clearly needs to be further defined (Bunz et al., 1998; Wouters et al., 1999).

The current studies also provide a framework for why mutations only affecting the apoptotic pathway do not necessarily correlate with a poor prognosis. These mutations might have prevented the occurrence of other lesions, such as in p53, which are in fact more harmful because they could mitigate the efficacy of anticancer agents. The observations reported in these two papers (Schmitt et al., 2002a, 2002b) also argue that the genomic instability that is concurrent with p53 deficiency in this system does not significantly contribute to tumor initiation and progression but rather is an accidental side effect that nevertheless may have major consequences for the efficacy of subsequent treatment.

The obvious question that remains is how senescence plays out against apoptosis and mitotic catastrophe in the eradication of various tumors by anticancer agents. The CTX-induced senescence was most pronounced in the absence of apoptosis. Hence, the relative role of senescence in drug treatment sensitivity likely depends on the cell type, the genetic lesions that drove tumor development, and last but not least, the anticancer agent itself. Studies in other systems support this (Bunz, 1999). Therefore, we must understand the interplay between these lesions and their impact on the various resistance mechanisms. Only then can we offer patients—after appropriate molecular characterization of the tumor—the tailored treatment that takes into account tumor-specific resistance mechanisms similar to those described by Lowe and collaborators. Induction of senescence offers at least an appealing new option to include as a strategy for intervention.

Acknowledgments

The author would like to thank Drs. Peeper and Van Lohuizen for discussion and critical reading of the manuscript.

Anton Berns

Division of Molecular Genetics and
Centre of Biomedical Genetics
The Netherlands Cancer Institute
Plesmanlaan 121
1066 CX Amsterdam
The Netherlands
E-mail: a.berns@nki.nl

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p53 leans on its siblings

Despite the common assumption that p53 by itself can induce apoptosis, results of a recent study implicate the homologous genes p63 and p73 in p53-mediated programmed cell death.

The p53 tumor-suppressor protein, first identified in 1979, acts as a major node in a complex network evolved to sense diverse cellular stresses including DNA damage and hyperproliferative signals (Ko and Prives, 1996). Once stabilized and activated by genotoxic stress, p53 can either activate or repress a wide array of different gene targets, which in turn can regulate cell cycle, cell death, DNA repair, angiogenesis, and other outcomes. p53 functions, including apoptosis, are thought to require its sequence-specific DNA binding and transcriptional activation activities, and a number of apoptosis-related genes are induced by p53 activation (Johnstone et al., 2002). Consequently, p53 is reported to be functionally inactivated in more than half of all human tumors, and murine models have confirmed p53's central role in tumorigenesis. Furthermore, the majority of tumor-associated p53 mutations occur in the core DNA binding domain and prevent interaction with target sequences. The cloning in the late 1990s of two p53-related genes, p63 and p73, caused a great excitement within the cancer biology community with the prospect of two additional tumor suppressors (Yang and McKeon, 2000). Initially the family resemblance was striking. p63 and p73 share with p53 an amino-terminal trans-

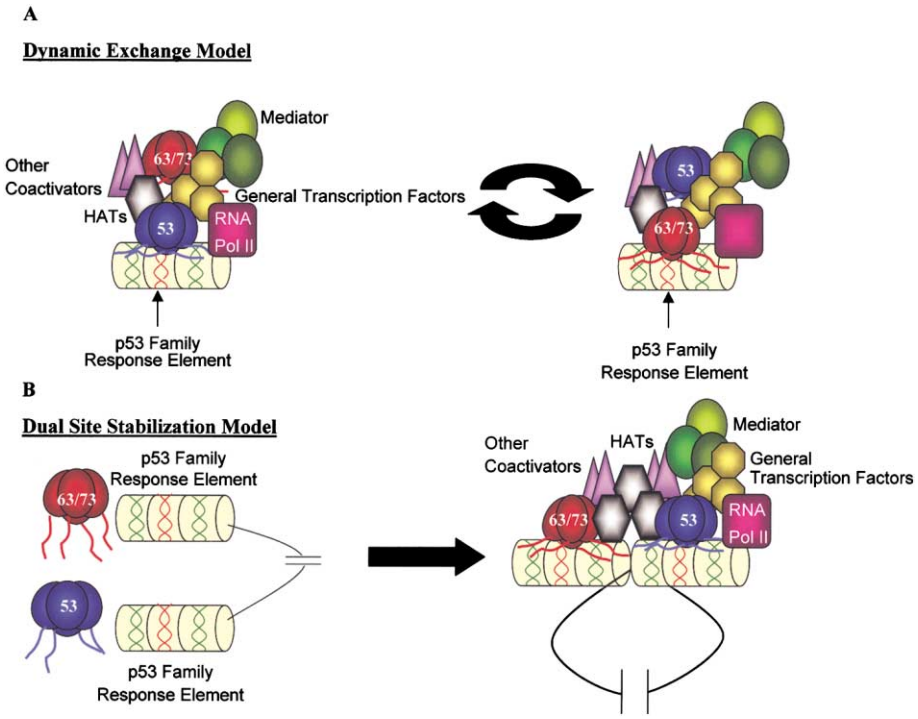

Figure 1. Two models for the role of p63 and p73 in p53-dependent apoptosis

activation domain and a highly conserved central DNA binding domain, as well as a carboxy-terminal tetramerization domain. Moreover, at least in overexpression assays, p63 and p73 were shown to transactivate many p53 target genes including p21/WAF-1 and Bax, and they were able to induce cell cycle arrest and apoptosis (Yang and McKeon, 2000).

In screening for tumor-associated mutations, the siblings began to differentiate themselves. Multiple reports failed to show that inactivating p63 or p73 mutations in a wide variety of tumor types were as common as in p53. Furthermore, mouse knockout studies indicated that loss of p73 or p63 did not predispose to cancer (Yang and McKeon, 2000). Thus, despite their strong family resemblance, it appeared that p53, p63, and p73 were not acting simply as redundant tumor suppressors.

However, a recent landmark paper from Tyler Jacks' laboratory reunites the p53 family (Flores et al., 2002). To investigate the role of p63 and p73 in p53-induced apoptosis, the Jacks group utilized a series of mouse embryo fibroblasts (MEFs) lacking either p63 or p73 singly or double-knockout cells lacking both p63 and p73 but retaining p53. Building on a previous model showing that adenovirus E1a-expressing MEFS are predisposed to apoptosis when subjected to DNA damage (Lowe et al., 1993), treatment of these cells with doxorubicin revealed that cells lacking p63 or p73 alone showed an intermediate resistance to apoptosis between that of p53 null and wild-type MEFs. Most interesting is their observation that $p63^{-/-}$;$p73^{-/-}$ double null cells were equally resistant to apoptosis as $p53^{-/-}$ MEFS, suggesting that p63 and p73 are required for p53-induced apoptosis. The same observation was made by examining apoptosis in the central nervous system of single- or double-knockout embryos following γ irradiation. Thus, p53 may be more dependent on its siblings than previously thought.

Following DNA damage, p53 is stabilized as a result of release from its negative regulator, the Mdm2 E3 ubiquitin ligase (Michael and Oren, 2002). Western blotting of the MEFs revealed that stabilization of p53 as well as p63 occurs normally in single- and double-null MEFs following doxorubicin treatment, indicating that in the absence of p63, p73, or both, factors functioning upstream of the p53 family are intact and that faulty upstream signaling cannot explain the drug resistance of $p63^{-/-}$;$p73^{-/-}$ cells.

As stabilization of p53 in the absence of p63 and p73 was found to be intact, the logical next step was to assess transcriptional activation within the p53 family MEF system. These experiments revealed striking differences in the activity of p53 when it lacks the support of the family members. Induction of *p21/WAF-1*, indispensable for p53-induced cell cycle arrest and *mdm-2,* important in the regulation of p53 protein levels, occurred normally in $p63^{-/-}$, $p73^{-/-}$, and double-null MEFs. Remarkably, however, loss of p63 and p73 severely affected the induction of *bax* and *PERP*, two genes thought to mediate p53-dependent apoptosis. This suggests that p53 target genes are differentially affected by the loss of p63 and p73 and that apoptosis-related targets may be specifically regulated within the entire p53 family.

To further characterize the mechanism of target gene disparity, the Jacks group performed chromatin immunoprecipitation (ChIP) assays to examine promoter element binding in vivo by p53 and p63. Indeed, following doxorubicin treatment, p53 was still bound to the p21 and mdm-2 promoters but did not engage with promoter elements of the apoptosis-related genes *bax*, *PERP*, or *NOXA*. This suggests that for certain genes, p63 and p73 are required for stable p53 promoter binding. Interestingly, ChIP analysis in $p53^{-/-}$ MEFs showed that p63 still bound to promoter sequences for *bax*, *PERP*, and *NOXA.* This strengthens the notion that the p63/p73 gene products are important for p53-dependent apoptosis but that if *bax*, *PERP*, and/or *NOXA* are necessary for apoptosis, binding by p63 alone is not sufficient. Moreover, in $p53^{-/-}$ MEFs, basal levels of <u>all</u> p53 target genes tested fall below detectable levels, suggesting reciprocally that p63 and p73 cannot transactivate certain target genes without p53's support. Taken together, these results demonstrate essential roles for p63 and p73 in p53-mediated apoptosis of MEFs and murine neuronal cells and imply that the p53 homologs work somehow to selectively stabilize the interaction of p53 with promoters of proapoptotic genes.

The results of Flores et al. pose many questions and point the way for future studies. Most intriguingly, the mechanism by which p53 and its relatives cooperate to produce cell death upon DNA damage remains to be fully elucidated. The authors' data are most consistent with p53 activating a single

apoptotic program that requires assistance from p63/p73, which is supported by their result that p53 binding to selective proapoptotic promoters in vivo is abrogated in the absence of p63 and p73. This in itself is a rather surprising result because the most obvious explanation, namely that p53 and its two siblings can interact with each other to form active cotetramers (thus producing a larger pool of activator), is argued against strongly by data showing that stable cotetramers between p53 and p63 or p73 cannot form in vitro (Davison et al., 1999) and that when overexpressed, full-length wild-type p53 and either p63 or p73 cannot be coimmunoprecipitated in vivo (Gaiddon et al., 2001).

Hence, to explain p53's inability to bind certain promoters in the absence of p63 and p73, more indirect mechanisms may be invoked. In vitro studies have shown that p53 has relatively high on and off rates for DNA binding, and p53/DNA complexes can be stabilized by protein-protein interactions. Thus, if strong binding by p53 to some sites in vivo requires extensive interaction with other factors, then one could envisage how p63 or p73 might play an indirect role in stabilization and ultimately in p53 apoptotic function. In one possible scenario, "the dynamic exchange model," p63 and/or p73 reside with p53 in a larger transcriptional complex in which either p53 or a sibling may engage the promoter element at any given moment. In this capacity, p63 and p73 may function to stabilize the complex but also may favor promoter activation by increasing the probability that an active tetramer is bound to the response element (Figure 1A).

An alternative model posits a requirement for two discrete binding sites within a target gene promoter (Figure 1B). Engagement of two or more such response elements by p53 and/or p63 and p73 within a single promoter may be required for maximal induction of transcription through stabilization of the association between DNA and p53 family proteins as well as complete recruitment of transcriptional machinery and other cofactors (Figure 1B). In fact, a number of p53 proapoptotic target promoters have been reported to possess at least two discrete widely spaced p53 binding sites such as Bax (Miyashita and Reed, 1995) and PERP (Attardi et al., 2000). Intriguingly, another proapoptotic gene, PIG3, was recently reported to be activated by p53 through a microsatellite sequence within its UTR distinct from its previously reported classical p53 consensus sequence, suggesting the existence of alternate modes of DNA binding and transcriptional regulation by p53 (Contente et al., 2002). Notably as well, Flores et al. indicated that their analysis of the PERP promoter suggested that p53 and p63 each engage most strongly with distinct promoter elements. This may represent an additional mechanism for p63 and p73 involvement in the induction of apoptosis. Nevertheless, having one versus two binding sites cannot be the sole defining factor for whether or not a promoter requires all p53 family members, since numerous additional genes, including *p21* (el-Deiry et al., 1995), possess two discrete p53 binding sites, and p21 was shown by Flores et al. to be induced in the absence of p63 and p73. Whatever the mechanism, the data presented by the Jacks group suggests that promoters vary in their levels of need for p63 or p73.

In addition to elucidating the means by which p63 and p73 facilitate apoptosis by p53, many questions remain to be addressed. Since there are multiple isoforms expressed by p63 and p73 which differ at their N and C termini, what are the domains of p63 or p73 proteins that are required to facilitate p53-promoter interactions? Do requirements for p53 family members vary with the source of stress signal? In addition to their role in apoptosis, do p63 and p73 have any part in the ability of p53 to regulate cell cycle, DNA repair, or angiogenesis? Finally, loss of p63 and p73 has discrete effects on certain tissue compartments: squamous epithelia in the case of p63 and neural cells for p73 (Yang and McKeon, 2000). Tumors of distinct histological origin may also show variations in p53 family relations. Since p53 knockout mice do not recapitulate the defects of family member knockouts, p63 and p73 most likely perform additional p53-independent functions. What is the nature of such functions, and how are they differentially regulated by these different genes?

Going forward, the paper by Flores et al. is revolutionary in that these data force us to consider p53 in the context of its family more than ever before. While this may add further complexity to an already complicated area, we can take comfort in the fact that p53 no longer stands apart but rather is yet another example of a truth we have long know about family: you can't live with them and you can't live without them.

Marshall Urist and Carol Prives[1]

Department of Biological Sciences
Columbia University
New York, New York 10027
[1]E-mail: clp3@columbia.edu

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