Cell Death and Differentiation (2006) 13, 913–915

www.nature.com/cdd

News and Commentary

# Clues from worms: a Slug at Puma promotes the survival of blood progenitors

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*Cell Death and Differentiation* (2006) **13,** 913–915.
doi:10.1038/sj.cdd.4401906; published online 17 March 2006

A critical decision in the lifetime of a cell is the selection of an appropriate response to a given stress. Where DNA has been damaged, the choice to live demands repair and recovery. The alternative, more drastic choices, to senesce or die are critical to stave-off cellular catastrophes, such as malignancy onset. What governs these decisions is one of the most fundamental questions in the field of stress response and cancer biology. A powerful candidate protein to orchestrate these decisions is the tumor suppressor p53.[1,2] Myriads of factors have been shown to influence p53 activation, among which are cell type and context, as well as the nature of the stress and its magnitude.[3] A molecular explanation of how cellular fate is decided however is yet to be precisely elaborated.

## P53-induced Apoptosis

Cell growth arrest is primarily induced via the cyclin-dependent kinase inhibitor, p21, whereas the apoptotic response in contrast appears to be more complex. The persistent claim that p53 promotes apoptosis through both transcriptional-dependent and -independent mechanisms has received new impetus over the last 2 years. The transcriptional-independent apoptotic activity of p53 is largely achieved by the transportation of p53 to the mitochondria, where it interacts with members of the Bcl-2 family. This leads to the permeabilization of the outer mitochondrial membrane and the release of cytochrome *c*.[4] The transcriptional activity of p53 has been advocated for many years and has been continuously supported by the identification of new apoptotic target genes. The contribution of candidate targets was evaluated by a genetic knockout approach. Commonly, these studies revealed a disappointing partial effect on p53 apoptotic activity. An important breakthrough in the transcriptional apoptotic pathway came with the finding that the elimination of *puma* alone is sufficient to protect thymocytes from ionizing radiation (IR)-induced death, to the same extent as the p53-deficient thymocytes.[5,6] This intriguing finding placed Puma as the major apoptotic factor in the apoptotic pathway of p53 acting at the mitochondria. More recently, Chipuk *et al*.[7] refined the contribution of Puma to the apoptotic activity p53 by showing that Puma relieves cytoplasmic p53 from the antiapoptotic Bcl-xL, allowing p53 to activate the proapoptotic Bax and induce mitochondrial outer membrane permeabilization and hence apoptosis. Thus, *puma* is the major target of p53 transcriptional-dependent apoptosis in thymocytes, and in turn contributes to the transcriptional-independent apoptotic action of p53 at the mitochondria (Figure 1).

## Slugging Puma Controls p53-induced Death

If the induction of *puma* by p53 in response to IR is sufficient to kill thymocytes, why are other cells, including hematopoietic progenitors, protected from the same insult? In fact, hematopoietic stem cells (HSC) are resistant to IR-induced apoptosis. Look and co-workers previously demonstrated that this protection depends on Slug, a zinc-finger transcriptional repressor. Slug is expressed in multiple subsets of hematopoietic progenitors but not in the more differentiated pro-B and T cells, correlating with their resistance or sensitivity to IR-induced death respectively.[8] Keeping in mind that this apoptotic pathway is p53-dependent, and Puma is the critical agent of death in the pathway of certain hematopoietic cells, Look and co-workers linked Slug to the p53 apoptotic pathway.[9] Applying comprehensive analysis of mice lacking *Slug*, singly or doubly with *p53* or *puma*, they have linked Slug to the p53 apoptotic pathway mediated by its downstream target *puma*. They have demonstrated that Slug is 'slotted' downstream of p53, being induced as a direct target gene, but acting upstream of *puma*. Strikingly, they found that Slug directly represses the expression of *puma*, thereby providing an explanation for the protection of hematopoietic progenitor cells from IR-induced death in a p53- and Puma-dependent manner (Figure 1).

These findings provide an elegant explanation for why HSC, but not more differentiated cells, are protected from genotoxic stress. Wu *et al*.[9] have also defined an experimental system in which fundamental questions can be addressed. First, what happens to the surviving HSC? Do they arrest while their damaged DNA is being repaired, or do some cells survive while others senesce or die? Second, how is the induction of *Slug* expression in HSC, but not in differentiated pro-B and T cells, regulated in response to identical genotoxic signals? Or in other words, how does p53 'know' when and where to induce *Slug*, or any other target gene for that matter? Two models have been proposed[10]: the 'p53 dumb', where p53 binds all target genes equally but other transcription factors (cell type or differentiation stage specific) determine the promoter specificity; and the 'p53 smart', where p53 binds differentially to different promoters, thereby establishing a specific expression spectrum. A comparative chromatin

immunoprecipitation (ChIP) of p53 at the *Slug* promoter combined with quantitative expression analysis between radio-sensitive and -resistant hematopoietic cells may help to distinguish between the two models. Third, how does Slug antagonize the p53 transcriptional induction of *puma*?

Wu *et al*.[9] have demonstrated that Slug binds and inhibits the promoter activity of *puma* in response to IR and further prevents p53 from inducing *puma* expression. Interestingly, the relevant Slug binding sites are in intron 1 of *puma*, where the p53 consensus sequence has also been identified.[11,12] Several models may explain how Slug inhibits p53 from inducing *puma* (Figure 1). First, Slug elevates HDAC expression[13] and interacts with it;[14] hence, it may recruit HDAC to the *puma* promoter, leading to altered chromatin structure, as recently shown for the repression of *Brca2* by Slug.[15] Second, the effect of HDAC may be to facilitate Slug-mediated elevation of a known co-repressor of p53, mSin3a,[13] which interacts with p53 and may be recruited to and repress the *puma* promoter, as was recently shown for the *p21* promoter.[16] Third, the co-binding of p53 and Slug to the same genomic region may interfere with p53 transcriptional activation by multiple mechanisms, such as interference with the proper assembly of the transcriptional machinery.[17] These models are consistent with the earlier kinetics of Slug induction as compared with Puma, allowing sufficient time for Slug accumulation. In fact, Slug appears to occupy the *puma* site even in unstressed hematopoietic progenitor cells and suppress its expression, supporting a role for Slug in the regulation of basal expression of *puma*.[9] In contrast, in unstressed, *Slug*-deficient mouse embryo fibroblasts (MEFs), *puma* expression is downregulated,[13] suggesting that at least in MEFs the presence of Slug is required to maintain basal levels of Puma. These studies clearly demonstrate the importance of context in the final expression profile of these two p53 targets. Wu *et al*.[9] have already shown that not all p53 target genes are affected by Slug, for instance *noxa*. It would be interesting to define how extensive the effect of Slug is on p53 target genes, and whether there are different spectrums of genes affected in cells undergoing for example growth arrest *versus* apoptosis.

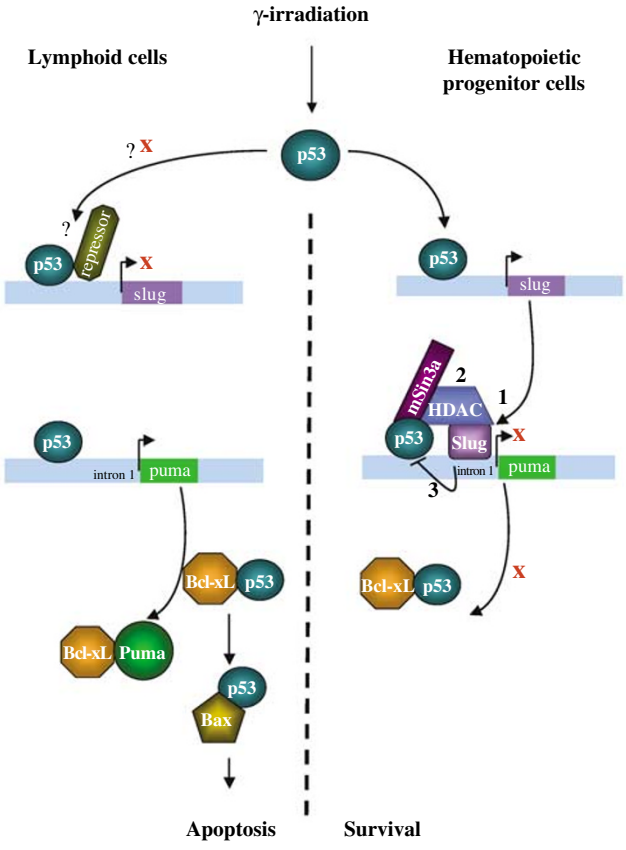


**Figure 1** A model of the effect of the Slug/Puma interplay on the response of hematopoietic cells to IR. The left-hand side depicts the pathway downstream to p53 in differentiated lymphoid cells, whereas on the right is the pathway in hematopoietic progenitor cells. In the lymphoid cells, *slug* is not induced by p53, for example because of the action of a repressor, or because p53 does not bind the promoter. *puma* on the other hand is induced and activates p53 by sequestering Bcl-xL to induce apoptosis. In the hematopoietic progenitor cells, Slug is induced by p53, which in turn blocks *puma* induction by one or more mechanisms: (1) Slug elevates HDAC and may recruit it to the *puma* promoter to alter chromatin structure; (2) Slug elevates mSin3a, which binds p53 and may recruit HDAC to the *puma* promoter; (3) By co-binding near p53 to intron 1, Slug may interfere with p53 transcriptional activity, such as with the assembly of the transcriptional machinery

## The Infinite Loops in the p53 Network

The regulation of p53 is heavily intertwined by multiple loops.[18] These recent findings define a new loop by which p53 monitors its apoptotic potency by controlling the level of Slug in the cells. Whereas in the hematopoietic system the interplay between Slug and Puma has an 'all or none' apoptotic effect, in other cell types this interplay is likely to have a more moderate effect. Low levels of Slug expression would permit Puma to facilitate the apoptotic action of p53 at the mitochondria, whereas high Slug expression would block this pathway permitting p53 to induce apoptosis in a transcriptional-dependent pathway but in a Puma-independent manner. The action of Noxa[6,19] and other p53 apoptotic target genes would then be dominating. It is difficult to judge at this stage how widely this Slug/Puma interplay affects p53-induced apoptosis in different cell types. It appears that two criteria must be met in order for this interplay to exert a dominant influence in a particular cell type or developmental stage. First, IR-induced death must depend on Puma, and second, the expression of Slug must be regulated in this context.

A regulatory loop involving two p53 target genes is not unique to Slug and Puma and has been previously described. The inhibition of p53 by its major negative regulator, Mdm2, is enhanced through phosphorylation of Mdm2 by the mitogen-activated kinase Akt. Mdm2 activation can be counteracted by dephosphorylation of the Akt sites by the phosphatase PP2A. The activity of PP2A requires the PP2AB' subunit, which is recruited to Mdm2 by the p53 proapoptotic target gene, *Cyclin G*.[20] Thus again, the ratios between different target genes of p53 can tilt the balance between survival and apoptosis.

## Slug and Cancer

In contrast to the controlled expression of Slug in normal cells, it is aberrantly expressed in a number of cancers, specifically t(17:9) leukemic cells,[21] rhabdomyosarcomas expressing the translocation of *Pax3-Fkhr*[22] and in breast cancer (correlating strongly with E-cadherin suppression[23]). A definitive role for Slug in the development of mesenchymal tumors (leukemias: acute B-cell lymphoblastic leukemia and acute myeloid leukemia; and sarcomas) has recently been demonstrated in mice carrying a tetracycline-repressible *Slug* transgene.[24] Importantly, as the postnatal expression of *Slug* and the effects of *Slug* deletion are similar in humans and mice (reviewed by Perez-Mancera PA *et al.* [24]), it may be valid to extrapolate from the role of Slug in the protection of blood progenitors from IR in mice, to its importance in mesenchymal human cancers. Drawing on the findings of Wu *et al.*,[9] it will be fascinating to establish whether cancers with deregulated Slug expression carry wild-type p53. It is tempting to speculate that in these tumors, Slug confers resistance to p53-mediated death via Puma suppression. Accordingly, Slug may protect certain tumor types from p53-induced apoptosis in response to oncogenic stress, or to genotoxic anticancer treatments. Such a conjecture may be exemplified in the HSC malignancy, chronic myelogenous leukemia (CML), where at least in the chronic phase p53 remains wild type. Surprisingly Bcr-Abl activity results in constitutive signaling to activate p53. The growth inhibitory effect of p53 in CML is counteracted by Bcr-Abl concomitantly activating Mdm2.[25] Interestingly, Slug is also upregulated by Bcr-Abl and has been shown to be essential for leukemogenesis by Bcr-Abl.[24] It is plausible therefore that induction of Slug in CML biases the cells to survive rather than die in response to activated p53.

These studies raise the potential use of Slug as a target for anticancer treatment. One approach is to downregulate Slug in the relevant cancer cells expressing wild type p53 with the aim of triggering p53-induced death. Unfortunately, data from animal studies indicate that the elimination of Slug is insufficient to halt the progression of these tumors, as cellular modifications provoked by Slug overexpression become Slug independent with time.[24] A different therapeutic application of Slug has been proposed[9] to elevate its expression with the aim of protecting hematopoietic progenitors and potentially committed hematopoietic cells in the context of radiotherapy and possibly chemotherapy. The success of such a strategy however would rely on the specificity of protection, where the activation of Slug in tumor cells could be counterproductive.

## A Lesson from Worms

A striking evolutionary parallel of the functions of the mammalian p53, Slug and Puma has been elaborated in the worm *Caenorhabditis elegans*. The worm ortholog of these genes CEP-1, CES-1 and EGL-1, respectively, coordinate with exquisite duplicity to dictate the fate of NSM sister neurons. CES-1 (the worm ortholog of Slug) is able to bind the enhancer element of *egl-1* (the BH3-only gene that is most closely related to *puma*) and repress its transcription. Under the influence of IR, CEP-1 (the p53 ortholog) is able to directly induce the expression of *egl-1* and consequently induce death in germ cells but not somatic cells.[9] These studies further demonstrate the extensive wealth of genetic clues encoded in the primitive archives of the worm that can be deciphered to illuminate the intricacies of our own cellular fate determination.

1. Vousden KH and Prives C (2005) Cell 120: 7–10.
2. Lozano G and Zambetti GP (2005) J. Pathol. 205: 206–220.
3. Sionov RV and Haupt Y (1999) Oncogene 18: 6145–6157.
4. Moll UM *et al.* (2005) Curr. Opin. Cell Biol. 17: 631–636.
5. Jeffers JR *et al.* (2003) Cancer Cell 4: 321–328.
6. Villunger A *et al.* (2003) Science 302: 1036–1038.
7. Chipuk JE *et al.* (2005) Science 309: 1732–1735.
8. Inoue A *et al.* (2002) Cancer Cell 2: 279–288.
9. Wu WS *et al.* (2005) Cell 123: 641–653.
10. Vousden KH (2000) Cell 103: 691–694.
11. Nakano K and Vousden KH (2001) Mol. Cell 7: 683–694.
12. Yu J *et al.* (2001) Mol. Cell 7: 673–682.
13. Bermejo-Rodriguez C *et al.* (2005) Genomics 87: 113–118.
14. Hemavathy K *et al.* (2000) Mol. Cell. Biol. 20: 5087–5095.
15. Tripathi MK *et al.* (2005) J. Biol. Chem. 280: 17163–17171.
16. Dannenberg JH *et al.* (2005) Genes Dev. 19: 1581–1595.
17. Ho J and Benchimol S (2003) Cell Death Differ. 10: 404–408.
18. Harris SL and Levine AJ (2005) Oncogene 24: 2899–2908.
19. Shibue T *et al.* (2003) Genes Dev. 17: 2233–2238.
20. Chen X (2002) Dev. Cell 2: 518–519.
21. Inukai T *et al.* (1999) Mol. Cell 4: 343–352.
22. Khan J *et al.* (1999) Proc. Natl. Acad. Sci. USA 96: 13264–13269.
23. Hajra KM and Fearon ER (2002) Genes Chromosomes Cancer 34: 255–268.
24. Perez-Mancera PA *et al.* (2005) Oncogene 24: 3073–3082.
25. Levav-Cohen Y *et al.* (2005) Biochem. Biophys. Res. Commun. 331: 737–749.