PIG-1-controlled Asymmetric Cell Divisions promote the Caspase-independent Apoptosis of Cells that Are Shed from the *C. elegans* Embryo

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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, suggesting that these cell deaths are caspase-independent. 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 cells are normally fated to die in wild-type embryos. To identify factors that promote caspase-independent cell death, we performed genetic screens and tested candidate genes for suppression of the *ced-3* shed cell phenotype. We observed that *pig-1* inactivation reduces the number of shed cells by more than 50%. *pig-1* encodes a serine-threonine kinase that governs the cleavage plane position in many cells that divide asymmetrically. *ced-3* shed cell detachment is also blocked by the inactivation of *gex-2* and *gex-3*, which are required for the actin-driven migration of epithelial cells during enclosure of the ventral epidermis. We propose that PIG-1-controlled asymmetric cell divisions promote a caspase-independent apoptotic program in the shed cells, which are then physically displaced from the embryo by migrating epithelial cells.

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