Fas, p53, and Apoptosis

In their report "Cell surface trafficking of Fas: A rapid mechanism of p53-mediated apoptosis" (*1*), Martin Bennett *et al.* describe the consequences of overexpression of the chimeric p53 estrogen receptor protein in human vascular smooth muscle cells. They found increased cell surface expression of Fas (*2*) after activation of p53 through the addition of 4-hydroxytamoxifen. They conclude that "p53 activation can regulate sensitivity to apoptosis by allowing cytoplasmic death receptors to redistribute to the cell surface."

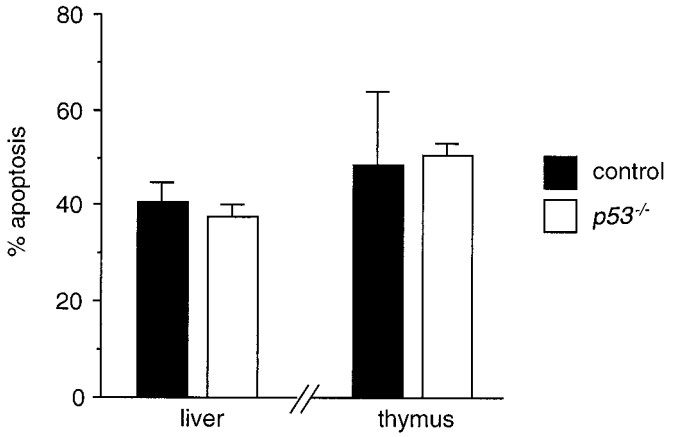

Fig. 1. Fas-induced apoptosis in the absence of p53. Apoptotic cells were detected by TUNEL staining in liver sections from $p53^{-/-}$ or control mice (both on C57BL/6 background) 2 hours after intravenous injection of 100 μg of anti-Fas (Jo2) antibody. Apoptosis was detected by phosphatidylinositol (PI) staining in thymocytes from $p53^{-/-}$ or control mice 24 hours after treatment in a culture of 1.0 μg/ml antibody (Jo2) against Fas. Values are means ± SD from at least two animals.

The physiological significance of crosstalk between Fas and p53 in apoptosis is best established by comparison between animals in which these molecules are expressed at normal levels and mutant animals that lack them altogether. We and others have established that Fas is not required for p53-activated cell death, because cells from mice deficient in Fas expression (*lpr*) or lacking functional FasL (*gld*) are unaffected in their response to p53-transduced apoptotic stimuli such as those elicited by γ-radiation or DNA-damaging drugs (*3*). We have also shown that p53 is not required for Fas-induced apoptosis, because Fas cell surface levels and Fas-ligation activated cell death are normal in mice lacking p53 (Fig. 1). A recent study showed that p53 can induce cell surface expression of Fas (*4*), but this result was based on overexpression of p53 in transformed cell lines and, like the data in the report (*1*), its relevance to normal physiology is therefore questionable.

Bennett *et al.* report that activation of overexpressed p53 causes an increase in cell surface expression of not only Fas, but also of the tumor necrosis factor TNF-R1 (*1*, p. 292). Perhaps other molecules might exhibit similarly aberrant cell surface expression under such nonphysiological conditions. If this is so, the results stated in the report (*1*) may not indicate the involvement of Fas in a "mechanism of p53-mediated apoptosis" (as stated in the title); they may instead be explained by the activation of overexpressed p53 (or the ensuing apoptotic process) having a nonspecific effect on cell surface protein traffic. Studies based on extreme overexpression such as the report by Bennett *et al.* should be viewed in the light of experiments performed under physiological conditions. Data from nontransformed cells in whole animals or tissue culture are consistent with the idea that pathways to apoptosis that are activated through p53 are distinct from those triggered by Fas ligation.

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References and Notes

1. M. Bennett *et al.*, *Science* **282**, 290 (1998).
2. Fas is also called CD95, which is a tumor necrosis factor (TNF-R) family member.
3. A. Strasser *et al.*, *EMBO J.* **14**, 6136 (1995); E. J. Fuchs *et al.*, *Cancer Res.* **57** 2550 (1997).
4. M. Miller *et al.*, *J. Exp. Med.* **188**, 2033 (1998).

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Response: We agree that overexpression studies (particularly those studying apoptosis) should be interpreted with caution. However, overexpression of p53 via the retrovirus system we used (*1*) resulted in concentrations of p53 expression that are below those seen after DNA damage by chemotherapeutic drugs or irradiation. This is not "extreme overexpression," and the cells were nontransformed. Thus, our experiments were close to physiological conditions. Although we observed increased surface expression of Fas and TNF-R1 after p53 activation, we did not see increased surface expression of a variety of other intracellular or membrane proteins, including the Fas ligand (FasL) and TNF-α. Thus, we concluded that p53 did not affect nonspecific surface protein trafficking. Also, the p53 effect on surface Fas expression that we observed was transient, associated with Fas signaling; basal surface Fas expression was reestablished if high expression of p53 was maintained. Thus, we would not expect to see reduced basal Fas expression in p53-null cells.

Our studies and those of O'Connor and Strasser (and references therein) are not directly comparable. We studied the specific interactions between p53 and Fas/FasL, through p53-induced apoptosis in *lpr* and *gld* cells, and not apoptosis that may be mediated only in part by p53. Although p53 is activated by γ-irradiation and DNA-damaging drugs, both of these treatments can induce apoptosis in the absence of p53. Thus, the fact that *lpr* and *gld* cells show similar apoptosis induced by these agents in other studies may be the result of an increased sensitivity of these cells to apoptosis that is not mediated by p53. Our studies also focused on the role of Fas/FasL in p53-induced apoptosis in vascular smooth muscle cells and fibroblasts, in which Fas is located predominantly intracellularly, and not in cells such as thymocytes, in which Fas is located predominantly on the surface. Fas trafficking induced by p53 will not be detected in cells in which Fas is not intracellular, and it will not be required in cells such as thymocytes or hepatocytes that express high concentrations of surface Fas. An open question is whether administration of the anti-fas antibody will induce apoptosis in vascular smooth muscle cells or fibroblasts in p53 wild-type or null animals. The answer may be a reflection of the intracellular location of Fas in these cells.

We showed that p53 can sensitize cells to Fas-mediated apoptosis and equally loss of p53 can reduce sensitivity to apoptosis. This does not mean that Fas-induced apoptosis cannot occur in the absence of p53. Nor does it mean that p53-induced apoptosis cannot occur in the absence of Fas/FasL or the adaptor molecule FADD. In contrast, we demonstrated that *lpr* and *gld* mouse embryo fibroblasts (MEFs) have impaired p53-induced apoptosis compared with wild-type MEFs. Similarly, antibodies to Fas/FasL, or expression of a dominant-negative FADD or crmA could reduce, but not abolish, p53-induced apoptosis. Some of the p53-induced apoptosis must have occurred through Fas/FasL, but other targets and mechanisms are also responsible. Similarly, we do not exclude the suggestion that some of the apoptosis we observed is dependent on p53 induction of target genes.

Actinomycin D, cycloheximide, and Brefeldin A reduced, but did not abolish, apoptosis. We know of no p53 mechanism or target that has been shown to be an absolute requirement for p53-induced apoptosis. This necessity has been shown for Bax, mdm2, and Fas explicitly (*2*), but the multitude of p53 transcriptional targets that regulate apoptosis also underscores this point. In contrast, we showed that optimal p53-induced apoptosis requires Fas/FasL in vascular smooth muscle cells (VSMCs) or MEFs. We did not show or state that Fas/FasL or FADD is an absolute requirement for p53-induced apoptosis, or conclude that this is the only or most important mechanism by which p53 induces apoptosis.

There is now increasing evidence that apoptosis induced by irradiation or chemotherapeutic agents requires Fas/FasL (*3*). In other studies, Fas/APO1-induced death is accompanied by massive translocation of the p53 from the cytoplasm to the nucleus in human B-lymphocytes (*4*). Bcl-2 inhibition of apoptosis is also associated with failure of p53 to translocate into the cell nucleus (*5*). Evidently, p53 and Fas may interact at multiple levels to induce apoptosis in many different cell types.

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