

## Extra Views

# Perp-etrating p53-Dependent Apoptosis

Rebecca A. Ihrie<sup>1</sup>

Laura D. Attardi<sup>1,2,\*</sup>

<sup>1</sup>Division of Radiation and Cancer Biology, Department of Radiation Oncology and

<sup>2</sup>Department of Genetics, Stanford University School of Medicine, Stanford, California 94305 USA

\*Correspondence to: Laura D. Attardi; CCSR South, Room 1255; 269 Campus Drive; Stanford University School of Medicine; Stanford, California 94305-5152 USA; Tel.: 650.725.8424; Fax: 650.723.7382; Email: attardi@stanford.edu

Received 12/17/03; Accepted 12/18/03

Previously published online as a *Cell Cycle* E-publication:  
<http://www.landesbioscience.com/journals/cc/abstract.php?id=722>

## KEY WORDS

p53, Perp, apoptosis, tetraspan, knockout mouse

## ACKNOWLEDGEMENTS

We would like to thank Steven Artandi and Amato Giaccia for helpful comments.

## ABSTRACT

The induction of apoptosis is a fundamental mechanism by which the p53 transcriptional activator protein suppresses tumor development. Recently, the roles of several p53 target genes in mediating the p53 apoptotic response have been queried through loss-of-function analysis with knockout mouse models. These studies have demonstrated that the p53 targets *Noxa*, *Puma*, and *Perp* play cell type-specific roles in p53-mediated apoptosis. *Perp*, a tetraspan protein localizing to the plasma membrane, rather than to mitochondria, is a novel type of p53 effector that may stimulate apoptosis through a different mechanism from the BH3-containing proteins *Noxa*, *Puma*, and *Bax*.

The p53 protein plays a central role in the tumor-free survival of mammals. Its crucial role as a tumor suppressor is underscored by its widespread inactivation in human cancers, as well as the striking phenotype of *p53* null mice, which universally develop early-onset cancer.<sup>1</sup> The p53 protein acts as a damage-control system, sensing a variety of cellular stresses and inducing cells to undergo either cell cycle arrest or programmed cell death to limit the propagation of damaged cells. p53 activates these responses at least in part through the transcriptional activation of target genes.<sup>2</sup> As a result, intense research efforts have focused on identifying target genes essential for p53 function. In particular, because the induction of apoptosis by p53 plays a major role in its anti-tumor activity, much emphasis has been placed on defining those transcriptional targets of p53 that are pivotal for this response. To date, a plethora of p53 target genes discovered in various systems have been implicated in apoptosis, either because of sequence similarity to known apoptotic regulators or because expression of these genes is sufficient to induce cell death. However, until recently, the requirement and relative contributions of most of these target genes to the p53 apoptotic response has remained uncertain because of a paucity of loss-of-function studies. Unequivocal elucidation of the role of these genes in p53-dependent apoptosis necessitates analysis in a system suited to the complexity of the p53 apoptotic response, which is executed through different assemblages of effectors according to the cellular context. Mouse knockout models are ideally suited to this purpose, as they afford the possibility to derive and examine cells from multiple lineages and to define the cell type-specific circuitry of the p53 apoptotic process.

The idea that the specific effector profile for p53-mediated apoptosis is cell-type dependent was first suggested several years ago through studies of *Bax*, a target gene that encodes a member of the Bcl-2 family of apoptotic regulators. *Bax* was shown to play an important role in DNA damage-induced apoptosis of neurons<sup>3</sup> and E1A oncogene-expressing mouse embryo fibroblasts (MEFs),<sup>4</sup> but not thymocytes or intestinal crypt cells (Table 1).<sup>5,6</sup> This concept has gained substantial support with a flurry of recent publications seeking to define additional critical mediators of p53-dependent apoptosis through the analysis of cells derived from knockout mice deficient for specific p53 apoptotic target genes. These studies, characterizing mouse knockouts in the genes *Noxa*,<sup>7,8</sup> *Puma*,<sup>8,9</sup> and *Perp*,<sup>10</sup> have demonstrated that the role of each target gene is dependent on the cell type and apoptotic stimulus. Thus the program of p53-mediated apoptosis is dictated by specific contextual signals, attesting to the complexity of p53 function. Moreover, these findings again emphasize the importance of studying p53-dependent apoptosis in the multiple cellular lineages that can be derived from a mouse model. In addition, the cells derived from these knockout mice typically exhibit partial defects in p53-dependent apoptosis, suggesting that the induction of apoptosis by p53 is mediated through multiple target genes acting in concert.

*Noxa* and *Puma*, like *Bax*, are members of the Bcl-2 family of proteins that act to promote mitochondrial dysfunction, a critical step in apoptosis. *Noxa* and *Puma* are BH3 (Bcl-2

Table 1 **CELL TYPE-SPECIFIC ROLES OF p53 TARGET GENES IN p53-DEPENDENT APOPTOSIS**

Gene	Oncogene-Expressing MEFs	Thymocytes	CNS	Intestinal Crypts
<i>Perp</i>	- (E1A)	+	+	ND
<i>Noxa</i>	+ (E1A)	-	ND	+
<i>Puma</i>	+ (E1A or myc)	+	+	ND
<i>Bax</i>	+ (E1A)	-	+	-

The roles of p53 apoptosis target genes in DNA damage-induced, p53-dependent cell death in various cell types, as established through recent studies of mouse knockouts, are summarized. + = required or partially required for apoptosis, - = not required for apoptosis, ND = not determined. The oncogene used in MEF analyses is indicated in parentheses for each genotype.

Homology 3 domain)-containing proteins, and stimulate apoptosis by inducing oligomerization and activation of Bax and Bak, which in turn causes permeabilization of the outer mitochondrial membrane to allow essential apoptotic-triggering molecules to be released from the mitochondria.<sup>7,11</sup> These recent studies illustrate that *Noxa*-deficient cells are partially defective in the DNA damage-induced p53 apoptotic response in intestinal crypt cells and E1A oncogene-expressing MEFs<sup>7,8</sup> but not in thymocytes (Table 1).<sup>8</sup> *Puma* knockout cells also have a deficiency in the p53 apoptotic response, and this defect was observed in thymocytes,<sup>8</sup> neurons,<sup>9</sup> and E1A or c-myc-expressing MEFs<sup>8,9</sup> after DNA damaging agent treatment or serum deprivation (Table 1). Thus, *Bax*, *Noxa* and *Puma* all participate in the p53 apoptotic response, but their prominence in the response depends on the specific cellular context. An important role for these Bcl-2 family members in p53-dependent cell death is not unexpected given the vital role of mitochondria in apoptosis and of these proteins in regulating mitochondrial integrity.

Is this the whole story? Despite a clear role for these Bcl-2 family proteins in apoptosis, it is unlikely that they can explain the complete p53 apoptotic response. A major missing piece of the puzzle is the signal that biases cells toward an apoptotic cell fate. Although target genes like *Bax*, *Noxa* and *Puma* are activated in a p53-dependent manner after cellular stress, they are induced to similar levels during arrest and apoptosis,<sup>7,12,13</sup> suggesting that their expression is not sufficient to dictate the induction of apoptosis. Moreover, forced overexpression of either Bax or Noxa is insufficient to trigger apoptosis in fibroblasts lacking an oncogene,<sup>4,7</sup> indicating that other factors are needed to specify this cell fate decision. Perp is an attractive candidate for directing this choice, as it represents a novel type of p53 target whose expression is highly upregulated during p53-mediated apoptosis compared to p53-induced arrest.<sup>12</sup> Thus Perp could be a critical factor in tilting the balance toward the apoptotic pathway. In recent work from our laboratory utilizing a *Perp* knockout mouse model, we have shown that Perp is indeed an important component of the p53-dependent apoptotic response. Perp was examined in three cell types: E1A-expressing MEFs, thymocytes, and neurons of the embryonic CNS.<sup>10</sup> *Perp*-deficient thymocytes and neurons have a compromised p53 cell death response, indicating Perp is essential for a complete p53 apoptotic response in multiple contexts. Significantly, although *Perp* is highly upregulated after DNA damage in all of the systems examined, the functional requirement for Perp in apoptosis is different in each of these cell types. This finding highlights the point that, while expression analysis is an acceptable starting point to implicate a gene product in the apoptotic process, genetic

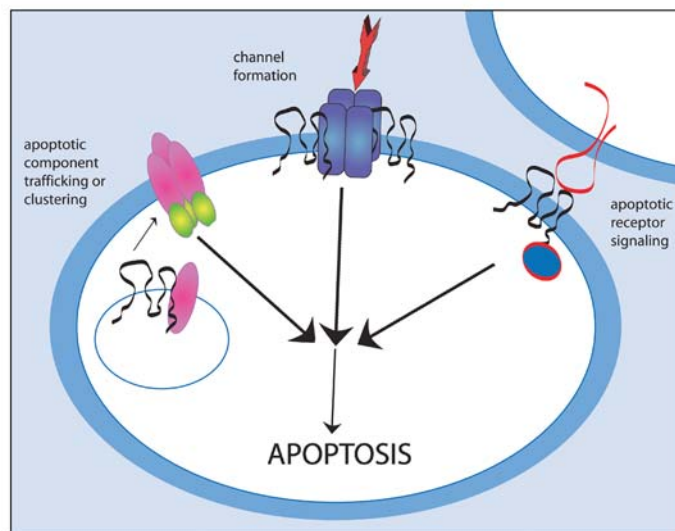


Figure 1. Possible mechanisms of action for Perp in apoptosis. The Perp tetraspan membrane protein (shown in black) may be required for the transport of other molecules involved in cell death to the plasma membrane, or may be involved in the proper assembly of protein complexes at the cell surface. Alternatively, Perp may be a constituent of a channel at the cell surface, which allows the transit of agents important for triggering apoptosis. Finally, Perp itself may act as the receptor for a "death ligand", in a manner analogous to TNF receptor superfamily members.

loss-of-function analyses in the mouse are critical to ascertain the functional implications of target gene induction. Furthermore, although many genes are able to induce apoptosis when overexpressed in cells, it is likely that a given gene will not be required for p53-dependent apoptosis in every cell type, or potentially even in the cell type in which it was originally identified. With respect to Perp, results in knockout cells demonstrate that this protein, although originally identified in E1A-MEFs and able to induce apoptosis upon overexpression in this system,<sup>12</sup> is not rate-limiting for p53-dependent cell death in this cell type. However, *Perp* does contribute to the p53 apoptotic response in thymocytes and, along with *Puma*, is one of a very limited number of p53 target genes shown to be required for a full apoptotic response in this system. Additionally, *Perp*-deficiency significantly compromises apoptosis in the developing CNS induced by either irradiation or hyperproliferative signals, suggesting that it is an important player in a cell type that appears to involve multiple target genes including *Puma* and *Bax*. This finding is particularly compelling because Perp is related to a tetraspan membrane protein with a fundamental role in the nervous system, peripheral myelin protein 22 (PMP-22).

Perp, as a tetraspan protein, represents a novel type of effector involved in p53-dependent apoptosis. Perp is a member of the Peripheral Myelin Protein 22/growth arrest specific 3 (PMP22/gas3) family, which includes PMP-22 and the epithelial membrane proteins 1, 2, and 3. Intriguingly, Perp, like PMP-22, appears to localize to the plasma membrane and secretory pathway rather than the mitochondria<sup>12</sup> and is therefore unlike the other genetically characterized p53 effectors involved in apoptosis. Perp also resembles PMP-22 family members with respect to cell death; overexpression of PMP-22, EMP-2 or Perp is sufficient to cause cell death.<sup>12,14,15</sup> Cell death induced by these proteins appears to rely at least in part on the classical apoptotic machinery, as caspase inhibitors or Bcl-2 coexpression

abrogates cell death in these overexpression experiments.<sup>12,14,16</sup> However, it is unclear how these tetraspan membrane proteins, including Perp, interface with the canonical apoptotic pathway, and therefore the exact mechanism by which they stimulate apoptosis remains to be elucidated.

The various roles played by other tetraspan proteins may offer clues to the mechanism of action of Perp. Tetraspan membrane proteins have been implicated in a multiplicity of cellular processes, including protein trafficking, receptor function, channel activity, and cell adhesion.<sup>17</sup> Stargazin, another tetraspan protein related to PMP-22, is essential for the delivery of specific receptor components to the plasma membrane.<sup>18</sup> It is easy to envision that Perp could act in an analogous manner, facilitating the shuttling of some critical death receptor protein to the cell surface, thus triggering apoptosis (Fig. 1). Alternatively, some tetraspan proteins act as receptors for extracellular ligands<sup>19</sup> or as channels, and either of these functions could be imagined to stimulate the apoptotic response. Thus Perp's role in apoptosis could be to receive and transmit the signal from a "death ligand" or to allow the transport of some ion or molecule crucial for apoptosis across the plasma membrane (Fig. 1). In line with the latter idea, tetraspan proteins in the PMP-22 family, while not known to be channels themselves, have been proposed to stimulate apoptosis by regulating ion channel activity. Overexpression of either PMP-22 or the EMP proteins induces cell death through a mechanism that involves association with the P2X(7) cation channel and the consequent induction of membrane blebbing.<sup>20</sup> Perp, too, potentially could interact with P2X(7) to induce membrane blebbing that contributes to activation of the apoptotic pathway. Finally, beyond these direct mechanisms of activating apoptosis, there also remains the possibility that Perp could act in a more indirect manner, by affecting the state of the cell through effects on proliferation status, differentiation or adhesion, and in this way could predispose the cell to undergoing apoptosis. Whatever its mechanism of action, the involvement of a membrane protein in apoptosis provides a means for cell-extrinsic signals to be sensed and translated into effects on the apoptotic process.

In the future, it will be important to distinguish between these possibilities to dissect the mechanism by which Perp acts, as well as to define the role of the Perp pathway with respect to the mitochondrial pathway. The Perp pathway could be envisaged to act either through known effectors of p53-mediated apoptosis or through a separate, parallel pathway. For instance, the Perp signal could impinge upon the mitochondrial pathway by exerting effects on the localization or function of the Bcl-2 family members Bax, Noxa or Puma. Alternatively, Perp signaling could be part of a discrete branch of p53-dependent apoptosis distinct from mitochondrial effectors. For example, while BH3-containing proteins act to cause mitochondrial membrane dysfunction, tetraspan proteins such as Perp could act in a pathway that causes permeabilization of the plasma membrane, providing another mechanism for inducing cell death after stress. Examination of double-knockout cells, lacking both Perp and a BH3-containing protein such as Bax, will allow us to determine whether these pathways are distinct and whether compromising two structurally dissimilar p53-regulated proteins is sufficient to recapitulate the resistance to cell death typically seen in a p53 null background. Through this analysis of different cell types derived from knockout mice, we ultimately will be able to unravel the complexity of p53-mediated apoptosis. This knowledge in turn will reveal how this network of apoptotic regulators fits into the framework of tumor suppressive mechanisms attributed to p53.

## References

- Attardi LD, Jacks T. The role of p53 in tumour suppression: Lessons from mouse models. *Cell Mol Life Sci* 1999; 55:48-63.
- Vousden KH, Lu X. Live or let die: The cell's response to p53. *Nat Rev Cancer* 2002; 2:594-604.
- Chong MJ, Murray MR, Gosink EC, Russell HR, Srinivasan A, Kapsetaki M, et al. Atm and Bax cooperate in ionizing radiation-induced apoptosis in the central nervous system. *Proc Natl Acad Sci USA* 2000; 97:889-94.
- McCurrach ME, Connor TM, Knudson CM, Korsmeyer SJ, Lowe SW. bax-deficiency promotes drug resistance and oncogenic transformation by attenuating p53-dependent apoptosis. *Proc Natl Acad Sci USA* 1997; 94:2345-9.
- Pritchard DM, Potten CS, Korsmeyer SJ, Roberts S, Hickman JA. Damage-induced apoptosis in intestinal epithelia from bcl-2-null and bax-null mice: Investigations of the mechanistic determinants of epithelial apoptosis in vivo. *Oncogene* 1999; 18:7287-93.
- Knudson CM, Tung KS, Tourtellotte WG, Brown GA, Korsmeyer SJ. Bax-deficient mice with lymphoid hyperplasia and male germ cell death. *Science* 1995; 270:96-9.
- Shibue T, Takeda K, Oda E, Tanaka H, Murasawa H, Takaoka A, et al. Integral role of Noxa in p53-mediated apoptotic response. *Genes Dev* 2003; 17:2233-8.
- Villunger A, Michalak EM, Coultas L, Mullauer F, Bock G, Ausserlechner MJ, et al. p53- and drug-induced apoptotic responses mediated by BH3-only proteins puma and noxa. *Science* 2003; 302:1036-8.
- Jeffers JR, Parganas E, Lee Y, Yang C, Wang J, Brennan J, et al. Puma is an essential mediator of p53-dependent and -independent apoptotic pathways. *Cancer Cell* 2003; 4:321-8.
- Ihrig RA, Reczek E, Horner JS, Khachatryan L, Sage J, Jacks T, et al. Perp is a mediator of p53-dependent apoptosis in diverse cell types. *Curr Biol* 2003; 13:1985-90.
- Wu X, Deng Y. Bax and BH3-domain-only proteins in p53-mediated apoptosis. *Front Biosci* 2002; 7:d151-6.
- Attardi LD, Reczek EE, Cosmas C, Demicco EG, McCurrach ME, Lowe SW, et al. PERP, an apoptosis-associated target of p53, is a novel member of the PMP-22/gas3 family. *Genes Dev* 2000; 14:704-18.
- Yu J, Zhang L, Hwang PM, Kinzler KW, Vogelstein B. PUMA induces the rapid apoptosis of colorectal cancer cells. *Mol Cell* 2001; 7:673-82.
- Wang CX, Wadehra M, Fisk BC, Goodglick L, Braun J. Epithelial membrane protein 2, a 4-transmembrane protein that suppresses B-cell lymphoma tumorigenicity. *Blood* 2001; 97:3890-5.
- Fabbretti E, Edomi P, Brancolini C, Schneider C. Apoptotic phenotype induced by overexpression of wild-type gas3/PMP22: Its relation to the demyelinating peripheral neuropathy CMT1A. *Genes Dev* 1995; 9:1846-56.
- Brancolini C, Marzintonto S, Edomi P, Agostoni E, Fiorentini C, Muller HW, et al. Rho-dependent regulation of cell spreading by the tetraspan membrane protein Gas3/PMP22. *Mol Biol Cell* 1999; 10:2441-59.
- Hemler ME. Tetraspanin proteins mediate cellular penetration, invasion, and fusion events and define a novel type of membrane microdomain. *Annu Rev Cell Dev Biol* 2003; 19:397-422.
- Chen L, Chetkovich DM, Petralia RS, Sweeney NT, Kawasaki Y, Wenthold RJ, et al. Stargazin regulates synaptic targeting of AMPA receptors by two distinct mechanisms. *Nature* 2000; 408:936-43.
- Flint M, Maidens C, Loomis-Price LD, Shotton C, Dubuisson J, Monk P, et al. Characterization of hepatitis C virus E2 glycoprotein interaction with a putative cellular receptor, CD81. *J Virol* 1999; 73:6235-44.
- Wilson HL, Wilson SA, Surprenant A, North RA. Epithelial membrane proteins induce membrane blebbing and interact with the P2X7 receptor C terminus. *J Biol Chem* 2002; 277:34017-23.