Controlling TRAIL-mediated caspase-3 activation.

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Commentary

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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 ~~the~~ 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 cystein 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 plateforms, such as ~~the~~ 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 ~~the~~ 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 ~~h~~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 N-terminal 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 ~~(not shown)~~ [1]. In addition, exogenous cytochrome c enabled caspase-3 activation ~~(not shown)~~ [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, ~~at the contrary of~~ 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 sequiterpene lactone, known to alter Ca^{2+} homeostasis by inhibiting endoplasmic reticulum Ca^{2+} ATPases. Indeed, Bax and Bak, have been shown to directly modulate endocytoplasmic reticulum Ca^{2+} 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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