

Controlling TRAIL-mediated caspase-3 activation

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Introduction

This commentary is addressed at the paper by Dr Jie and his co-workers in the current issue of *Leukemia* ('Differential involvement of Bax and Bak in TRAIL-mediated apoptosis of leukemic T cells'). In this paper, the authors show the preferential use of Bax over Bak for the induction of mitochondrial apoptotic events in leukemic cell exposed to TRAIL 1 . These findings are consistent with previous studies involving Bax in TRAIL-mediated apoptosis in the colon carcinoma cell line HCT116 2 –4 . The redundant function of Bak and Bax was initially demonstrated in lymphocytes subjected to various apoptotic stimuli 5 –8 . Hence, simultaneous Bax and Bak gene inactivation results in a profound effect on the control of lymphocyte survival upon growth factor or glucose deprivation 9 . Whereas, Bak inactivation, alone, appears to have no effect, Bax inactivation, however, slightly affected lymphocyte survival 9 . These data could therefore suggest that Bax and Bak may not be so redundant after all. Accordingly, the use of Jurkat clones deficient for both Bak and Bax by Jie and collaborators, revealed that Bax and Bak could exhibit differential functions, at least in the studied cell type system, as only Bax re-expression could restore caspase-3 processing and sensitization to TRAIL-induced cell death 1 .

Loss of function mutations of Bax are often found both in colon carcinomas 10 and in leukemias 11 ,12 . Therefore, since both death domain containing receptors and chemotherapeutic agents may require Bax for the triggering of the apoptotic process 2 ,13 , this information may be important for future cancer therapeutic approaches using TRAIL or TRAIL receptor agonists either alone or in combination with chemotherapeutic agents.

Regulating caspase activation

Like other death domain containing receptors, agonistic TRAIL receptors share common apoptotic signalling components with antitumor drugs such as caspases 14 ,15 . Cell death triggered by TRAIL proceeds either directly from DISC (Death-Inducing Signalling Complex) formed upon TRAIL binding to its cognate agonistic receptors (TRAIL-R1 or TRAIL-R2), or indirectly via an amplification loop involving the mitochondria 16 . Both pathways, ultimately lead to the activation of caspase-3, the main caspase responsible for the execution of apoptosis. To date, caspases form a large family of cysteine proteases of 13 members which function can be subdivided into two main subsets as initiator caspases or executioner caspases (recent review 17). Comprehension of caspase activation has dramatically changed during the last months. It was previously assumed that executioner caspases, such as caspase-3, -6 or -7, were activated by proteolytic cleavage by initiator caspases such as caspase-2, -8, -9 or -10 18 , releasing active dimer fragments capable in turn to process various substrates as the PARP (Poly-ADP-Ribose-Polymerase). While executioner caspases are dimeric, initiator caspases are monomeric, and until recently, their activation was thought to occur upon assembly into large protein platforms, such as DISC. This assembly was thought to trigger proteolytic activation by "close proximity"19 . However, it has been shown recently that dimerization alone was sufficient to induce initiator caspase activation 20 –22 . These informations have important consequences for the interpretation of experimental results relating to caspase activation, as it implies that procaspase cleavage is not an absolute requirement for caspase activation. Caspase activities have also been shown to be involved in various apoptotic-independent cellular signalling pathways, like inflammation or differentiation 23 –25 . Last but not least, caspase substrate specificity may also significantly be influenced depending on how caspases are activated. We have shown recently that caspase-8 activation and substrate specificity could be altered by cFLIP, a caspase-8 inhibitor, at the level of DISC, restricting its activity to a limited subset of proteins located at a close proximity 21 .

Post-mitochondrial control of caspase activation

Mitochondria are believed to play a major role in apoptosis, and mitochondrial permeability transition is observed in a large number of apoptotic events 26 . Death receptor-induced mitochondrial activation is mainly triggered in a caspase-8 dependent manner via clivage of Bid 27 , which in turn induces Bax translocation from the cytosol to the mitochondria 28 . Bax translocation is believed to be crucial for cytochrome c release from the mitochondrial intermembrane space 29 . The mechanisms involving proapoptogenic factor release from the mitochondria are still unclear and controversial.

Both Bak and Bax have been shown to trigger the release of apoptogenic factors from the mitochondria, and to play important regulatory functions upon TRAIL-induced caspase activation 30 . Bax-induced mitochondrial potential reduction is thought to be dependent on its oligomerization conformation state controlled by its subcellular localization and co-activation by cleaved Bid 31 . In line with these comments is the observation made by Jie and collaborators in this issue of *Leukemia* , which demonstrate that full p20 caspase-3 fragment processing is required for TRAIL-mediated apoptosis execution in a Bax-dependent fashion 1 . Therefore, how can caspase-3 activation be inhibited at the p20 level ? Several inhibitors of apoptosis proteins, such as IAPs, have been shown to bind to and inhibit caspases downstream mitochondria. Amongst these IAPs, XIAP inhibits caspase-3 via its BIR2 domain 32 . IAPs, though, are counteracted by other proteins such as Smac/DIABLO, Omi/HtrA2 or GSPT1/eRF3 in mammals 33 –36 . These proapoptogenic factors, also released from the mitochondria, facilitate cytochrome c-mediated activation of caspase-9 and –3 in the cytosol. They share a conserved Nterminal IAP-binding motif necessary and sufficient to relieve IAP's inhibition. However, caspase-3 p20 processing inhibition, in the clonal Bak^{-/-}; Bax^{-/-} Jurkat cell lines described in Jie's study, upon TRAIL stimulation, seems to be independent of XIAP, as the use of Smac agonistic peptides did not relieve caspase-3 inhibition 1 . In addition, exogenous cytochrome c enabled caspase-3 activation 1 . Accordingly, Smac-induced cytochrome c release has been shown to be independent of Bax in human carcinoma cells 37 , therefore the data provided by Jie and collaborators suggest that other inhibitory mechanisms may be involved. Other IAP members, known to display E3 ubiquitin-ligase activity, due to their RING domain 38 , could regulate caspase-3 processing and activation. Indeed c-IAP-1 or c-IAP-2 could contribute to this process, since their E3 ligase activity has been shown to be unaffected by binding to Smac/Diablo, contrary to XIAP 39 . In addition, caspase-3 p12 and p17 subunit half-lives have been shown to be regulated by ubiquitination-mediated proteasome-induced degradation 40 . Thus, the ubiquitin proteasome pathway could play a central role in the regulation of TRAIL-induced cell death 41 , 42 , and in particular in this clonogenic system described by Jie and collaborator.

Therefore, given the importance of Bax in TRAIL-induced apoptosis in certain cell types, and in particular in leukemias, understanding how caspase-3 activation is tuned 43 should prove useful. Thus, as Bax expression/function can be altered in many tumors, it becomes clear that treatment that would permit to bypass Bax-deficiency will be welcomed. Interestingly, caspase-3 has been shown to be activated by thapsigargin, in Bax-deficient cell lines 44 . Thapsigargin is a sesquiterpene lactone, known to alter Ca²⁺ homeostasis by inhibiting endoplasmic reticulum Ca²⁺ ATPases. Indeed, Bax and Bak, have been shown to directly modulate endoplasmic reticulum Ca²⁺ stores 45 , boosting the release of cytochrome c from mitochondria 46 , suggesting that mitochondria and endoplasmic reticulum may well be the gatekeepers of apoptosis control 47 . Future experiments may thus shed light on the molecular mechanisms involved and provide new therapeutic approaches to circumvent Bax deficiencies.

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