

Death-associated proteins: from gene identification to the analysis of their apoptotic and tumour suppressive functions

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Aberrations of apoptosis are implicated in many diseases, including cancer, autoimmune disease, cardiovascular disease and neurodegeneration. The cell's apoptotic machinery is, therefore, an important potential target for the development of new therapies. Our laboratory has used a strategy called technical knockout (TKO) to identify novel genes involved in apoptosis. TKO is based on random inactivation of gene expression with antisense cDNA libraries, followed by selection of those cells that survive in the continuous presence of an apoptotic stimulus. Using this approach, we have isolated five novel genes, including a serine/threonine kinase, a nucleotide-binding protein and a homologue of the p220 translation initiation factor. Expression of one of these genes (DAP kinase) is lost in some cancers, and this loss appears to increase the metastatic potential of some tumours.

APOPTOSIS (programmed cell death), a genetically controlled process by which cells commit suicide, has been the subject of intensive and extensive research over the past few years. Aberrations of this process are implicated in a wide variety of diseases and pathologies. While loss of apoptotic responses can lead to cancer or autoimmune diseases, an increased apoptotic rate is implicated in neurodegenerative diseases, brain ischaemia, and myocardial infarction. Various approaches have been, and are being, used to identify and study genes that function as positive mediators of apoptosis. Some strategies have taken advantage of the existence of cell-death mutants in invertebrate model organisms, such as *Caenorhabditis elegans* and *Drosophila*

melanogaster, and then have identified the mammalian homologues of the mutated genes^{1,2}. Other approaches have applied the yeast two-hybrid selection screen to systems in which apoptosis is triggered by members of the tumour necrosis factor (TNF) family of cytokines and the ligand to Fas/APO-1 (Refs 3–6). This screen led to the identification of adaptor proteins and enzymes that interact with the corresponding cell-surface receptors. Here, we address a functional approach to gene cloning (named technical knockout, TKO), aimed at isolating intracellular death-promoting genes by knocking them out with antisense RNA⁷. The TKO screen has led to the isolation of novel apoptotic genes, called death-associated protein (DAP) genes. Other groups have used similar functional screens with a few technical variations, resulting in the isolation of several other death-promoting genes, including those coding for Requiem, kinesin heavy chain, ALG-2 and ALG-3 (Refs 8–10).



The TKO strategy: hunting for genes according to their function

The TKO strategy directly targets functionally relevant genes involved in cell death, by virtue of the phenotypic change caused by their inactivation. Apoptosis is triggered by exposing HeLa cells to interferon γ (IFN- γ) – a cytokine that induces death in a variety of cell systems¹¹. At the morphological level, cell death triggered by IFN- γ displays typical apoptotic hallmarks, such as membrane blebbing, cytoskeletal disorganization and nuclear chromatin condensation

followed by its fragmentation¹². TKO allows gene expression to be inactivated in an unbiased manner by transfecting cells with antisense cDNA expression libraries. TKO relies on the assumption that the specific antisense RNA-mediated inactivation of a death-promoting gene will confer some growth advantage on cells that are continuously exposed to the lethal effects of IFN- γ . This advantage can then be used as a powerful means of forward selection to rescue the relevant cDNA.

The cDNA library itself was designed to yield high levels of antisense RNAs by virtue of the fact that: (1) it was cloned into an Epstein-Barr virus-based episomal vector that allows a high copy number of episomes per cell, and (2) the expression vector contains an IFN response element to elevate further the expression of antisense RNAs during selection in the presence of IFN- γ . The vector also contains a gene conferring resistance to the drug hygromycin B, which allows selection for survival of only those cells that contain the vector, thus eliminating untransfected cells. In addition, the episomal nature of the vector simplifies rescue of the antisense cDNAs from surviving clones⁷. As depicted in Fig. 1, the cDNA library (prepared from a mixture of untreated and IFN- γ -treated HeLa cells) is transfected into HeLa cells. The entire cell population is then exposed to IFN- γ and hygromycin B for 3–4 weeks. Finally, surviving cells are isolated, and episomal vectors rescued from these cells are individually tested in a second round of transfection and selection in HeLa cells, to confirm the phenotype.

Use of the TKO approach in our laboratory has yielded antisense fragments to seven non-overlapping groups of cDNAs, corresponding to seven different genes (Table 1). Sequence analysis indicated that five groups corresponded to novel genes, which we named *DAP* genes (Table 1; *DAP-2* is also called *DAP* kinase). Two groups represented known genes, encoding (1) thioredoxin, which is involved in redox regulation of intramolecular disulphide bonds, and (2) cathepsin-D, which is a lysosomal aspartyl protease.

The molecular and functional characterization of the novel *DAP* genes has revealed that the genes rescued by TKO code for proteins that have a variety of biochemical activities, thus suggesting that the TKO approach identified various elements whose functional organization along cell-death pathway(s) should be investigated in the future. The properties of the *DAP*s are described below.

What do we know about the structure and function of the *DAP* genes?

DAP-3

DAP-3, localized to chromosome 1, band q21 (Ref. 13), codes for a protein of 46 kDa that carries a potential P-loop motif, suggesting that it is a nucleotide-binding protein. The gene is transcribed into a single 1.7 kb mRNA, which is ubiquitously expressed¹⁴. The notion that *DAP-3* functions as a major positive mediator of cell death was supported by two independent lines of evidence. One of them is based on the original assay shown in Fig. 2, which in fact led to the identification of this gene, that is, protection from cell death conferred by reduced expression of the protein. The assay in this experiment was based on neutral red dye uptake into viable cells. It showed that transfection with a vector expressing antisense *DAP-3* RNA caused a significant increase in the fraction of cells that remained viable in the continuous presence of IFN- γ . Western blot analysis, performed by using polyclonal antibodies against *DAP-3*, confirmed that expression of the antisense RNA indeed reduced the steady-state levels of *DAP-3* in the continuous presence of IFN- γ (Ref. 14).

The second line of evidence came from overexpression of the full-

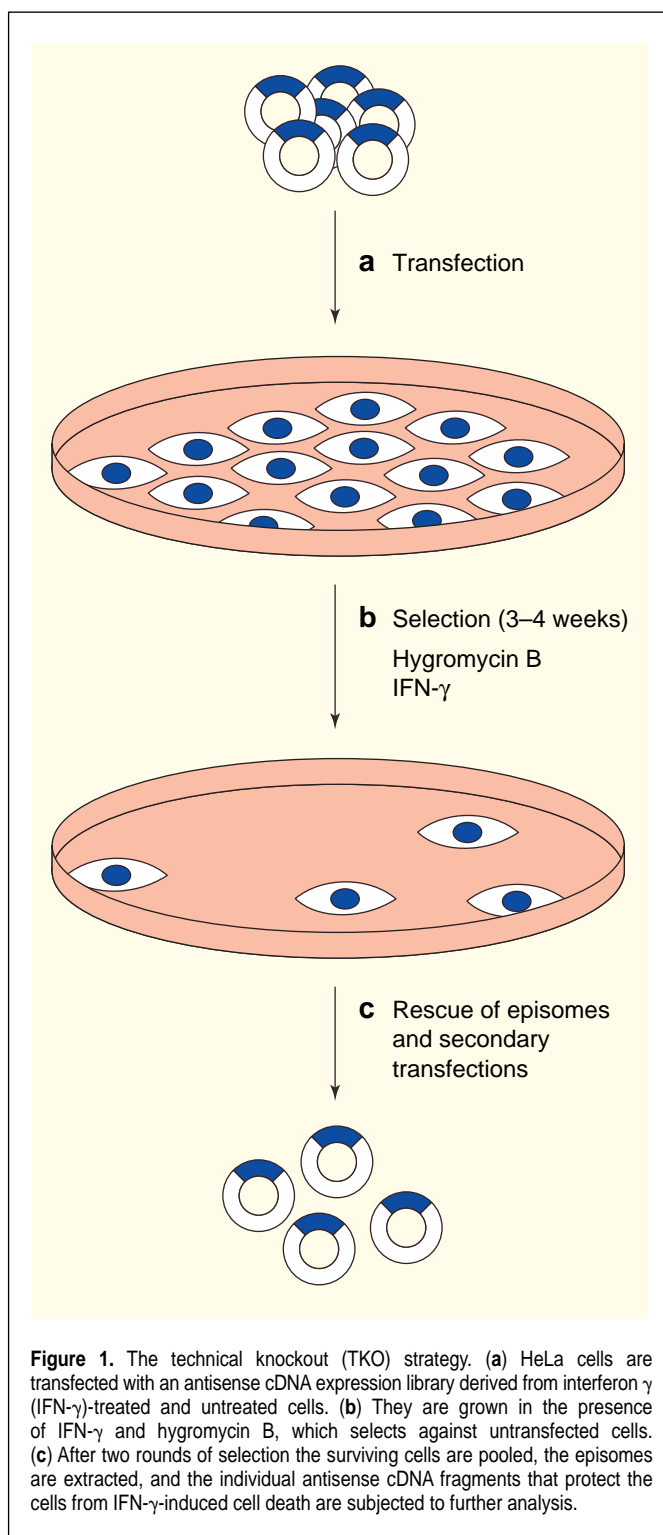


Figure 1. The technical knockout (TKO) strategy. (a) HeLa cells are transfected with an antisense cDNA expression library derived from interferon γ (IFN- γ)-treated and untreated cells. (b) They are grown in the presence of IFN- γ and hygromycin B, which selects against untransfected cells. (c) After two rounds of selection the surviving cells are pooled, the episomes are extracted, and the individual antisense cDNA fragments that protect the cells from IFN- γ -induced cell death are subjected to further analysis.

length sense cDNA. Conceptually, it is well accepted now that overexpression of a single gene that is part of a molecular pathway that leads to cell death is often itself sufficient to trigger the entire cell-death process, even in cases where the normal regulation of the gene during apoptosis does not involve elevation of expression^{15–17}. We

Table 1. DAP genes isolated by the technical knockout approach

Gene name	Size of mRNA (kb)	Size of protein (kDa)	Molecular and biochemical features	Chromosomal assignment
DAP-1	2.4	15	Proline rich, phosphorylation site for cdk	5p15.2
DAP kinase	6.3	160	Calmodulin binding, serine/threonine kinase, cytoskeleton binding, ankyrin repeats, death domain	9q34
DAP-3	1.7	46	Nucleotide-binding motif	1q21
DAP-4	4.0	N.D.	N.D.	Unknown
DAP-5	3.8	97	Homologue of eIF4G	11p15
Cathepsin D	2.2	Precursors: 52 and 48; mature: 30 and 14	Lysosomal aspartyl protease	11p15
Thioredoxin	0.7	12	Dithiol-disulphide active site	9q31

found that ectopic expression of DAP-3 from a constitutive promoter induced the death of HeLa cells within 48–72 h, leading to a significant reduction in the number of stable drug-resistant clones after two weeks. Thus, the finding that overexpression of *DAP-3* induced cell death provides independent support for this gene being a positive mediator of cell death.

DAP kinase

DAP kinase, initially termed DAP-2, was identified as a kinase once the full-length cDNA had been isolated and the deduced amino acid sequence compared with the protein databases¹². DAP kinase (160 kDa in size), is a Ca^{2+} -calmodulin-dependent serine/threonine kinase with a unique structure (Fig. 3). The protein has eight ankyrin repeats, which mediate protein-protein interactions and might serve as an interaction domain for DAP kinase with upstream or downstream effectors. Also, two P-loop motifs reside in the protein outside the kinase domain^{12,18}. The C-terminus contains a typical death-domain module, followed by a serine-rich stretch of amino acids. The death domain of DAP kinase contains all the boxes of homology and the conserved amino acids characteristic of analogous domains in other death domain-containing proteins¹⁹. Many of these other proteins, including the p55 TNF receptor (see the review by A. Hata, Y. Shi and J. Massagué in this issue), the Fas/APO-1 receptor, DR3-5, FADD/MORT-1, RIP and TRADD, have a direct involvement in programmed cell death¹⁹. Being a motif composed of several α -helical regions, the death domain of DAP kinase might prompt association with other protein partners crucial for the function of this novel type of kinase.

Subsequent biochemical assays have confirmed that DAP kinase is a Ca^{2+} -calmodulin-dependent kinase¹⁸. *In vitro* kinase assays show that DAP kinase can phosphorylate itself and other substrates, and

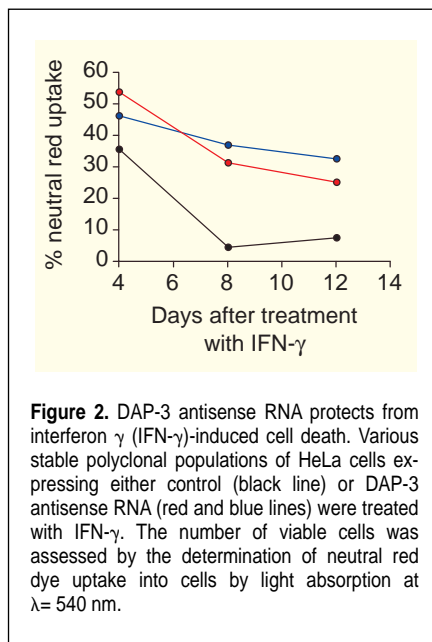
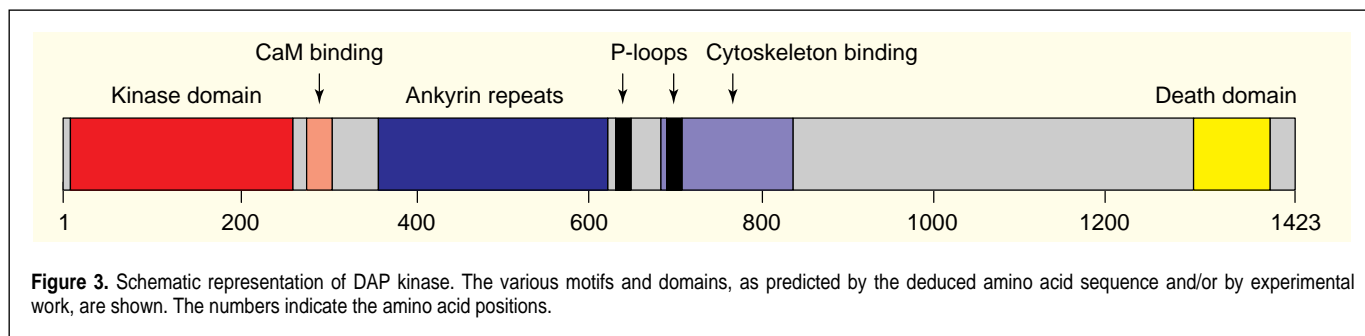


Figure 2. DAP-3 antisense RNA protects from interferon γ (IFN- γ)-induced cell death. Various stable polyclonal populations of HeLa cells expressing either control (black line) or DAP-3 antisense RNA (red and blue lines) were treated with IFN- γ . The number of viable cells was assessed by the determination of neutral red dye uptake into cells by light absorption at $\lambda = 540$ nm.

that phosphorylation is enhanced by Ca^{2+} -calmodulin. DAP kinase activity is abolished by the substitution of a conserved lysine by an alanine residue within the kinase domain (K42A), thus generating a loss-of-function mutant. Conversely, the removal of the calmodulin-binding domain generates a constitutively active kinase (gain-of-function mutant)¹⁸. Overexpression of DAP kinase induces HeLa cell death (as for DAP-3), and the death-promoting function of DAP kinase is fully dependent on its catalytic activity: the constitutively active kinase, lacking the calmodulin-binding domain, leads to death of the largest numbers of cells in these assays, whereas the catalytically inactive K42A mutant kinase cannot induce cell death¹⁸. Furthermore, the K42A mutant DAP kinase protects HeLa cells from IFN- γ -induced cell death, probably by competing with the function of the endogenous kinase. Immunostaining for DAP kinase indicates that the protein is localized to the cytoskeleton, specifically in association with the actin microfilament system. Interestingly, the correct intracellular localization of DAP kinase is also required for the death-promoting functions of this protein¹⁸.

DAP-5

DAP-5 codes for a 97 kDa protein that is highly homologous to translation initiation factor 4G (eIF4G; p220): the overall amino acid identity is 27% (48% similarity)²⁰. p220 functions as a scaffold for assembling several initiation factors that direct ribosomes to RNA at the cap site. The N-terminal part of p220 binds eIF4E, the well-known cap-binding protein, whereas the middle region of the protein binds the translation initiation factors eIF3 and eIF4A. The cap-binding end of p220 is cleaved during lytic infection by RNA viruses, thus inhibiting cap-dependent translation. Strikingly, DAP-5 lacks the N-terminal region that is necessary for cap-dependent



translation. Thus, DAP-5 might represent a naturally occurring form of the cleaved p220, linking translational control to the process of cell death²⁰.

What is the biochemical function of DAP-5? One angle on this issue was studied by the group of Sonenberg who independently isolated this gene and named it p97. This group has shown that DAP-5/p97 binds to eIF3 and eIF4A and that, like the cleaved p220, it fails to bind the cap-binding protein eIF4E (Ref. 21). Assays based on transient transfections showed that overexpression of DAP-5/p97 repressed both cap-dependent and cap-independent translation from viral internal ribosomal entry sites (IRES), suggesting that DAP-5/p97 might function as a repressor of translation that titrates out eIF3 and eIF4A from cells. However, this possibility is not compatible with the abundant expression of DAP-5 in many cell types and tissues. An alternative working model proposes that DAP-5/p97 might be converted into a putative death-promoting active form, driving the cap-independent translation of certain crucial cellular mRNAs required for cell death. Whether cellular IRESs exist that can bind DAP-5/p97 remains to be determined.

Another line of research recently resulted, quite surprisingly, in the independent cloning of the gene encoding DAP-5. Yamanaka *et al.*²² developed an elegant strategy to clone novel targets for the RNA-editing enzyme Apobec-1. Apobec-1 is the catalytic subunit of the editosome complex, which converts cytidine to uridine via its cytidine deaminase activity. By overexpressing Apobec-1 in the liver of transgenic mice, this group identified DAP-5 as a target for the enzyme and have mapped multiple sites along the DAP-5 RNA that undergo specific editing²². This work opens up the possibility that Apobec-1-mediated editing of DAP-5 RNA might regulate DAP-5 function.

DAP-1

DAP-1 is a 15 kDa, proline-rich basic protein. It has two potential cdk phosphorylation sites; phosphorylated DAP-1 displayed a different pattern of mobility on gels¹². Immunostaining and biochemical fractionation established that this protein is localized in the cytoplasm. Yet, no clues have so far been provided concerning the biochemical function of this protein. At the cellular level, the death-promoting effects of the protein were prominent, and overexpression of the full-length protein potentiated the killing effects of IFN- γ (T. Raveh and A. Kimchi, unpublished).

DAP kinase and cancer

Selection against positive mediators of apoptosis is an advantageous step in the multi-step process of tumorigenesis. The best-characterized example is the *TP53* tumour suppressor gene: malignant cells often

have loss-of-function mutations or deletions of *TP53*, providing mechanisms by which tumours lose apoptotic control²³. It therefore seems logical to assume that functional *DAP* genes might also be lost during tumorigenesis. So far, we have concentrated on the potential role of DAP kinase in cancer, because its gene maps to human chromosome 9, band q34.1 (Ref. 24), a region prone to translocations in human leukaemias and to loss of heterozygosity (LOH) in bladder carcinomas²⁵⁻²⁷. The levels of expression of DAP kinase mRNA and protein are below the detection limits in 70% of B-cell lymphoma and leukaemia cell lines tested, and in 30% of cell lines derived from bladder carcinomas, breast carcinomas, and renal cell carcinomas²⁸. This is in sharp contrast to the finding that DAP kinase mRNA is widely expressed in all tested human and murine tissues, as well as in many immortalized cell lines established from normal cells.

What are the mechanisms responsible for the loss of expression of DAP kinase? Gene silencing by methylation is well documented and has been demonstrated for various tumour suppressor genes, such as *CDKN2A* (p16), *VHL* and *RB*, during tumorigenesis (reviewed in Ref. 29). In cell lines derived from two cases of bladder carcinoma and one case of B-cell lymphoma in which DAP kinase is not expressed, treatment of cells with the DNA methylation inhibitor 5'-aza-2'-deoxycytidine restores expression of DAP kinase. However, in some other cases, this treatment does not restore DAP kinase expression, indicating that DNA methylation is only one of several mechanisms involved in shutting off DAP kinase expression²⁸. These experiments provided the first hint that DAP kinase inactivation might be a causative factor in the formation of tumours, and directed us to experimental animal model systems.

The animal model system used to test the involvement of DAP kinase in tumorigenesis was based on the finding that highly metastatic clones originating from a Lewis lung-cell carcinoma were DAP kinase negative, whereas the poorly metastatic counterparts of these lung carcinoma cells expressed normal DAP kinase levels³⁰. Transfection of highly metastatic clones with the gene for DAP kinase yields clones expressing the transgene at physiological levels, with no changes in the overall pattern of their growth *in vitro*. The metastatic activity of these genetically manipulated cells can be examined by intravenous injection of the cells into syngeneic mice. Strikingly, restoration of physiological levels of DAP kinase in the highly metastatic Lewis carcinoma cells suppresses their ability to form lung metastases in mice³⁰. Conversely, rare lung lesions, which are spontaneously selected in mice following injection of the original poorly metastatic cells, lose endogenous DAP kinase expression at high frequency³⁰. Altogether, these experiments suggest that loss of DAP kinase expression provides a positive selective advantage during the formation of lung metastases. The transgene also delays local

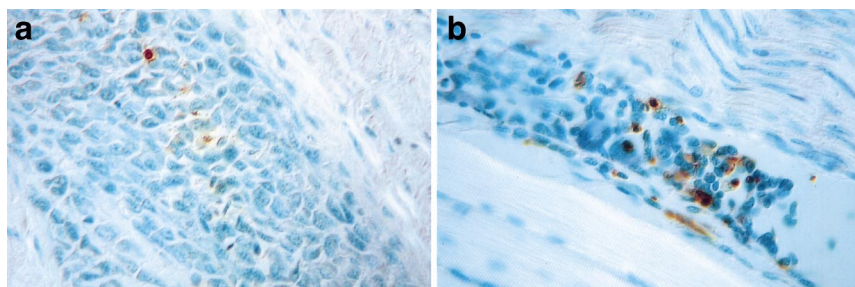


Figure 4. Exogenous expression of DAP kinase increases the apoptotic index. *In situ* TUNEL staining of tumour sections from Lewis lung-cell carcinoma cells that are (a) null for DAP kinase expression or (b) transfected with DAP kinase. The brown staining corresponds to apoptotic cells. The incidence of apoptotic cells is higher in (b), thus demonstrating that the reintroduction of DAP kinase into these cells increases the apoptotic index of this tumour. Reproduced, with permission, from Ref. 30. © 1997 Macmillan Magazines Ltd.

tumour growth, but with lower efficiency than the suppression of the metastatic activity.

In situ TUNEL staining performed on histological sections of local tumours reveals that the apoptotic index of the slow-growing local tumours formed by DAP kinase-transfected cells is higher than the value measured in the tumour mass formed by the control clone (Fig. 4). These results indicate that expression of DAP kinase re-sensitizes the tumour cells to apoptotic stimuli, a property that they had originally managed to circumvent by losing DAP kinase. In assays in which the cells are exposed *in vitro* to different apoptotic stimuli, they are extremely sensitive to TNF- α or to detachment from the extracellular matrix³⁰. Based on these experiments, we suggest that

DAP kinase-mediated suppression of metastasis results, at least in part, from increased sensitivity to various death-inducing stimuli. We propose that loss of DAP kinase expression provides a novel mechanism that links suppression of apoptosis to metastasis.

Cathepsin D: a novel twist in the 'protease connection' with cell death

As it is well established that proteases are positive mediators of apoptosis, it did not come as a total surprise that a protease, cathepsin D, was identified by the TKO approach³¹. However, the proteases identified to date as involved in apoptosis are the caspases of the cysteine protease family³², whereas cathepsin D is a lysosomal aspartyl protease. In secondary transfections, the rescued cathepsin D antisense cDNA fragment

protects HeLa cells from IFN- γ and Fas/APO-1 induced cell death. The death-promoting effects of cathepsin D are dependent on its protease activity, because pepstatin A, a known peptide inhibitor of cathepsin D, protects HeLa cells from IFN- γ and Fas/APO-1 induced cell death. In addition, it has been demonstrated that pepstatin A can also protect U937 cells from apoptosis induced by TNF- α ³¹.

How and where does a housekeeping lysosomal protease fit into the apoptotic scheme? To answer this question, we asked whether cathepsin D displays an altered pattern of regulation during apoptosis. We found that indeed this is the case, both at a transcriptional and post-translational level. First, the levels of cathepsin D mRNA are elevated three- to fourfold after treatment of HeLa cells with

Glossary

Apoptotic index – A number indicating the frequency of apoptotic cells in the general cell population or tissue.

Ankyrin repeat – A 38-residue motif originally identified in ankyrin. It mediates protein–protein physical interactions.

Cap-binding protein – A protein that binds to the 5'-cap of eukaryotic mRNA.

Cdk phosphorylation site – A consensus site on proteins that is phosphorylated by cyclin-dependent kinases.

Death domain – A protein motif composed of several α -helical structures, found in many proteins that participate in apoptosis. This motif mediates interactions between death domain-containing proteins, forming homodimers or heterodimers.

Editosome – A multiprotein complex that binds to mRNAs and carries out the function of post-transcriptional RNA editing.

Fas/APO-1/CD95 – A cell-membrane receptor of the tumour necrosis factor (TNF) family of receptors. Initiation of signalling through Fas induces apoptosis in several types of cells.

Interferon γ (IFN- γ) – A cytokine belonging to a family of small secreted proteins (the IFN family). IFN- α and IFN- β species are produced by cells when challenged by viruses. IFN- γ is preferentially produced by activated T cells. Binding of IFNs to their cognate cell-surface receptors activates specific early signalling pathways involving JAK kinases and STAT transcription factors, leading to various cellular responses, such as suppression of viral replication, inhibition of cell proliferation and cell death.

Internal ribosomal entry site (IRES) – A pyrimidine-rich stretch of 5'UTR sequences of mRNA that forms secondary structures and directs ribosomes to internal ATG translation initiation sites.

Loss of heterozygosity (LOH) – Loss of genetic material from one region of a pair of chromosomes. If an individual is heterozygous for a mutation in a tumour suppressor gene, loss of the functional copy of the gene (and therefore heterozygosity in the region) can lead to tumour growth.

P loop – A protein motif that coordinates the interaction of proteins with a nucleotide.

TUNEL – TdT-mediated dUTP-biotin nick end labelling. This method transfers a marker to the ends of DNA strands, thus indicating the extent of DNA fragmentation in a desired system.

IFN- γ . Furthermore, western blots revealed that the processing of the protease is altered. Normally, cathepsin D is produced as an inactive prepro-form of 52 kDa. The protein is then cleaved at the N-terminus, resulting in a proteolytically active 48 kDa form localized to pre-lysosomal vesicles. Finally, it is cleaved again to its mature double-chain form of 14 and 30 kDa, which is localized to the lysosome. After treatment with IFN- γ , the 48 kDa intermediate form accumulates and predominates in the apoptotic cells at the expense of the mature double-chain form³¹. This suggests that changes in the intracellular localization of this protease might occur; perhaps this is mirrored by alterations in the profile of its substrates.

Wu and colleagues have now demonstrated that cathepsin D has a role in p53-dependent apoptosis induced by the DNA-damaging drug adriamycin³³. Two p53 DNA-binding sites have been identified in the cathepsin D promoter: they bind p53 *in vitro*, and appear to mediate transactivation by p53 of a reporter gene driven by the cathepsin D promoter. It appears from this work that cathepsin D also mediates apoptotic signals that involve DNA damage and are p53 dependent, thus further extending the spectrum of apoptotic signals that use this aspartic protease in response to genotoxic signals.

Cathepsin D can therefore be added to the growing list of proteases that function as positive mediators of apoptosis. How its function is related to the cascade of caspase activation during apoptosis is an issue that remains to be studied.

Prospects for the future

The TKO screen for positive mediators of IFN- γ -induced cell death has resulted in an eclectic combination of genes, and, so far, we do not know whether or how they interact functionally. By virtue of the mode of their rescue, each of these genes should have a rate-limiting function in the apoptotic process. In some cases, we have already strengthened support for this hypothesis by using additional tools, such as dominant-negative mutants (for example, the K42A DAP kinase mutant), or small peptide inhibitors (for example, pepstatin A), each of which displayed a similar rate-limiting effect to the original antisense cDNA fragment. In addition, the death-promoting effects of DAP overexpression provide further independent support for this idea.

Does the rate-limiting function of DAPs provide sufficient proof that they are intrinsic components of apoptotic pathways? One way to address this question is to look for regulation of these genes in response to the apoptotic stimulus that initiated the process. Obviously, the regulation could take place at different levels of gene expression/function such as transcription, translation, post-translational modifications, or association with other proteins. The apoptosis-related regulation pattern of cathepsin D, mentioned above, provides a classic example addressing this point. This important issue should be extensively studied with respect to each of the isolated DAP genes. If they are involved directly in apoptotic pathways, their functional positions with respect to each other should be determined. For example, can we rescue the death-promoting effects initiated by overexpressing one of the DAP genes, by co-transfecting the cells with an antisense cDNA fragment or a dominant-negative mutant corresponding to another DAP gene? Also the cross-interaction of DAP genes with the known caspases and with members of the bcl-2 family should be worked out in detail.

Is mediation of cell death the sole function of DAP genes? As these genes are expressed ubiquitously in many cell types and tissues, and are highly conserved from an evolutionary standpoint, they might have roles in addition to the single function by which they were isolated.

The outstanding questions

- Will the next rounds of selection with the same cDNA library yield the same DAP genes or is the system still far from being fully exhausted?
- Why were genes belonging to the caspase or bcl-2 families not rescued?
- Where along the apoptotic pathways can we functionally place the DAP genes and cathepsin D? Are they downstream, upstream or in parallel to the caspases?
- Where else can the TKO strategy be applied? Which types of positive selection might be as powerful as selection of surviving cells?
- What are the prospects for using functional gene cloning, like TKO selection, in the rescue and identification of novel tumour suppressor genes?

Finally, what is the connection of the DAP genes to pathologies in which aberrant apoptosis plays a role? As the DAP genes are positive mediators of cell death, it is theoretically possible that their aberrant expression might have a role in diseases such as immune deficiencies, neurodegenerative diseases and cancer. To date the only connection between a DAP gene and a pathology is that of DAP kinase and cancer. Perhaps an additional indication for the involvement of a DAP gene in malignancy comes from the recent isolation of DAP-5 during positional cloning while searching for a gene that lies in the proximity of an inserted viral sequence causing myeloid leukaemia in mice³⁴. The relevance and prevalence of these phenomena in human malignancies remains to be determined by screening freshly isolated human tumours at different stages.

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Focus on cancer

There's something for cancer researchers in almost every issue of *MMT*. As well as cutting-edge news, literature reports and meeting reports, we publish a wide variety of reviews of relevance to cancer. Here's a collection of recent reviews to whet your appetite...

Carreau, M. and Buchwald, M. (1998) Fanconi's anaemia: what have we learned from the genes so far? *Mol. Med. Today* 4, 201–206

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Articles planned for future issues include: the role of mucins in cellular interactions and immune regulation, molecular understanding of testicular tumours, the oncostatin M signalling pathway, and more.

