



## Controlling TRAIL-mediated caspase-3 activation.

Olivier Micheau, Delphine Mérino

### ► To cite this version:

Olivier Micheau, Delphine Mérino. Controlling TRAIL-mediated caspase-3 activation.. Leukemia, 2004, 18 (10), pp.1578-80. 10.1038/sj.leu.2403497 . inserm-00527121

**HAL Id: inserm-00527121**

**<https://inserm.hal.science/inserm-00527121>**

Submitted on 18 Oct 2010

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## Commentary

Controlling TRAIL-mediated caspase-3 activation :

D. Mérino<sup>#</sup> and O. Micheau<sup>#</sup>

<sup>#</sup>U517 INSERM, Faculté de Médecine et de Pharmacie, 7 Bd Jeanne d'Arc, 21000 Dijon, France.

### 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 <sup>1</sup>. These findings are consistent with previous studies involving Bax in TRAIL-mediated apoptosis in the colon carcinoma cell line HCT116 <sup>2-4</sup>. The redundant function of Bak and Bax was initially demonstrated in lymphocytes subjected to various apoptotic stimuli <sup>5-8</sup>. Hence, simultaneous Bax and Bak gene inactivation results in a profound effect on the control of lymphocyte survival upon growth factor or glucose deprivation <sup>9</sup>. Whereas, Bak inactivation, alone, appears to have no effect, Bax inactivation, however, slightly affected lymphocyte survival <sup>9</sup>. 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 <sup>1</sup>.

Loss of function mutations of Bax are often found both in colon carcinomas <sup>10</sup> and in leukemias <sup>11,12</sup>. Therefore, since both death domain containing receptors and chemotherapeutic agents may require Bax for the triggering of the apoptotic process <sup>2,13</sup>, 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 <sup>14,15</sup>. 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 <sup>16</sup>. 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 <sup>17</sup>). 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 <sup>18</sup>, 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 ~~the~~ DISC. This assembly was thought to trigger proteolytic activation by “close proximity”<sup>19</sup>. However, it has been shown recently that dimerization alone was sufficient to induce initiator caspase activation <sup>20-22</sup>. 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 <sup>23-25</sup>. 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 <sup>21</sup>.

### **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 <sup>26</sup>. Death receptor-induced mitochondrial activation is mainly triggered in a caspase-8 dependent manner via clivage of Bid <sup>27</sup>, which in turn induces Bax translocation from the cytosol to the mitochondria <sup>28</sup>. Bax translocation is believed to be crucial for cytochrome c release from the mitochondrial intermembrane space <sup>29</sup>. 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 <sup>30</sup>. Bax-induced mitochondrial potential reduction is thought to be dependent on its holigomerization conformation state controlled by its subcellular localization and co-activation by cleaved Bid <sup>31</sup>. 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 <sup>1</sup>. 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 <sup>32</sup>. IAPs, though, are counteracted by other proteins such as Smac/DIABLO, Omi/HtrA2 or GSPT1/eRF3 in mammals <sup>33-36</sup>. 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~~) <sup>1</sup>. In addition, exogenous cytochrome c enabled caspase-3 activation (~~not shown~~) <sup>1</sup>. Accordingly, Smac-induced cytochrome c release has been shown to be independent of Bax in human carcinoma cells <sup>37</sup>, 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 <sup>38</sup>, 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<sup>39</sup>. In addition, caspase-3 p12 and p17 subunit half-lives have been shown to be regulated by ubiquitination-mediated proteasome-induced degradation<sup>40</sup>. Thus, the ubiquitin proteasome pathway could play a central role in the regulation of TRAIL-induced cell death<sup>41,42</sup>, 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<sup>43</sup> 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<sup>44</sup>. Thapsigargin is a sesquiterpene lactone, known to alter  $\text{Ca}^{2+}$  homeostasis by inhibiting endoplasmic reticulum  $\text{Ca}^{2+}$  ATPases. Indeed, Bax and Bak, have been shown to directly modulate endoplasmic reticulum  $\text{Ca}^{2+}$  stores<sup>45</sup>, boosting the release of cytochrome c from mitochondria<sup>46</sup>, suggesting that mitochondria and endoplasmic reticulum may well be the gatekeepers of apoptosis control<sup>47</sup>. Future experiments may thus shed light on the molecular mechanisms involved and provide new therapeutic approaches to circumvent Bax deficiencies.

Correspondence : O Micheau, U517 INSERM, Faculté de Médecine et de Pharmacie, 7  
Bd Jeanne d'Arc, 21000 Dijon, France; Tel: + 33 3 80 39 34 68 Fax: 33 3 80 39 34 34;  
E-mail: [omicheau@u-bourgogne.fr](mailto:omicheau@u-bourgogne.fr)

**Acknowledgements:** We are grateful to Eric Solary and Sophie Launay for helpful  
advices and valuable discussions.

**Keywords :** Apoptosis, Caspase, Proteasome, Bax, TRAIL

**References :**

- 1 Jie H, Godstein LA, Gastman BR, Rabinovitz A, Wang GQ, Fang B, Rabinowich  
H. Differential involvement of Bax and Bak in TRAIL-mediated apoptosis of  
leukemic T cells. *Leukemia* 2004.
- 2 LeBlanc H, Lawrence D, Varfolomeev E, Totpal K, Morlan J, Schow P, Fong S,  
Schwall R, Sinicropi D, Ashkenazi A. Tumor-cell resistance to death receptor--  
induced apoptosis through mutational inactivation of the proapoptotic Bcl-2  
homolog Bax. *Nat Med* 2002; **8**: 274-281.
- 3 Deng Y, Lin Y, Wu X. TRAIL-induced apoptosis requires Bax-dependent  
mitochondrial release of Smac/DIABLO. *Genes Dev* 2002; **16**: 33-45.
- 4 Kim M, Park SY, Pai HS, Kim TH, Billiar TR, Seol DW. Hypoxia Inhibits Tumor  
Necrosis Factor-Related Apoptosis-Inducing Ligand-Induced Apoptosis by  
Blocking Bax Translocation. *Cancer Res* 2004; **64**: 4078-4081.
- 5 Lindsten T, Ross AJ, King A, Zong WX, Rathmell JC, Shiels HA, Ulrich E,  
Waymire KG, Mahar P, Frauwirth K, Chen Y, Wei M, Eng VM, Adelman DM,

- Simon MC, Ma A, Golden JA, Evan G, Korsmeyer SJ, MacGregor GR, Thompson CB. The combined functions of proapoptotic Bcl-2 family members bak and bax are essential for normal development of multiple tissues. *Mol Cell* 2000; **6**: 1389-1399.
- 6 Zong WX, Lindsten T, Ross AJ, MacGregor GR, Thompson CB. BH3-only proteins that bind pro-survival Bcl-2 family members fail to induce apoptosis in the absence of Bax and Bak. *Genes Dev* 2001; **15**: 1481-1486.
- 7 Plas DR, Rathmell JC, Thompson CB. Homeostatic control of lymphocyte survival: potential origins and implications. *Nat Immunol* 2002; **3**: 515-521.
- 8 Degenhardt K, Sundararajan R, Lindsten T, Thompson C, White E. Bax and Bak independently promote cytochrome C release from mitochondria. *J Biol Chem* 2002; **277**: 14127-14134.
- 9 Knudson CM, Tung KS, Tourtellotte WG, Brown GA, Korsmeyer SJ. Bax-deficient mice with lymphoid hyperplasia and male germ cell death. *Science* 1995; **270**: 96-99.
- 10 Rampino N, Yamamoto H, Ionov Y, Li Y, Sawai H, Reed JC, Perucho M. Somatic frameshift mutations in the BAX gene in colon cancers of the microsatellite mutator phenotype. *Science* 1997; **275**: 967-969.
- 11 Brimmell M, Mendiola R, Mangion J, Packham G. BAX frameshift mutations in cell lines derived from human haemopoietic malignancies are associated with resistance to apoptosis and microsatellite instability. *Oncogene* 1998; **16**: 1803-1812.



- 12 Meijerink JP, Mensink EJ, Wang K, Sedlak TW, Sloetjes AW, de Witte T, Waksman G, Korsmeyer SJ. Hematopoietic malignancies demonstrate loss-of-function mutations of BAX. *Blood* 1998; **91**: 2991-2997.
- 13 Naumann U, Weller M. Retroviral BAX gene transfer fails to sensitize malignant glioma cells to CD95L-induced apoptosis and cancer chemotherapy. *Int J Cancer* 1998; **77**: 645-648.
- 14 de Vries EG, Timmer T, Mulder NH, van Geelen CM, van der Graaf WT, Spierings DC, de Hooge MN, Gietema JA, de Jong S. Modulation of death receptor pathways in oncology. *Drugs Today (Barc)* 2003; **39 Suppl C**: 95-109.
- 15 Creagh EM, Conroy H, Martin SJ. Caspase-activation pathways in apoptosis and immunity. *Immunol Rev* 2003; **193**: 10-21.
- 16 Thorburn A. Death receptor-induced cell killing. *Cell Signal* 2004; **16**: 139-144.
- 17 Martinon F, Tschopp J. Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases. *Cell* 2004; **117**: 561-574.
- 18 Nicholson DW. Caspase structure, proteolytic substrates, and function during apoptotic cell death. *Cell Death Differ* 1999; **6**: 1028-1042.
- 19 Salvesen GS, Dixit VM. Caspase activation: the induced-proximity model. *Proc Natl Acad Sci U S A* 1999; **96**: 10964-10967.
- 20 Boatright KM, Renatus M, Scott FL, Sperandio S, Shin H, Pedersen IM, Ricci JE, Edris WA, Sutherlin DP, Green DR, Salvesen GS. A unified model for apical caspase activation. *Mol Cell* 2003; **11**: 529-541.

- 21 Micheau O, Thome M, Schneider P, Holler N, Tschopp J, Nicholson DW, Briand C, Grutter MG. The long form of FLIP is an activator of caspase-8 at the Fas death-inducing signaling complex. *J Biol Chem* 2002; **277**: 45162-45171.
- 22 Renatus M, Stennicke HR, Scott FL, Liddington RC, Salvesen GS. Dimer formation drives the activation of the cell death protease caspase 9. *Proc Natl Acad Sci U S A* 2001; **98**: 14250-14255.
- 23 Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Mol Cell* 2002; **10**: 417-426.
- 24 Sordet O, Rebe C, Plenchette S, Zermati Y, Hermine O, Vainchenker W, Garrido C, Solary E, Dubrez-Daloz L. Specific involvement of caspases in the differentiation of monocytes into macrophages. *Blood* 2002; **100**: 4446-4453.
- 25 Fernando P, Kelly JF, Balazsi K, Slack RS, Megeney LA. Caspase 3 activity is required for skeletal muscle differentiation. *Proc Natl Acad Sci U S A* 2002; **99**: 11025-11030.
- 26 Kroemer G, Reed JC. Mitochondrial control of cell death. *Nat Med* 2000; **6**: 513-519.
- 27 Yamada H, Tada-Oikawa S, Uchida A, Kawanishi S. TRAIL causes cleavage of bid by caspase-8 and loss of mitochondrial membrane potential resulting in apoptosis in BJAB cells. *Biochem Biophys Res Commun* 1999; **265**: 130-133.
- 28 Werner AB, de Vries E, Tait SW, Bontjer I, Borst J. TRAIL receptor and CD95 signal to mitochondria via FADD, caspase-8/10, Bid, and Bax but differentially

- regulate events downstream from truncated Bid. *J Biol Chem* 2002; **277**: 40760-40767.
- 29 Jurgensmeier JM, Xie Z, Deveraux Q, Ellerby L, Bredesen D, Reed JC. Bax directly induces release of cytochrome c from isolated mitochondria. *Proc Natl Acad Sci U S A* 1998; **95**: 4997-5002.
- 30 Kandasamy K, Srinivasula SM, Alnemri ES, Thompson CB, Korsmeyer SJ, Bryant JL, Srivastava RK. Involvement of proapoptotic molecules Bax and Bak in tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced mitochondrial disruption and apoptosis: differential regulation of cytochrome c and Smac/DIABLO release. *Cancer Res* 2003; **63**: 1712-1721.
- 31 Roucou X, Montessuit S, Antonsson B, Martinou JC. Bax oligomerization in mitochondrial membranes requires tBid (caspase-8-cleaved Bid) and a mitochondrial protein. *Biochem J* 2002; **368**: 915-921.
- 32 Huang Y, Rich RL, Myszka DG, Wu H. Requirement of both the second and third BIR domains for the relief of X-linked inhibitor of apoptosis protein (XIAP)-mediated caspase inhibition by Smac. *J Biol Chem* 2003; **278**: 49517-49522.
- 33 Du C, Fang M, Li Y, Li L, Wang X. Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. *Cell* 2000; **102**: 33-42.
- 34 Hegde R, Srinivasula SM, Zhang Z, Wassell R, Mukattash R, Cilenti L, DuBois G, Lazebnik Y, Zervos AS, Fernandes-Alnemri T, Alnemri ES. Identification of Omi/HtrA2 as a mitochondrial apoptotic serine protease that disrupts inhibitor of apoptosis protein-caspase interaction. *J Biol Chem* 2002; **277**: 432-438.

- 35 Hegde R, Srinivasula SM, Datta P, Madesh M, Wassell R, Zhang Z, Cheong N, Nejme J, Fernandes-Alnemri T, Hoshino S, Alnemri ES. The polypeptide chain-releasing factor GSPT1/eRF3 is proteolytically processed into an IAP-binding protein. *J Biol Chem* 2003; **278**: 38699-38706.
- 36 Martins LM, Iaccarino I, Tenev T, Gschmeissner S, Totty NF, Lemoine NR, Savopoulos J, Gray CW, Creasy CL, Dingwall C, Downward J. The serine protease Omi/HtrA2 regulates apoptosis by binding XIAP through a reaper-like motif. *J Biol Chem* 2002; **277**: 439-444.
- 37 Hasenjager A, Gillissen B, Muller A, Normand G, Hemmati PG, Schuler M, Dorken B, Daniel PT. Smac induces cytochrome c release and apoptosis independently from Bax/Bcl-x(L) in a strictly caspase-3-dependent manner in human carcinoma cells. *Oncogene* 2004; **23**: 4523-4535.
- 38 Suzuki Y, Nakabayashi Y, Takahashi R. Ubiquitin-protein ligase activity of X-linked inhibitor of apoptosis protein promotes proteasomal degradation of caspase-3 and enhances its anti-apoptotic effect in Fas-induced cell death. *Proc Natl Acad Sci U S A* 2001; **98**: 8662-8667.
- 39 Creagh EM, Murphy BM, Duriez PJ, Duckett CS, Martin SJ. Smac/Diablo antagonizes Ubiquitin ligase activity of inhibitor of apoptosis proteins. *J Biol Chem* 2004.
- 40 Chen L, Smith L, Wang Z, Smith JB. Preservation of caspase-3 subunits from degradation contributes to apoptosis evoked by lactacystin: any single lysine or lysine pair of the small subunit is sufficient for ubiquitination. *Mol Pharmacol* 2003; **64**: 334-345.

- 41 Kim S, Choi K, Kwon D, Benveniste EN, Choi C. Ubiquitin-proteasome pathway as a primary defender against TRAIL-mediated cell death. *Cell Mol Life Sci* 2004; **61**: 1075-1081.
- 42 Zhang HG, Wang J, Yang X, Hsu HC, Mountz JD. Regulation of apoptosis proteins in cancer cells by ubiquitin. *Oncogene* 2004; **23**: 2009-2015.
- 43 Sun XM, Butterworth M, MacFarlane M, Dubiel W, Ciechanover A, Cohen GM. Caspase activation inhibits proteasome function during apoptosis. *Mol Cell* 2004; **14**: 81-93.
- 44 He Q, Montalbano J, Corcoran C, Jin W, Huang Y, Sheikh MS. Effect of Bax deficiency on death receptor 5 and mitochondrial pathways during endoplasmic reticulum calcium pool depletion-induced apoptosis. *Oncogene* 2003; **22**: 2674-2679.
- 45 Nutt LK, Chandra J, Pataer A, Fang B, Roth JA, Swisher SG, O'Neil RG, McConkey DJ. Bax-mediated Ca<sup>2+</sup> mobilization promotes cytochrome c release during apoptosis. *J Biol Chem* 2002; **277**: 20301-20308.
- 46 Nutt LK, Pataer A, Pahler J, Fang B, Roth J, McConkey DJ, Swisher SG. Bax and Bak promote apoptosis by modulating endoplasmic reticular and mitochondrial Ca<sup>2+</sup> stores. *J Biol Chem* 2002; **277**: 9219-9225.
- 47 Scorrano L, Oakes SA, Opferman JT, Cheng EH, Sorcinelli MD, Pozzan T, Korsmeyer SJ. BAX and BAK regulation of endoplasmic reticulum Ca<sup>2+</sup>: a control point for apoptosis. *Science* 2003; **300**: 135-139.