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The DAP genes: deciphering molecular networks underlying apoptosis by a novel genetic approach

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A major challenge in the field of apoptosis is to identify the genes which constitute the molecular networks underlying this important process. A powerful genetic selection which can be applied in cultured cells was developed in our laboratory for trapping pro-apoptotic genes on the basis of their function. The approach, named TKO, was based on transfections with anti-sense cDNA libraries which were used as "virtual mutagens" for trapping the relevant apoptotic genes. Five novel pro-apoptotic genes, highly conserved in evolution, were identified by this genetic screen and were further studied in detail. They were named DAP genes, for Death Associated Proteins.

DAP genes differ in their specific biochemical mode of action, and operate within different cellular compartments (Fig.1). DAP-kinase which was identified as a novel prototype of calcium/calmodulin-regulated serine/treonine kinase, carrying ankyrin repeats and the death domain, was localized to actin microfilaments. DAP-3, was identified as a nucleotide-binding protein which is localized to endosomal vesicles. DAP-5 was identified as a component of the translation machinery, and DAP-1, a small RGD-containing protein, was found in a soluble cytosolic fraction within the cell. A known aspartic protease, cathepsin D, was also unpredictably selected, indicating that this particular lysosomal protease is actively recruited to the apoptotic process.

Structure/function studies of DAP-kinase revealed that the death-promoting effects depend on the status of the catalytic activity, the presence of the death domain, and on the binding of the kinase to the cytoskeleton. An auto-inhibitory domain was identified at the C-terminal tail of the protein. In vitro phosphorylation assays identified MLC as a major substrate, raising a working model which links the kinase function to the process of membranal blebbing. By the use of gain and loss of function mutants and other strategies it was found that various apoptotic signals impinge on DAP-kinase, and that several molecular arms emanate from this protein thus placing this novel kinase in a central junction within the apoptotic network (Fig.2).

One of the functional arms of DAP-kinase in primary embryonic fibroblasts leads to p53 activation in a manner

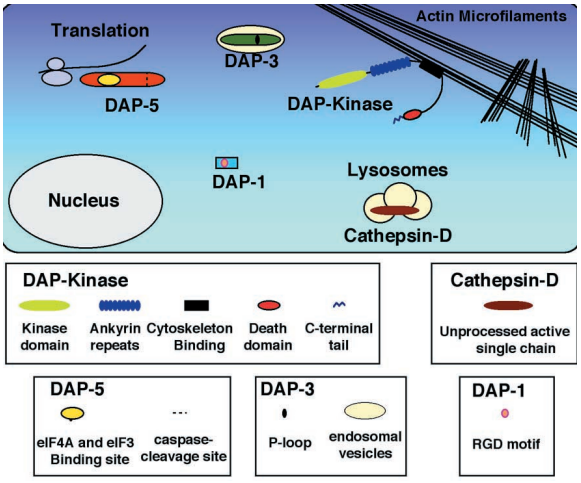

Fig. 1. *A scheme illustrating the intracellular localization of DAP proteins and details on their structural motifs.*

which is $p19^{ARF}$-dependent, resulting in suppression of oncogenesis. Another strong support for the tumor suppressor functions of DAP-kinase emerged from animal model studies where restoration of the gene into lung carcinoma cells which had lost it, strongly reduced their metastatic activity. A recent study performed on patient's carcinoma tumors revealed the occurrence of DAP-kinase deficiencies including promoter inactivation by DNA methylations.

The isolation of DAP5, a member of the eIF4G family of translation initiation factors, highlighted the importance of translational control in apoptosis. This 97kDa protein binds to eIF3 and eIF4A, but lacks the eIF4E-binding site necessary for cap-dependent translation. We found that DAP5/p97 is regulated during apoptosis at two levels: **a.** It undergoes a specific caspase-dependent cleavage generating an active DAP5/p86 truncated protein. **b**. It continues to be preferentially translated in the apoptotic cells, in the absence of eIF4G proteins, through an IRES element identified in its 5'UTR. In cell free systems, DAP-5 was capable of stimulating translation through DAP5's IRES. This raised a working model according to which a positive feedback loop keeps feeding DAP5 protein in

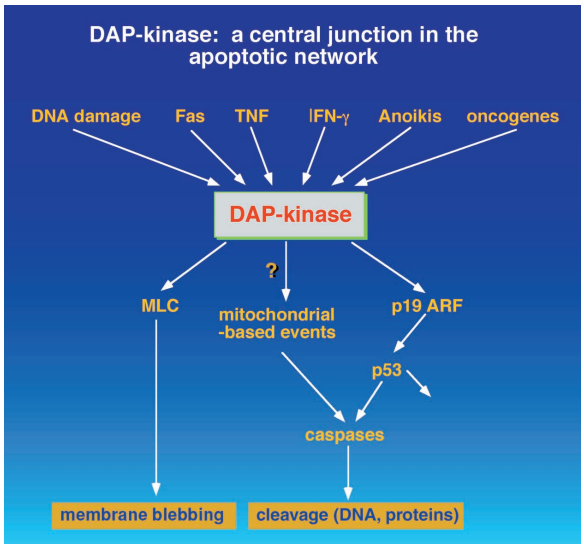

Fig. 2. *A working model illustrating the position of DAP-kinase within apoptotic networks*

the apoptotic cells and that this protein may be capable of mediating cap-independent translation of IRES-containing mRNAs.

In conclusion, the use of this genetic approach provided a set of novel genes which display a rate-limiting function in the apoptotic network. In addition, new gene families were discovered such as the DAP-kinase family of pro-apoptotic kinases.One of the members, DRP-1, shows an interesting pattern of cross talk with DAP-kinase and opens up an entire new pathway of signaling. This powerful approach of functional gene cloning will be applied in the future for the genetic dissection of other biological processes such as cell cycle arrest, cellular senescence and metastasis.

References

Deiss, L., Galinka,H., Berissi, H., Cohen O. and Kimchi, A. (1996). Cathepsin D protease mediates programmed cell death induced by interferon-γ Fas/APO1, and TNF-α .EMBO J. 15, 3861-3870.

Tiefenbrun,N., Melamed, D., Levy, N. Resnitzky,D., Hoffmann I., Reed, S.I. and Kimchi, A. (1996). Interferon-α suppresses cyclin D3 and cdc25A genes leading to a reversible G0-like arrest. MCB. 16,3934-44.

Raveh,T., Hovanessian, A., Meurs, E., Sonenberg, N. and Kimchi, A. (1996). Double-stranded RNA-dependent protein kinase mediates c-Myc suppression induced by type I interferons JBC, 271, 25479-25484.

Cohen, O*., Feinstein, E*. and Kimchi, A.. (1997). DAP-K is a Ca^{2+}/CAM- dependent, cytoskeletal associated kinase, with cell death-inducing functions that depend on its catalytic activity. EMBO J. 16, 998-1008.

Levy-Strumpf, N., Deiss, L.P., Berissi, H. and Kimchi, A. (1997). DAP-5, a novel homologue of the translation initiation factor 4G, isolated as a positive modulator of interferon-γ -induced programmed cell death. Mol. Cell. Biol. 17, 1615-1625.

Kissil, J.L., Feinstein, E., Cohen, O., Jones, P.A., Tsai, Y.C.,Knowles, M.A., and Kimchi, A. (1997). DAP-kinase Loss of Expression in various carcinoma Carcinoma and B-cell lymphoma cell Lines: Possible Implications for Role as Tumor Suppressor Gene. Oncogene 15, 403-407.

Inbal, B., Cohen, O., Polak-Charcon S., Kopolovic, J., Vadai, E., Eisenbach, L. and Kimchi, A. (1997). DAP-kinase is a tumor suppressor gene that links the control of apoptosis to metastatsis. Nature 390, 180-184.

Kimchi, A. (1998). DAP genes: novel apoptotic genes isolated by a functional approach to gene cloning. Biochimica et Biophysica Acta : BBA- Reviews on cancer. 1377, F13-F33.

Kissil, J.L. and Kimchi, A.. (1998). Death associated proteins: From gene identification to the analysis of their apoptotic and tumor suppressive functions. Molec. Med. Today June 268-274.

Cohen, O., Inbal, B., Kissil, J.L. Raveh, T., Berissi, H. Spivak,T. Feinstein, H. and Kimchi, A. (1999). DAP-kinase participates in TNF- α and Fas-induced apoptosis and its function requires the death domain. J Cell Biol. 146,141-148.

Kissil, J.L., Cohen, O.Raveh, T. and Kimchi, A. (1999). Structure/function analysis of an evolutionary conserved protein, DAP3, which mediates TNF-α and Fas-induced cell death. EMBO J. 18, 353-362.

Inbal, B*, Shani, G.*, Cohen, O., Kissil, J.L. and Kimchi, A. (2000). DAP-kinase-related protein-1 (DRP-1), a novel serine/threonine kinase involved in apoptosis. Mol. Cell. Biol.20, In press..

Raveh, T., Droguett, G .,Cohen O., Horwitz, M.S., DePinho, R.A., Kimchi, A. (2000). DAP-kinase activates a p19ARF/p53-mediated apoptotic checkpoint to suppress oncogenic transformation. Submitted.

Henis-Korenblit, S.*, Levy-Strumpf, N.* Goldstaub,D. and Kimchi, A. (2000). A novel form of DAP5 accumulates in apoptotic cells as a result of caspase cleavage and IRES-mediated translation. Mol. Cell. Biol. 20, In press.

Raveh, T., Berissi, H. Eisenstein, M,. Spivak,T. and Kimchi, A.. (2000). A functional genetic screen identifies regions at the C-terminal tail and death domain of DAP-kinase that are critical for its pro-apototic activity. Proc. Natl. Acad.Sci. USA, In press.

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