Cell Extrusion Is a Caspase-Independent Mechanism for Programmed Cell Elimination in *C. elegans*

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Programmed cell death plays critical roles in metazoan development and in the removal of damaged, virus-infected or cancerous cells. Most developmental cell deaths in the *C. elegans* soma require the caspase CED-3. However, a small number of cells die in mutants completely lacking *ced-3* activity. We observed that *ced-3* (but not wild-type) embryos contain on average six "shed cells" that detach from the developing animal and die in the extra-embryonic space of the egg. To test if other caspases are required for appearance of the *ced-3* shed cells, we constructed a strain with deletion mutations in all four *C. elegans* caspase genes: *ced-3*, *csp-1*, *csp-2* and *csp-3*. These embryos also contain shed cells, indicating that caspases are not required for the deaths of these cells. Surprisingly, the caspase-independent shed cells exhibit many of the hallmarks of apoptotic cells (*e.g.*, TUNEL-reactive DNA fragmentation and phosphatidylserine exposure), indicating that apoptosis can occur in the absence of caspases in *C. elegans*.

Using time-lapse microscopy, we determined the cellular identities of the shed cells in *ced-3* embryos and established that these are cells fated to die in wild-type embryos. Normally, these cells undergo *ced-3*-mediated programmed cell death and are engulfed by neighboring cells. However, in the absence of *ced-3*, they can be eliminated by a caspase-independent extrusion mechanism. To identify factors required for cell extrusion, we performed screens for mutations that block cell shedding in *ced-3* embryos. We observed that a null mutation of the gene *pig-1*, which governs the asymmetry of cell divisions in many neuronal cell lineages, reduces the number of shed cells by 75%. *pig-1* encodes an AMPK-related serine-threonine kinase that is homologous to the mammalian protein MELK. In mammals, most AMPK-related kinases are activated via phosphorylation by the LKB1:STRAD:MO25 tumor suppressor complex. Inactivation of *par-4/LKB1* or *strd-1/STRAD* also blocks cell shedding in *ced-3* animals, suggesting that PIG-1/MELK is a downstream substrate of the PAR-4/LKB1 kinase.

Mutations in human *LKB1* cause Peutz-Jeghers syndrome (PJS), which is characterized by the appearance of hamartomatous polyps in the intestine. Under normal conditions, epithelial cells are constitutively extruded from the mammalian small intestine at a rate of ~1400 enterocytes per villus per day. Based on our observations of *C. elegans*, we suggest that LKB1 activates MELK in a kinase pathway that makes enterocytes competent for elimination by extrusion and that *LKB1* mutations cause a cell-extrusion defect that contributes to polyp formation in PJS patients. The similarity between cell shedding in the *C. elegans* embryo and in the mammalian gastrointestinal tract also suggests that caspase activity is not required for either physiological rates of cell shedding in the mammalian intestine or the apoptotic appearance of shed enterocytes.

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