Investigating the pro-apoptotic function of ced-9
Kaitlin Driscoll, Peter Reddien, Brad Hersh and Bob Horvitz
HHMI, Dept. of Biology, MIT, Cambridge, MA 02139 USA

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 ced-9 function can decrease cell death.

In a genetic screen for mutations that enhance a defect in programmed 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 programmed cell death like cancer, autoimmunity and neurodegeneration.

Poster
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