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Commentary

Silencing Bcl-X_L in Cancer Therapy

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Commentary to:

Enhancing TRAIL-Induced Apoptosis by Bcl-X_L siRNA

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There are two main signaling pathways that lead to apoptosis: an "extrinsic" and an "intrinsic" pathway. The Tumor Necrosis Factor (TNF)-related apoptosis-inducing ligand (TRAIL) triggers the extrinsic pathway and is a promising agent for development as a cancer therapeutic[1] because it appears to specifically kill transformed and cancer cells, whereas most normal cells appear to be resistant to TRAIL.[2,3] TRAIL binding to either of two pro-apoptotic TRAIL death receptors, TRAIL R1 (DR4)[4] or TRAIL R2 (KILLER/DR5),[5] induces the formation of a death-inducing signaling complex (DISC), which consists of one of the TRAIL receptors, the adaptor protein FADD,[6] and an initiator caspase, procaspase-8. Once the DISC is formed, procaspase-8 is auto-processed and activated by induced proximity.[7] The activated caspase-8 can directly activate the executioner caspase, caspase-3, without the involvement of the mitochondrial pathway (type I pathway), or it can cleave Bid. Truncated cleaved Bid (tBid) exposes a new amino-terminal glycine that undergoes post-translational myristoylation.[8] The myristoylated Bid translocates to the mitochondria where tBid inserts into the membrane[9] followed by Bax translocation, Bak oligomerization, and cytochrome c release (type II pathway).[10]

The intrinsic pathway in response to DNA damage, such as that caused by radio- and chemotherapeutic agents[11,12] involves the participation of mitochondria, which release cytochrome *c*.[8] The released cytosolic cytochrome *c* induces oligomerization of Apaf-1 and recruitment of procaspase-9 into a large complex known as the apoptosome.[13] After activation in the apoptosome, caspase-9 activates the effector caspases,[13,14] thereby initiating apoptosis.

The Bcl-2 family proteins are the major regulators of mitochondrial apoptotic homeostasis.[15] Several members of the Bcl-2 family (including Bcl-2, Bcl- X_L, MCL-1, A1 and BAG-1) promote survival while the other members (including Bcl-X_S, Bad, Bax, and Bak) promote cell death. The relative ratios of these pro- and anti-apoptotic members (i.e., homodimers:heterodimers) of the Bcl-2 family, rather than the expression level of any single Bcl-2 family protein, have been shown to determine the ultimate apoptotic sensitivity or resistance of cells to diverse stimuli.[16] Anti-apoptotic proteins including Bcl-2 or Bcl-X_L are frequently overexpressed in human tumors,[17] thereby blocking death signal propagation through mitochondria.[18] Accordingly, targeted knock-down or silencing of the anti-apoptotic Bcl-2 family has potential to facilitate cancer cell apoptosis induced by various apoptotic stimuli. Until recently, the repression of these genes has been mediated by anti-sense oligonucleotides. The oligonucleotides targeting Bcl-X_L could sensitize tumor cells to various chemotherapeutic agents,[19] agonistic anti-FAS Ab,[20] or to TRAIL.[21,22] Recently small interfering RNAs (siRNAs) that are 21- to 23 nucleotides of dsRNA[23] have emerged for repression of these genes. For example, Bcl-X_L protein expression was silenced by Bcl-X_L-specific synthetic siRNA in 5-FU-resistant cancer cells and proliferation of the cells became inhibited by 5-FU.[24]

In the present study, Zhu et al show that the combination of Bcl-X_L siRNA and TRAIL inhibited cell proliferation and sensitized TRAIL-induced apoptosis in human cancer cells with both acquired (DLD1-TRAIL/R, a TRAIL-resistant derivative selected from human colon cancer DLD-1 parental cells) and intrinsic (human ovarian cancer SKOV3 cells, which express high levels of Bcl-X_L and have K441R polymorphism in the death domain of DR4[25]) TRAIL resistance. The results show that although there is detectable cytochrome-c release into cytosol by Bcl-X_L siRNA alone, TRAIL alone, or the combination of Bcl- X_L siRNA and TRAIL in both cell lines, there was no release of Smac/DIABLO[26,27] from mitochondria in any of the treatment groups. Smac/DIABLO is normally localized to mitochondria but is released into the cytosol during the early stages of apoptosis, where it promotes caspase activity by inhibiting IAPs, particularly the X-linked inhibitor of apoptosis (XIAP).[28,29] Overexpression of Bcl-X_L prevented mitochondrial release of Smac/DIABLO and subsequent inactivation of the XIAP protein.[30] Overexpression of the IAP family proteins is associated with poor responsiveness to apoptosis-inducing

therapies.[31,32] Therefore, it is possible that the outcome of treatment using the combination of Bcl-X_L siRNA and TRAIL may be affected by the expression level of endogenous XIAP in cancer cells.

To have broad applications for cancer therapy, the downregulation of Bcl-X_L should have minimal cytotoxicity toward normal cells. Although severe clinical side effects related to the transient downregulation of Bcl-X_L or Bcl-2 in normal cells and other normal tissues have not been reported, there was a report that normal cells underwent apoptosis upon treatment with Bcl-X_L or Bcl-2/Bcl-X_L anti-sense oligonucleotides.[33] To avoid this potential side effect, Bcl-X_L siRNA should either be delivered to the target cells in a tumor-specific manner or the target Bcl-X_L should be specifically expressed in the tumors being treated. Recently, there have been reports on schemes that could be used to express siRNAs in a tissue specific manner.[34,35] By modifying these schemes, Bcl-X_L siRNA could be expressed specifically in cancer cells and thus, unwanted cytotoxicity toward normal cells could be circumvented and therapeutic sensitization of cancer cells toward TRAIL or other cancer chemotherapeutics could be achieved.

References

1. French LE, Tschopp J. The TRAIL to selective tumor death. Nat Med 1999; 5:146-47.
2. Ashkenazi A, Dixit VM. Death receptors: Signaling and modulation. Science 1998; 281:1305-08.
3. Walczak H, Miller RE, Ariail K, Gliniak B, Griffith TS, Kubin M, Chin W, Jones J, Woodward A, Le T, Smith C, Smolak P, Goodwin RG, Rauch CT, Schuh JC, Lynch DH. Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand in vivo. Nat Med 1999; 5:157-63.
4. Pan G, Ni J, Wei YF, Yu G, Gentz R, Dixit VM. An antagonist decoy receptor and a death domain-containing receptor for TRAIL. Science 1997; 277:815-18.
5. Wu GS, Burns TF, McDonald ER, 3rd, Jiang W, Meng R, Krantz ID, Kao G, Gan DD, Zhou JY, Muschel R, Hamilton SR, Spinner NB, Markowitz S, Wu G, El-Deiry WS KILLER/DR5 is a DNA damage-inducible p53-regulated death receptor gene. Nat Genet 1997; 17:141-43.
6. Kischkel FC, Lawrence DA, Chuntharapai A, Schow P, Kim KJ, Ashkenazi A. Apo2L/TRAIL-dependent recruitment of endogenous FADD and caspase-8 to death receptors 4 and 5. Immunity 2000; 12:611-20.
7. Muzio M, Stockwell BR, Stennicke HR, Salvesen GS, Dixit VM. An induced proximity model for caspase-8 activation. J Biol Chem 1998; 273:2926-30.
8. Luo X, Budihardjo I, Zou H, Slaughter C, Wang X. Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. Cell 1998; 94:481-90.
9. Zha J, Weiler S, Oh KJ, Wei MC, Korsmeyer SJ. Posttranslational N-myristoylation of BID as a molecular switch for targeting mitochondria and apoptosis. Science 2000; 290:1761-65.
10. Korsmeyer SJ, Wei MC, Saito M, Weiler S, Oh KJ, Schlesinger PH. Pro-apoptotic cascade activates BID, which oligomerizes BAK or BAX into pores that result in the release of cytochrome c. Cell Death Differ 2000; 7:1166-73.
11. Green DR, Evan GI. A matter of life and death. Cancer Cell 2002; 1:19-30.
12. Wang X. The expanding role of mitochondria in apoptosis. Genes Dev 2001; 15:2922-33.
13. Zou H, Li Y, Liu X, Wang X. An APAF-1.cytochrome c multimeric complex is a functional apoptosome that activates procaspase-9. J Biol Chem 1999; 274:11549-56.
14. Srinivasula SM, Ahmad M, Fernandes-Alnemri T, Alnemri ES. Autoactivation of procaspase-9 by Apaf-1-mediated oligomerization. Mol Cell 1998; 1:949-57.
15. Cory S, Adams JM. The Bcl2 family: Regulators of the cellular life-or-death switch. Nat Rev Cancer 2002; 2:647-56.
16. Oltvai ZN, Milliman CL, Korsmeyer SJ. Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. Cell 1993; 74:609-19.
17. Jansen B, Zangemeister-Wittke U. Antisense therapy for cancer-the time of truth. Lancet Oncol 2002; 3:672-83.
18. Scaffidi C, Fulda S, Srinivasan A, Friesen C, Li F, Tomaselli KJ, Debatin KM, Krammer PH, Peter ME. Two CD95 (APO-1/Fas) signaling pathways. EMBO J 1998; 17:1675-87.
19. Luo D, Cheng SC, Xie H, Xie Y. Effects of Bcl-2 and Bcl-XL protein levels on chemoresistance of hepatoblastoma HepG2 cell line. Biochem Cell Biol 2000; 78:119-26.
20. Kondo S, Shinomura Y, Kanayama S, Higashimoto Y, Kiyohara T, Zushi S, Kitamura S, Ueyama H, Matsuzawa Y. Modulation of apoptosis by endogenous Bcl-xL expression in MKN-45 human gastric cancer cells. Oncogene 1998; 17:2585-91.
21. Kim K, Nakagawa H, Fei P, Rustgi AK, El-Deiry WS. Targeting Bcl-xL in esophageal squamous cancer to sensitize to chemotherapy plus TRAIL-induced apoptosis while normal epithelial cells are protected by blockade of caspase 9. Cell Death Differ 2004; 11:583-37.
22. Zangemeister-Wittke U. Antisense to apoptosis inhibitors facilitates chemotherapy and TRAIL-induced death signaling. Ann NY Acad Sci 2003; 1002:90-94.
23. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature 2001; 411:494-8.
24. Zhu H, Guo W, Zhang L, Davis JJ, Teraishi F, Wu S, Cao X, Daniel J, Smythe WR, Fang B. Bcl-XL small interfering RNA suppresses the proliferation of 5-fluorouracil-resistant human colon cancer cells. Mol Cancer Ther 2005; 4:451-56.
25. Kim K, Fisher MJ, Xu SQ, el-Deiry WS. Molecular determinants of response to TRAIL in killing of normal and cancer cells. Clin Cancer Res 2000; 6:335-46.
26. 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.
27. Verhagen AM, Ekert PG, Pakusch M, Silke J, Connolly LM, Reid GE, Moritz RL, Simpson RJ, Vaux DL. Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. Cell 2000; 102:43-53.
28. Wu G, Chai J, Suber TL, Wu JW, Du C, Wang X, Shi Y. Structural basis of IAP recognition by Smac/DIABLO. Nature 2000; 408:1008-12.
29. Liu Z, Sun C, Olejniczak ET, Meadows RP, Betz SF, Oost T, Herrmann J, Wu JC, Fesik SW. Structural basis for binding of Smac/DIABLO to the XIAP BIR3 domain. Nature 2000; 408:1004-8.
30. Sun XM, Bratton SB, Butterworth M, MacFarlane M, Cohen GM. Bcl-2 and Bcl-xL inhibit CD95-mediated apoptosis by preventing mitochondrial release of Smac/DIABLO and subsequent inactivation of X-linked inhibitor-of-apoptosis protein. J Biol Chem 2002; 277:11345-51.
31. Ng CP, Bonavida B. X-linked inhibitor of apoptosis (XIAP) blocks Apo2 ligand/tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis of prostate cancer cells in the presence of mitochondrial activation: Sensitization by overexpression of second mitochondria-derived activator of caspase/direct IAP-binding protein with low pI (Smac/DIABLO). Mol Cancer Ther 2002; 1:1051-8.
32. Tamm I, Kornblau SM, Segall H, Krajewski S, Welsh K, Kitada S, Scudiero DA, Tudor G, Qui YH, Monks A, Andreeff M, Reed JC. Expression and prognostic significance of *IAP*-family genes in human cancers and myeloid leukemias. Clin Cancer Res 2000; 6:1796-803.
33. Olie RA, Hafner C, Kuttel R, Sigrist B, Willers J, Dummer R, Hall J, Stahel RA, Zangemeister-Wittke U. Bcl-2 and bcl-xL antisense oligonucleotides induce apoptosis in melanoma cells of different clinical stages. J Invest Dermatol 2002; 118:505-12.
34. Shinagawa T, Ishii S. Generation of Ski-knockdown mice by expressing a long double-strand RNA from an RNA polymerase II promoter. Genes Dev 2003; 17:1340-5.
35. Xia H, Mao Q, Paulson HL, Davidson BL. *siRNA*-mediated gene silencing in vitro and in vivo. Nat Biotechnol 2002; 20:1006-10.