—Photogravure—

Involvement of Bcl-2 Family Proteins in p53-induced Apoptosis

Kei Tobiume
Department of Molecular Oncology, Institute of Gerontology, Nippon Medical School

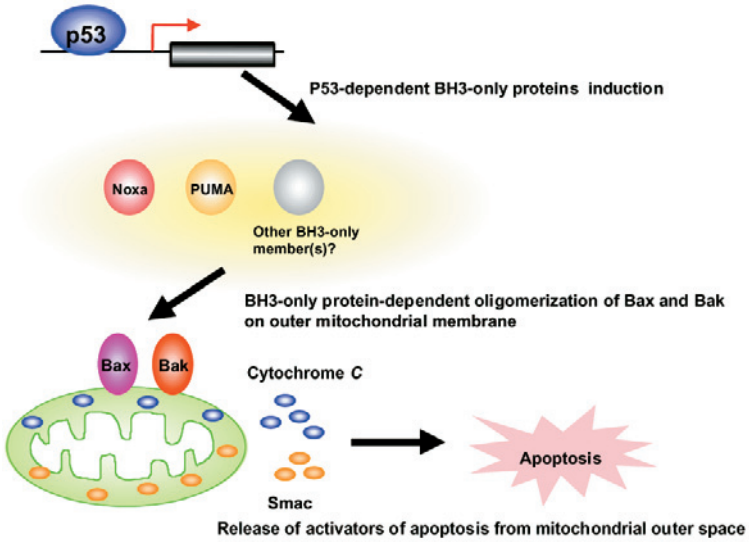

Fig. 1

Abstract

Mutations in the *p53* tumor suppressor gene occur in more than 50% of human cancers. In response to various cellular stresses, such as DNA damage, the p53 protein rapidly accumulates by posttranscriptional mechanism(s) and activates the expression of genes that play a major role in cellular responses leading to cell cycle arrest, DNA repair and apoptosis as a transcriptional activator. In particular, the induction of apoptosis is considered to be an important function in tumor suppression by p53. Recently, two BH3-only members of the Bcl-2 family, Noxa and PUMA, have been identified as p53 target genes[1,2]. Furthermore, the analysis of mice doubly deficient in multidomain Bcl-2 family proteins, Bax and Bak, revealed that apoptosis induced by the BH3-only protein is completely dependent on Bax and Bak[3]. More recently, it was demonstrated using gene knockout mice[4,5] that Noxa and PUMA function as the effectors of p53-induced apoptosis. These analyses revealed that p53-induced apoptosis is regulated by these Bcl-2 family proteins. In this photogravure, the regulation of these Bcl-2 family proteins in p53-induced apoptosis was visualized by fluorescent protein fusion and immune fluorescence methods.

Fig. 1 Schema of p53-dependent apoptosis.
The tumor suppressor gene product p53 activates the mRNA expression of Bcl-2 homology domain 3(BH3)-only proteins, Noxa and PUMA. Proapoptotic Bcl-2 family proteins, including genetically apical BH3-only proteins and downstream muitidomain proapoptotic Bcl-2 proteins, induce apoptosis by releasing the inducers of apoptosis such as cytochrome *c* and Smac from the mitochondria to the cytosol, which results in the activation of the executioner of apoptosis, caspases[6].

Fig. 2 p53 induces apoptosis.
Green fluorescent protein-tagged Bax (GFP-Bax)-expressing HeLa cells were infected with p53-expressing recombinant adenovirus (Ad-p53). Forty-eight hours after infection, cells were fixed and stained with the anti-cytochrome *c* antibody and Alexa 568-conjugated secondary antibody. Fluorescence was detected under Olympus IX-70 microscope.
The left panel shows the emergence of cells with Bax aggregated in the mitochondrial outer membrane. The right panel shows a diffuse cytochrome *c* staining pattern in the same cells. Both are characteristic of apoptotic cells.

Fig. 3 p53 expression induces the release of apoptotic inducers from the mitochondria to the cytosol. Cyan fluorescent protein-tagged Smac (CFP-Smac)-expressing HeLa cells were infected with Ad-p53. Forty-eight hours after infection, cells were fixed and stained with the anti-cytochrome *c* antibody and Alexa 568-conjugated secondary antibody.

Correspondence to Kei Tobiume, Department of Molecular Oncology, Institute of Gerontology, Nippon Medical School, 1–396 Kosugi, Nakahara-ku, Kawasaki, Kanagawa 211–8533, Japan
E-mail: tobi5472@nms.ac.jp
Journal Website (http://www.nms.ac.jp/jnms/)

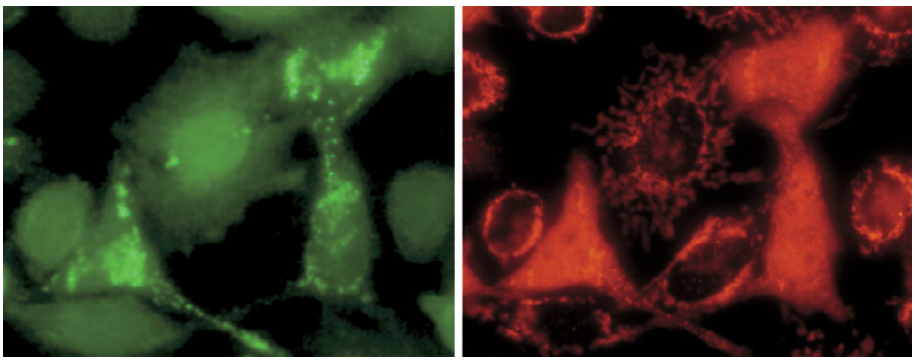

Fig. 2

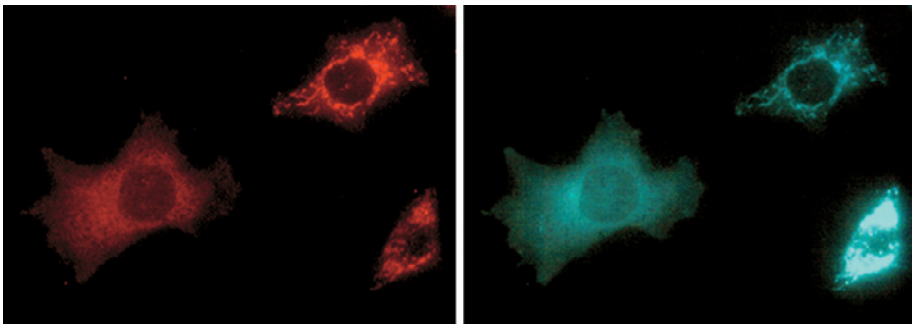

Fig. 3

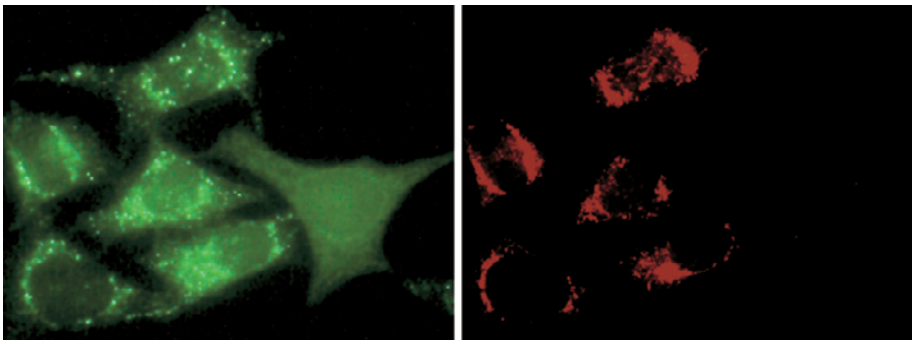

Fig. 4

The left panel shows the emergence of the cell with diffuse cytochrome *c* staining. The right panel shows a diffuse localization of CFP-Smac in the same cell.

Fig. 4 Noxa expression induces the translocation of Bax from the cytosol to the mitochondria. GFP-Bax-expressing HeLa cells were transfected with the expression vector for HA-tagged Noxa (pEF-Noxa). After 12 hours, cells were fixed and stained with the anti-HA antibody and Alexa 568-conjugated secondary antibody.

The left panel shows the emergence of cells with Bax agrregated in the mitochondrial outer membrane only in Noxa-expressing cells. The right panel shows HA-tagged Noxa-expressing cells in the same view.

References

1. Oda E, Ohki R, Murasawa H, Nemoto J, Shibue T, Yamashita T, Tokino T, Taniguchi T, Tanaka N: Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. Science 2000; 288: 1053–1058.
2. Nakano K, Vousden KH: PUMA, a novel proapoptotic gene, is induced by p53. Mol Cell 2001; 7: 683–694.
3. Cheng EH, Wei MC, Weiler S, Flavell RA, Mak TW, Lindsten T, Korsmeyer SJ: BCL-2, BCL-X(L) sequester BH3 domain-only molecules preventing BAX- and BAK-mediated mitochondrial apoptosis. Mol Cell 2001; 8: 705–711.
4. Shibue T, Takeda K, Oda E, Tanaka H, Murasawa H, Takaoka A, Morishita Y, Akira S, Taniguchi T, Tanaka N: Integral role of Noxa in p53-mediated apoptotic response. Genes Dev 2003; 17: 2233–2238.
5. Villunger A, Michalak EM, Coultas L, Mullauer F, Bock G, Ausserlechner MJ, Adams JM, Strasser A: p53- and drug-induced apoptotic responses mediated by BH3-only proteins puma and noxa. Science 2003; 302: 1036–1038.
6. Danial NN, Korsmeyer SJ: Cell death: critical control points. Cell 2004; 116: 205–219.