Investigating the pro-apoptotic function of *ced-9*

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Programmed cell death is a fundamental process that is required for proper development and tissue homeostasis in many organisms. Genetic analyses of *C. elegans* led to the discovery of the core components of the apoptosis pathway. One component, *ced-9,* is known to have an anti-apoptotic function, as *ced-9(null)* animals are maternal-effect lethal due to massive amounts of cell death and gain-of-function mutations in *ced-9* prevent normal programmed cell deaths from occurring. However, *ced-9* also has a pro-apoptotic function, which has not been well characterized. This function was discovered because weak *ced-3* loss-of-function animals have more undead cells in a *ced-9(null)* background, indicating that decreasing c*ed-9* function can decrease cell death.

In a genetic screen for mutations that enhance a defect in programed cell death of weak *ced-3(n2472)* mutants, we recovered a *ced-9(n3377)* allele that not only enhances the *ced-3* cell-death defect but also has a recessive cell-death defect on its own. Evidence suggests that *ced-9(n3377)* has a loss of *ced-9* killing function rather than a gain of protective function. First, *ced-9(n3377)* confers a recessive increase in cell survival, which is different from the dominant gain of protective function *ced-9(n1950)* allele. Second, *ced-9(n3377)* acts different from the gain-of-function allele in relation to CED-4 localization. CED-9 normally localizes to mitochondria, where it binds CED-4 and prevents CED-4 from activating CED-3. Upon EGL-1 binding to CED-9, CED-4 is released and localizes to the perinuclear membrane. In *ced-9* gain-of-function animals CED-4 is localized to mitochondria, even when EGL-1 is over-expressed. By contrast, in *ced-9(n3377); ced-3(n2427)* animals CED-4 is localized to the perinuclear membrane, as it is in *ced-9(null);ced-3(n2427)* animals. This finding is consistent with the increase in undead cells in *ced-9(n3377)* animals being from a loss of *ced-9* killing function rather than a slight gain of protective function.

Currently we are performing genetic screens to obtain additional loss of *ced-9* killing function alleles and to identify suppressors of the *ced-9(n3377)* cell-death defect. These screens might identify genes that regulate the *ced-9* killing function as well as unknown components of the cell-death pathway. We are also using molecular and biochemical techniques with *ced-9(n3377)* to evaluate if the *ced-9* pro-apoptotic function is mediated through the core apoptotic pathway components and/or its regulation of mitochondrial fusion and fission. Ideally, these experiments will lead to a better understanding of the apoptosis pathway and possibly novel therapeutic targets for diseases caused by misregulation of programed cell death like cancer, autoimmunity and neurodegeneration.

Poster
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