p53—An examination of sibling support in apoptosis control

While p53 family members have distinct nonoverlapping functions, the involvement of p63 and p73 in p53-mediated apoptosis is controversial. Results of a recent study indicate that at least in thymocytes, p53-dependent apoptosis occurs independently of p63 and p73.

The p53 tumor suppressor belongs to a gene family that includes p63 and p73 (Melino et al., 2003; Yang et al., 2002). The three paralogs encode proteins with remarkable similarity at the structural and functional level. Each member of the family functions as a sequence-specific transcription factor, each member is induced by different forms of stress including DNA damage, and each member has been implicated in pathways leading to cell death. p63 and p73 are more similar to each other than to p53, and both can activate expression of genes containing p53-responsive elements. Despite all these similarities, it has become evident that these genes are involved in different biological processes and that they are not functionally redundant. One indication of this is provided by the distinct patterns of expression exhibited by the family members. In contrast with the ubiquitous expression of p53 mRNA, p63 is expressed in the basal or progenitor layers of many epithelial tissues and p73 is expressed in the epidermis, sinuses, inner ear, and brain as well as in developing neurons. Another indication of their individuality is provided by knockout mouse studies. p63 null mice are born with striking developmental malformations, including the lack of limbs and epithelial structures. These mice die within a day of birth due to desiccation and maternal neglect. p73 null mice are characterized by chronic infections and developmental defects, including hippocampal dysgenesis, hydrocephalus, and abnormalities in the pheromone sensory pathway. Both phenotypes are very unlike that of p53 knockout mice, which are without gross developmental defects. Unlike p53 null mice, p73 null mice do not develop tumors, and in contrast to p53, p73 and p63 are rarely mutated in human cancers. While p63 and p73 can activate a subset of p53 target genes independently of p53, p63 and p73 can also regulate specific sets of target genes. It is also notable that viral oncoproteins such as SV40 large T antigen, adenovirus E1B, and human papilloma virus E6, which bind and inactivate p53 in order to promote cell cycle entry, do not target p73 and p63. Altogether, these data indicate that p53, p63, and p73 have diverged significantly from a common ancestral gene and that their products have distinct nonoverlapping functions.

In spite of these differences, provocative findings suggest that functional interactions—either collaborative or inhibitory—can take place among family members. Heterotypic interactions are complicated by the multiple isoforms of p63 and p73 that are expressed. The p63 and p73 genes contain two separate promoters that are used to generate transcripts encoding proteins with (TAp63 or TAp73) or without (ΔNp63 or ΔNp73) an N-terminal transactivation domain. Alternative splicing at the 3′ end of the p63 and p73 RNA transcripts generates additional complexity by creating both TA and ΔN proteins with different C termini. The different isoforms appear to act differently either in promoting or repressing p53-dependent functions including apoptosis. Perhaps the best example of interference occurring in a physiological setting was provided by Pozniak et al. (2000), who demonstrated that ΔNp73β was the predominant p73 isoform expressed in postnatal day 10 mouse brain and in sympathetic neurons. In p73$^{-/-}$ mice, all isoforms of p73 are deleted, and p53-dependent apoptosis of developing neurons is greatly enhanced. They concluded that ΔNp73β plays a critical antiapoptotic function in neurons by antagonizing p53-dependent neuronal apoptosis.

An intriguing example of cooperation amongst family members was provided by Flores et al. (2002), who investigated γ radiation-induced apoptosis in the developing central nervous system of embryos mutant for p53 family proteins. p53 has been shown to be required for radiation-induced apoptosis in the developing nervous system. Comparison of wild-type, p53 null, p63 null, p73 null, and doubly mutant p63/p73 null embryos revealed that wild-type embryos were radiation-sensitive, that the single mutant p63$^{-/-}$ and p73$^{-/-}$ embryos were partially resistant, and that the p63/p73 double null embryos were as resistant as the p53$^{-/-}$ embryos. The authors concluded that p63 and p73 are required for p53-dependent neuronal apoptosis in irradiated embryos. The same study reported a requirement for p73 and p63 in p53-dependent apoptosis in E1A-expressing mouse embryo fibroblasts (MEFs) treated with various genotoxic agents, including doxorubicin, cisplatin, and γ radiation. In addition, p63 and p73 were required for activation of certain proapoptotic p53 target genes in the E1A transformed MEFs. Chromatin immunoprecipitation (ChIP) analyses indicated a failure of p53 to bind proapoptotic promoters in the absence of p63 and p73. These data suggested that p63 and p73 may be required for the stable association of p53 with a subset of its target promoters and give rise to models involving the assembly of transcriptional complexes containing all three family members (reviewed in Urist and Prives, 2004).

A report by Senoo et al. (2004) in this issue of *Cancer Cell* provides much-needed clarity on the generality of the concept that p53 requires p63 and p73 for its apoptotic function by showing that at least in immature T cells, p53 functions perfectly well on its own. Senoo et al. (2004) used fetal hematopoietic (liver) cells derived from wild-type, p73 null, p63 null embryos or p63/p73 double null embryos to reconstitute lymphocytes in sublethally irradiated Rag2$^{-/-}$ mice. The fetal liver cell adoptive transfer technique is a widely accepted procedure to study gene function in lymphocytes that avoids any systemic defect imparted by loss or aberrant expression of a gene. Any T or B cells observed in Rag2$^{-/-}$ mice have the genotype of the donor animal, since Rag2$^{-/-}$ mice lack the V(D)J recombination machinery required for lymphocyte maturation. Senoo et al. (2004) show that overall numbers of lymphocytes in the reconstituted thymi were similar, that the proportion of CD4^{+}, CD8^{+}, double positive thymocytes were similar, and the number and proportion of lymphocytes in the spleen were similar. Importantly, Rag2-reconstituted wild-type, p63 null,

p73 null, and p63/p73 null thymocytes were sensitive to irradiation in contrast to p53 null thymocytes, which were resistant. In addition, CD4+ purified mature T cells derived from Rag2$^{-/-}$ mice having single or combination losses of p63 and p73 underwent apoptosis (activation-induced cell death) following TCR stimulation similar to that seen in wild-type CD4^{+} T cells. Senoo et al. (2004) conclude that p63 and p73 are not required for radiation-induced death in immature T cells (a p53-dependent process) and that p63 and p73 are also not required for activation-induced cell death in mature peripheral T cells (a p53-independent process).

Senoo et al. (2004) claim that p53 can act independently of p63 and p73 in apoptosis control, and their findings challenge the notion that p63 and p73 largely mimic and facilitate the action of p53. At least in immature thymocytes, this certainly appears to be correct. In the irradiated CNS of developing embryos and in irradiated E1A-transformed MEFs, however, p53 is dependent on its sibling coactivators to promote apoptosis. How does one begin to explain these disparate findings?

The response of fibroblasts and immature thymocytes to radiation-induced damage is very different. Lowe et al. (1993) showed clearly that early passage p53$^{+/+}$ and p53$^{-/-}$ MEFs do not lose viability when treated with various chemotherapeutic agents or γ radiation. It is only after E1A is expressed that these cells exhibit p53-dependent apoptosis. Hence, the requirement for p63 and p73 in E1A-transformed MEFs to facilitate p53 function may be a requirement imposed by E1A expression. Thymocytes, on the other hand, readily undergo p53-dependent apoptosis in response to irradiation in the absence of E1A and require neither p63 nor p73.

Another possibility relates to cell type specificity. Animal studies indicate that p53-dependent apoptosis is restricted to certain tissues from irradiated mice (spleen, thymus, small intestine, developing nervous system). This could reflect tissue differences in DNA repair, p53 expression and activation, intracellular death/survival pathways, or selective transactivation/repression of p53 target genes in different organs (Bouvard et al., 2000; Fei et al., 2002). Promoter selectivity could reflect differences in the affinity of various promoters for p53, such that some are responsive only to high levels of p53 or to certain modified forms of p53. Promoter selectivity might also be regulated by the interaction of p53 with an ever-growing number of proteins that act as transcriptional cofactors by selectively stabilizing the interaction of p53 with specific promoters (Gudkov and Komarova, 2003; Oren, 2003; Vousden and Lu, 2002).

Future experiments will likely characterize the p53-containing transcriptional complexes that assemble on different p53-responsive promoters in vivo in specific tissues and under specific stress conditions. This will lead to a better understanding of critical p53 modifications, transcriptional coactivators, and obligatory histone modifications that are involved in p53-dependent transcriptional activity. The concept that ΔN-isoforms of p63 and p73 antagonize p53 function in different tissues needs to be investigated more widely. In addition, future experiments will continue to identify critical p53-dependent proapoptotic target genes; these may be differentially regulated in different cells and tissues with correspondingly different coactivator requirements. The different requirement for p63 and p73 in p53-dependent apoptosis in neurons and thymocytes provides an entry point to investigate the complexity of DNA damage responses in the whole organism.

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Selected reading

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