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Investigating the pro-apoptotic function of the BCL-2 homolog *ced-9.*

Programmed cell death is a conserved process essential for proper development and tissue homeostasis in metazoans. Either insufficient or excessive cell death can result in diseases such as cancer or neurodegenerative disorders, respectively. In *C. elegans* the evolutionarily conserved process of caspase-mediated apoptosis is inhibited by *ced-9* (BCL-2 in mammals), and a strong *ced-9* loss-of-function mutation leads to maternal-effect lethality as a consequence of ectopic cell death. It has been proposed based on protein interaction and localization studies that CED-9 acts to prevent cell death by sequestering the pro-apoptotic protein CED-4 (APAF-1 in mammals) to mitochondria. In cells fated to die, EGL-1 (a BH3-only protein) binds CED-9 causing a conformational change in CED-9 that results in the release of CED-4. After its release from CED-9, CED-4 localizes to the perinuclear membrane where it activates the caspase CED-3. Interestingly, in addition to this anti-apoptotic role, *ced-9* appears to have a pro-apoptotic function: *ced-9(lf)* enhances the partial cell-death defect observed in animals with a weak loss-of-function mutation in the pro-apoptotic caspase gene *ced-3*. This presumptive pro-apoptotic role for *ced-9* remains poorly understood. Although a strong *ced-9* loss-of-function mutation leads to maternal-effect lethality, animals carrying a strong loss-of-function mutation in *ced-9* and a weak loss-of-function mutation in a cell-death promoting gene -- *ced-4* or *ced-3* -- are viable and show an enhanced cell-death defect over the cell-death promoting gene single mutants, again suggesting a pro-apoptotic role for *ced-9*. In animals carrying *ced-9(n1653ts),* a temperature-sensitive allele that causes lethality at non-permissive temperatures, some CED-4 protein is localized to the perinuclear membrane at the permissive temperature; additionally, animals carrying the *ced-9* allele *n3377,* which carries a missense mutation in the CED-9-CED-4 binding pocket, show CED-4 localization to the perinuclear membrane similar to that seen in null mutants of *ced-9*. These observations suggest that CED-4 localization to the perinuclear membrane alone is not sufficient to cause cell-death. *ced-9(n3377)* was isolated from a screen for enhancers of the cell-death defect caused by a weak loss-of function allele of *ced-3*, *ced-3(n2427)*. When *ced-9(n3377)* was crossed into a wild-type background, *ced-9(n3377)* caused a recessive cell-death defect on its own and did not show the maternal-effect lethality that is characteristic of *ced-9* null alleles. This observation suggests that *ced-9*'s killing function might be dependent on its interaction with CED-4. By investigating the mechanism of *ced-9*'s pro-apoptotic function we hope to further our understanding of *ced-9*'s role in cell-death regulation. This work might provide insight into diseases resulting from the perturbation of normal cell death, such as cancer and neurodegenerative disorders.