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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