3* function. We have determined the identities of eight cells that can be eliminated via extrusion (or shedding) from *ced-3* mutant embryos. In wild-type embryos, these cells undergo *ced-3*-mediated apoptosis followed by engulfment. Thus, the canonical programmed cell death pathway and cell shedding function redundantly to ensure the elimination of a subset of cells fated to die.

One of the cells that can be shed from *ced-3* embryos is ABplpappap, the sister cell of which produces the RMEV neuron and the excretory cell. We predicted that ABplpappap might survive and adopt the fate of its sister cell in animals defective in both canonical programmed cell death and cell shedding. To identify factors required for cell shedding, we screened mutagenized *ced-3* animals for ectopic excretory cells, using the transgenic reporter P*pgp-12::gfp* to facilitate visualization of excretory-like cells. Three of our isolates with ectopic excretory cells contain mutations in *pig-1*, which encodes an AMPK-related serine-threonine kinase. A null mutation of *pig-1* reduces the number of shed cells in *ced-3* embryos by 75%, indicating that *pig-1* is required generally for the generation of shed cells. Most mammalian AMPK-related kinases are activated via phosphorylation by the LKB1:STRAD:MO25 tumor suppressor complex. Inactivation of *par-4/LKB1*, *strd-1/STRAD* or *mop-25.1* and *mop-25.2* (paralogs of *MO25*) also blocks cell shedding in *ced-3* animals. Additionally, the conserved T-Loop threonine (T169) of PIG-1, the predicted phosphorylation target of PAR-4/LKB1, is required for PIG-1 function, suggesting that the PAR-4 complex directly activates PIG-1 to regulate cell shedding.

We are currently using SNP mapping and whole-genome DNA sequence determination to identify the genes mutated in other mutant strains with ectopic excretory cells. Through biochemical, genetic and cell biological experiments, we will determine how these genes cooperate with *pig-1*. Our goal is a mechanistic understanding of the cell shedding process.

Poster
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