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Deciphering the molecular basis of programmed cell death: from single genes to global views of protein networks

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Molecular networks that underlie programmed cell death are composed of hundreds of proteins that communicate with each other, mainly through post-translational modifications. The main goals of our laboratory are: 1.to identify novel proteins that serve as central 'nodes' in these cell death networks; 2.to draw a 'static map' delineating each component's position in the network and its links to a precise sub-cellular change; and 3.to introduce a functional dimension into the global analysis of the network's performance.

Over the past years, we have identified and characterized several new components of the cell death network - the so called DAP (Death-Associated Protein) genes. One of them, DAP-kinase (DAPk), is a Ca2+/calmodulin regulated Ser/Thr kinase. We found that DAPk is regulated by a unique auto-inhibitory phosphorylation on Ser308 within the calmodulin regulatory domain. In parallel, we have functionally linked DAPk to two death related sub-cellular events i.e., membrane blebbing and the formation of autophagic vesicles. The blebbing function is attributed to DAPk's position at the actin cytoskeleton, where it phosphorylates the regulatory light chain of myosin II, thereby activating myosin contractility. A third functional arm of DAPk, which predominates in some cellular settings is linked to p53 activation in a p19ARF dependent manner. While searching for potential substrates in this pathway, a novel short form of p19ARF was discovered which localizes to the mitochondria, alters the normal mitochondrial morphology and reduces the inner membrane potential. Currently, major efforts in the lab are directed towards the unbiased discovery of additional DAPk substrates and interacting proteins via two independent proteomics-based approaches involving biochemical and/or affinity purification strategies followed by mass spectrometric analysis.

In addition, we are also analyzing a DAPk knockout mouse, generated in our lab, to understand the contribution of DAPk to various physiological and pathological conditions. We are currently generating lung and breast cancer-prone mice lacking DAPk by crossing DAPk KO mice to Ras or Myc transgenic mice. DAPk is the founding member of a family of death-associated kinases, which includes DRP-1 and ZIP-kinase (ZIPk). These family members share homology in their catalytic domains, yet differ in their extra-catalytic structures and intracellular localization. Recently we demonstrated the existence of physical and functional cross-talk between DAPk and ZIPk. We found that trans-phosphorylation of ZIPK by DAPk controls the shuttling of the former between nuclear and cytosolic compartments and amplifies the death promoting signals.

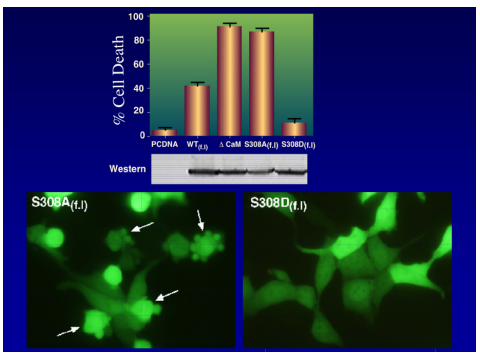

Fig. 1 *The blebbing phenotype and ultimate cell death appearing in human 293 cells after transient transfection with activated DAPk that carries Ser to Ala mutation at position 308, but not with the inactive Ser to Asp mutant.*

Additional functional modules within the apoptotic network were discovered in our laboratory with the elucidation of the structural/functional aspects of the other DAP genes that we isolated. DAP3 is a mitochondrial GTP-binding protein, which we discovered to contribute to the mitochondrial fragmentation that occurs during cell death. The discovery of DAP5 highlighted the contribution of

translational processes to cell death. DAP5 (p97/NAT1), a member of the eIF4G translation initiation factor family, supports cap-independent translation via specific IRESes. A novel unbiased screen for DAP5 mRNA targets has been implemented in our laboratory, and several potential mRNA candidates are currently undergoing validation for their physiological roles in cells.

Currently, one of the challenges is to integrate the molecular information emerging from the various DAP genes into a coherent global view of the network. In parallel, we determine the 'functional weight' of individual nodes, by applying a high-throughput strategy of siRNA-mediated silencing analysis. We expect that this multi silencing approach (MSA) will yield novel principles that delineate a network's performance in general.

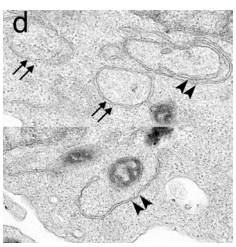

Fig. 2 *Transmission electron micropgraphs of DAPk-induced autophagic cell death. Typical autophagic vesicles containing cytoplasmic material (double arrows), ER or mitochondrion (double arrowheads) are shown.*

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