

## —Photogravure—

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

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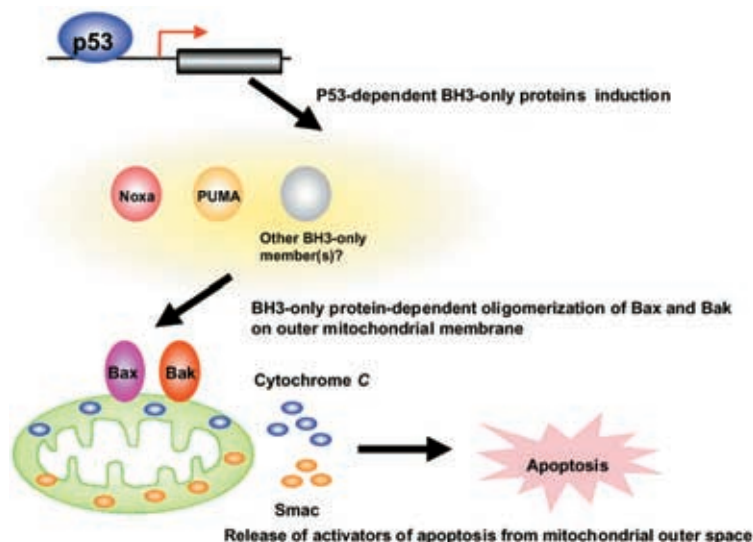


Fig. 1

**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<sup>1,2</sup>. 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<sup>3</sup>. More recently, it was demonstrated using gene knockout mice<sup>4,5</sup> 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 multidomain 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<sup>6</sup>.

**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.

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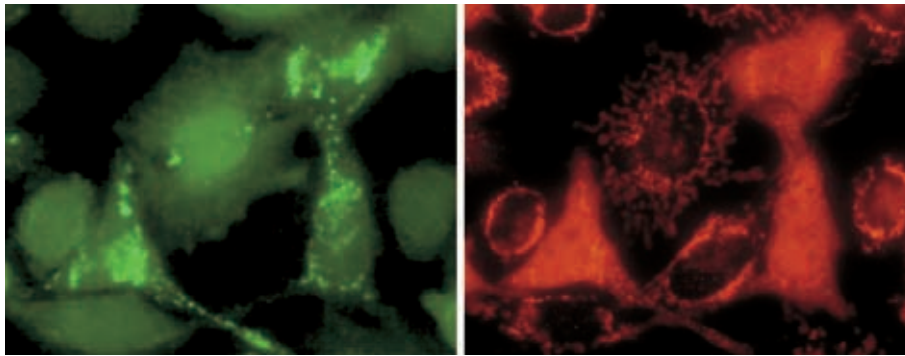


Fig. 2

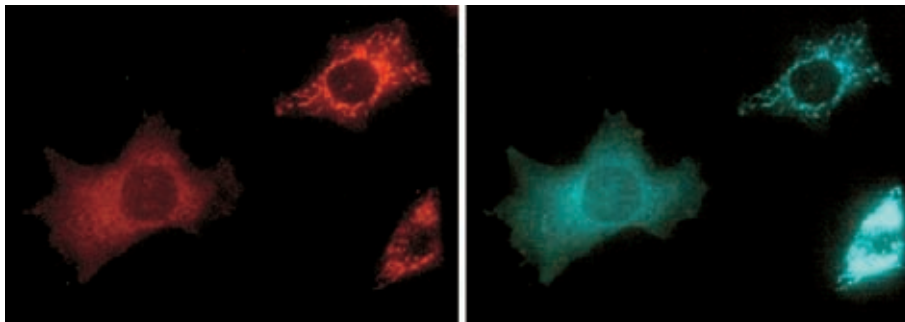


Fig. 3

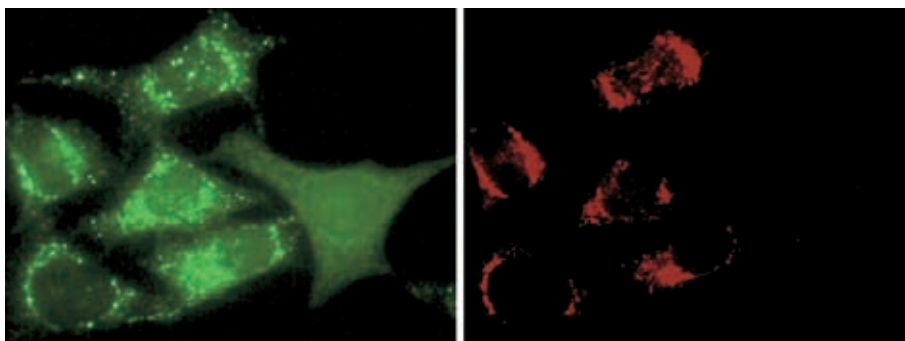


Fig. 4

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 aggregated in the mitochondrial outer membrane only in Noxa-expressing cells. The right panel shows HA-tagged Noxa-expressing cells in the same view.

#### References

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