Programmed Cell Death: from single genes and molecular pathways towards systems level studies

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Our lab studies programmed cell death, a fundamental process in cell biology, by proceeding from single gene studies towards global network analysis. Every cell has a built-in molecular network of 150-200 proteins, which once activated by the appropriate input signals, leads to cell death. It is now recognized that the cell has several routes by which to die, including type I apoptosis, type II autophagic cell death and type III programmed necrosis, and that different death stimuli can activate different combinations of these death programs. Each of these cell death modules is characterized by a set of morphological features (Fig. 1) which can be assessed by specific molecular markers (Fig. 2), and is regulated and executed by individual signaling pathways. Certain protein nodes in these pathways are shared by one or more module, representing points of integration or inter-modular cross-talk. By performing large scale anti-sense RNA screens in mammalian cells, we previously identified several new components of the cell death network. These genes, the DAPs (Death Associated Proteins), were further characterized at the structural and functional levels. Although DAP genes were originally selected from the same cellular stress settings, they actually differ substantially in their biochemical properties, intracellular localization and the molecular pathways in which they take part. We currently employ multiple approaches, including biochemistry, cell biology, proteomics-based strategies, 3D protein structure analysis, advanced microscopy and systems biology to elucidate the functions of the individual DAPs, to map their positions within the cell death network, and to analyze network functionality and integration as a whole. Our ultimate goal is to

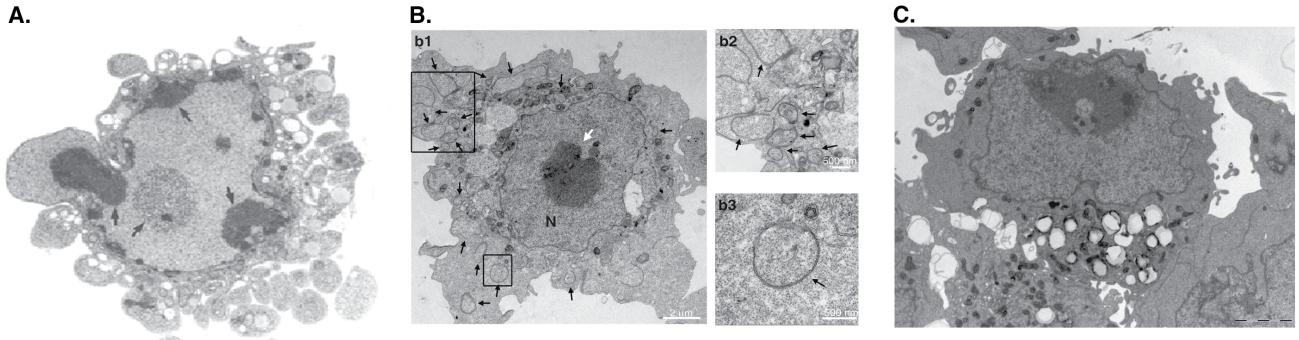

Fig. 1 *Electron micrographs of cells undergoing various types of programmed cell death. A. Apoptosis. Note the membrane blebs, fragmented cytoplasm, and condensed chromatin (arrows). B. Autophagy. Note the double-membrane enclosed autophagosomes (arrows). Boxed regions in b1 are shown at higher magnification in b2 and b3. C. Programmed necrosis. Note the extensive vacuolization and swollen organelles.*

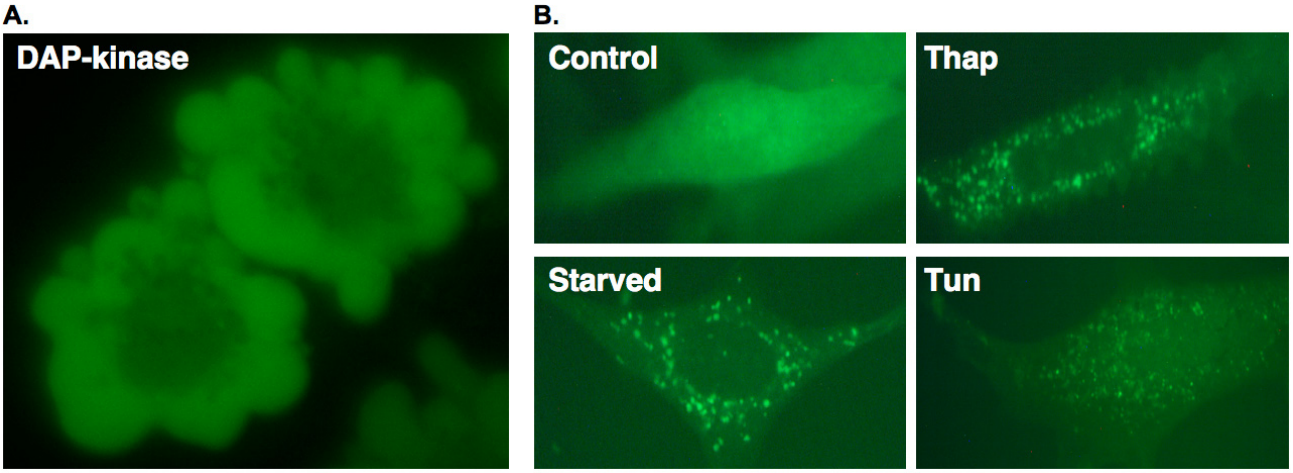

Fig. 2 *Fluorescent images of dying cells. A. GFP expressing 293T cells transfected with DAPk. Note extensive membrane blebbing. B. Primary fibroblasts derived from GFP-LC3 transgenic mice in which autophagy was activated by amino acid starvation or the ER stress inducers, thapsigargin (Thap) and tunicamycin (Tun). The diffuse localization of GFP-LC3 in non-treated cells becomes punctate during autophagy, a reflection of its recruitment to the growing autophagosome.*

integrate our findings with previously known data about cell death, in order to define a global cell death network, in which the functional significance of each death pathway, and the inter- and intra-modular interactions among individual proteins or protein complexes within specific pathways, can be predicted for any given death stimulus (e.g., DNA damaging drugs, cytokines, ER stress).

DAP-Kinase

DAP-kinase (DAPk), a Ca2+/calmodulin (CaM) regulated, Ser/Thr kinase, is one of the most extensively studied proteins in our laboratory. We have shown that, depending on the cell type and death trigger, DAPk can regulate various forms of programmed cell death, including apoptosis, autophagic cell death and programmed necrosis (Fig. 3). These pathways, and the specific morphologic phenotypes associated with them, comprise the multiple functional arms of DAPk. In order to characterize these functional arms, we have focused on identifying DAP-kinase substrates and interacting proteins, by two complementary approaches: the educated guess approach and unbiased, proteomics-based screens. In this manner, we have identified several important substrates/interactors, including the myosin II regulatory light chain, phosphorylation of which mediates the process of membrane blebbing, Protein Kinase D (PKD), whose activation by DAPk leads to the subsequent activation of JNK and caspase-independent cell death, and smARF, a small isoform of p19ARF that is produced by internal initiation of translation. smARF localizes to the mitochondria, and disrupts mitochondrial membrane potential, ultimately leading to caspase-independent autophagic cell death. smARF interacts with p32, a protein which is localized to the mitochondrial matrix, and is stabilized by this interaction. In addition, we are investigating the signaling pathways that activate DAPk by death stimuli, in particular, those that mediate dephosphorylation of a critical Ser residue within its CaM binding domain. Analysis of the DAPk knock-out mouse, generated in our lab, has enabled an understanding of how DAPk regulates physiologically relevant death programs, such as that which occurs during ER stress.

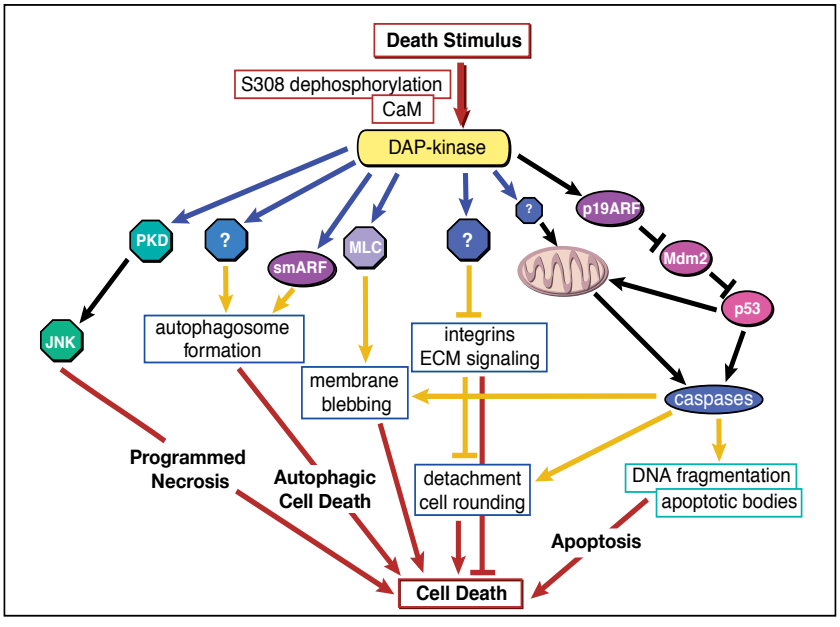

Fig. 3 *The DAPk death signaling network. Once activated by death stimuli, DAPk regulates numerous pathways through direct phosphorylation of multiple substrates (blue arrows). These pathways result in different phenotypes (yellow arrows), culminating in various types of cell death (red arrows).*

The DAPk Family

DAPk has two closely related family members, ZIP-kinase (ZIPk, also known as DAPK3) and DRP-1 (also known as DAPK2). They share with DAPk several common functional and structural features, including active participation in the autophagic process. In addition, they all possess a highly ordered basic loop located at the surface of the upper lobe of the catalytic domain, named the 'fingerprint' of the DAPk family (Fig. 4). This basic loop mediates heterodimerization between DAPk and ZIPk, which results in trans-phosphorylation and subsequent functional activation of ZIPk as well as homodimerization of DRP-1 (Fig. 4). Currently, we are studying the hierarchy among these kinases, with particular emphasis on elucidating the specific roles that each serves in the various death modules. We are also investigating several interesting isoforms of these genes that appear in genomic databases. Interestingly, we found that the murine ZIPk underwent specific sequence divergence from a conserved consensus found in all vertebrates. This divergence led to changes in protein properties concomitant with adaptive compensatory mechanisms, which developed to preserve the ultimate function of murine ZIPk in cell death.

DAP1- a Stress Response Modulator

DAP1 is a small (~15Kd), ubiquitously expressed phosphoprotein rich in prolines and lacking known functional motifs. The gene is highly conserved through evolution in all multicellular organisms. A bioinformatics search revealed a human homolog of DAP1, named DAP1-Like (DAP1-L). DAP1 and DAP1-L segregate into completely separate branches in a phylogenetic tree. In a screen applying different cellular stress inducers to cells, DAP1 displayed a tight regulatory profile involving changes in expression levels and post translational modifications, implicating its importance as a basic modulator of cellular stress responses.

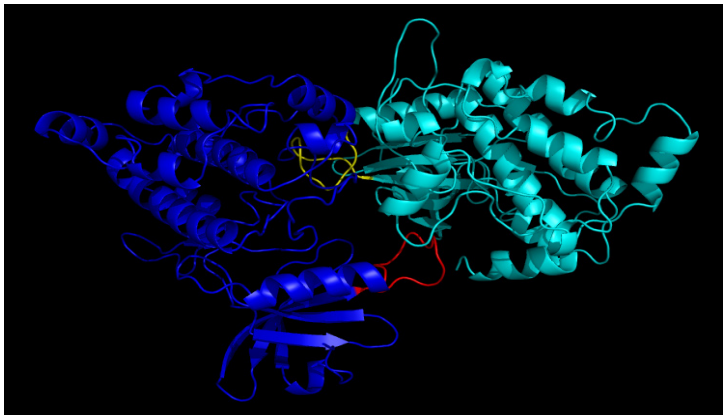

Fig. 4 *The fingerprint of the DAPk family. 3D ribbon diagram generated from the X-ray crystal structure of the DRP-1 catalytic domain in homodimeric conformation. The basic loop in each dimer is indicated in red or yellow (PDB 2A2A).*

DAP5 and Cap-Independent Protein Translation

DAP5 is a translation initiation factor of the family, which directs IRES-dependent translation under stress conditions when cap-dependent translation is compromised. As such, it provides a mechanism to maintain translation of itself and specific target mRNAs under circumstances when overall protein translation is suppressed, such as during apoptosis. Several novel DAP5 mRNA targets were recently identified in our laboratory by screening cDNA arrays with mRNAs that interact with the DAP5 protein or by examining the outcome of knock-down of DAP5 on the expression levels of specific proteins. Analysis of the profile of DAP5 targets indicates that by driving IRES-mediated translation of target mRNAs, and as a consequence, changing the relative steady state levels of critical proteins, DAP5 can either promote or inhibit the process of cell death.

The Global Cell Death Network

The complicated non-linearity of protein connectivity within each functional cell death module in the programmed cell death (PCD) network, and the potential inter-modular interactions, further emphasize the need for new strategies capable of dissecting the architecture of the PCD network, and converting it from a static to a dynamic map. To this end, we developed a new platform for dissecting the network's complexity based on single and double sets of RNAi-mediated perturbations of apoptotic and autophagic genes. By applying this strategy to cells exposed to a death inducing drug, we discovered an unexpected high degree of positive and negative inter-modular connectivity occurring through several apoptotic and autophagic genes. This high throughput function-based analysis provides a powerful tool for identifying new principles delineating the structure/function organization of the protein network underlying cell death. Different basic questions can be addressed using this system, such as, what is the minimal number of perturbations that will lead to the collapse of the network (i.e.,~100% reduction in cell death) in different combinations? Additionally, by changing the cell death inducing agent, it will be possible to determine to what extent the functional weight of the examined nodes change as a function of the death stimulus, and more. Answering these questions will shed light on the dynamics of the PCD network, revealing basic principles that might be used as a model system for other protein networks in mammalian cells.

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