Published in final edited form as:
Oncogene. 2008 October 27; 27(50): 6507–6521. doi:10.1038/onc.2008.315.

The p53 family and programmed cell death

E. Christine Pietsch[1], **Stephen M. Sykes**[2], **Steven B. McMahon**[3], and **Maureen E. Murphy**[1]

1*Division of Medical Sciences, Fox Chase Cancer Center, 333 Cottman Avenue, Philadelphia PA, 19111.*

2*Brigham and Women's Hospital, 1 Blackfan Circle, Boston, MA 02115.*

3*Kimmel Cancer Center, Thomas Jefferson Medical College, 233 S. 10th St. Philadelphia, Pennsylvania 19107*

Abstract

The p53 tumor suppressor continues to hold distinction as the most frequently mutated gene in human cancer. The ability of p53 to induce programmed cell death, or apoptosis, of cells exposed to environmental or oncogenic stress constitutes a major pathway whereby p53 exerts its tumor suppressor function. In the past decade we have discovered that p53 is not alone in its mission to destroy damaged or aberrantly proliferating cells: it has two homologues, p63 and p73, that in various cellular contexts and stresses contribute to this process. In this review, the mechanisms whereby p53, and in some cases p63 and p73, induce apoptosis are discussed. Whereas other reviews have focused more extensively on the contribution of individual p53-regulated genes to apoptosis induction by this protein, in this review we focus more on those factors that mediate the decision between growth arrest and apoptosis by p53, p63 and p73, and on the post-translational modifications and protein-protein interactions that influence this decision.

Keywords

p53; p63; p73; apoptosis; transcription; mitochondria

The p53 family

The tumor suppressor p53 is vital in maintaining cellular genomic integrity and controlled cell growth (Levine, 1997; Bargonetti and Manfredi, 2002; Fridman and Lowe, 2003). Loss or gain of p53 function results in the aberrant growth of cells. Hence, both the cellular expression and activity of p53 are tightly regulated. p53 protein has a very short half life and thus is usually present at extremely low levels within cells. In response to stress, DNA damaging agents, and chronic mitogenic stimulation, p53 is transiently stabilized and activated. Depending on cell type, cell environment and oncogenic alterations, p53 activation leads to inhibition of cell cycle progression, induction of senescence, differentiation, or apoptosis (Vousden and Lu, 2002). Over a decade after the identification of the tumor suppressor p53, two p53-related genes, p63 and p73, were identified (Kaghad *et al.*, 1997; Schmale and Bamberger, 1997; Osada *et al.*, 1998; Trink *et al.*, 1998; Yang *et al.*, 1998; Zeng *et al.*, 2001). Like p53, both p63 and p73 possess an amino-terminal transactivation domain (TAD), a DNA binding domain (DBD), and a carboxyl-terminal oligomerization domain (OD) (Murray-Zmijewski *et al.*, 2006). While p63 and p73 demonstrate relatively little homology with p53 in their TAD and OD, both share

Address correspondence to: Maureen E. Murphy, Division of Medical Sciences, Fox Chase Cancer Center, 333 Cottman Avenue, Philadelphia PA 19111. Phone: 215-7285684. FAX 215-728-4333. Email: Maureen.Murphy@FCCC.edu; or: Steven B. McMahon, Kimmel Cancer Center, Thomas Jefferson Medical College, 233 S. 10th St. Philadelphia, Pennsylvania 19107. Phone: 215-503-9066. Email: steven.mcmahon@mail.jci.tju.edu.

approximately 60% similarity with the p53 DBD, including conservation of essential DNA contact residues (DeYoung *et al.*, 2007) (see Figure 1). This similarity allows p63 and p73 to regulate p53 target genes and, similar to p53, to induce cell cycle arrest and apoptosis. However, studies of knockout mice have demonstrated that, even though these proteins clearly share some activities with p53, each of these proteins also has functions that are very distinct. *TP53* null mice are viable and develop normally (Donehower *et al.*, 1992). In contrast, p63 knockout mice show severe developmental defects, including failure to develop limbs, skin and other epithelial tissue; these mice do not survive beyond a few days after birth (Mills *et al.*, 1999). p73 knockout mice exhibit neuro-developmental (hippocampal dysgenesis and hydrocephalus) and inflammatory (chronic infections and excessive inflammation) defects (Yang *et al.*, 2000).

Isoforms of p53 family members

The *p53*, *p63* and *p73* genes are located on chromosomes 17 (17p13.1), 3 (3q27–29) and 1 (1q36), respectively, and all three genes are now known to express many differentially spliced isoforms (Muller *et al.*, 2006; Murray-Zmijewski *et al.*, 2006). All three genes encode two primary transcripts that are controlled by separate promoters (P1 and P2) (Figure 1). The P1 promoter of each gene is embedded in a non-coding region of exon 1. The P2 promoter of p63 and p73 is located in intron 3 while the P2 promoter of p53 is located in intron 4. Transcripts generated from the P1 promoter produce proteins that contain the TAD, the DBD, and the OD (TAp53, TAp63, and TAp73). In contrast, transcripts generated from the P2 promoter are missing the amino terminal TAD (Δ133p53, ΔNp63, and ΔNp73). These ΔN-terminal variants are generally regarded as dominant negative versions of p53 family members, as these can occupy promoter-binding sites but fail to transactivate gene expression. For both p53 and p73 additional ΔN variants can be generated by alternative splicing of the N-terminus or alternative initiation of translation (Δ40p53, Δex2p73, and Δex2/3p73) (Muller *et al.*, 2006; Murray-Zmijewski *et al.*, 2006). Additional complexity to p53 family member isoforms is added by virtue of the fact that P1 and P2 transcripts can be spliced at the C-terminus into several splice variants (α, β, γ, etc.). For p53, transcription from the P1 and P2 promoters and alternative splicing of intron 9 can generate at least six p53 isoforms, including three TA variants (p53, p53β & γ) and three ΔN variants (Δ133p53, Δ133p53β & γ). For p63 three TA variants (TAp63α, TAp63β, TAp63γ) and three ΔN variants (ΔNp63α, β & γ) have been identified. Finally, for p73, eight different isoforms from the p73 P1 promoter and P2 promoter elements have been described (TA and ΔN p73α, β, γ, δ, ε, ζ, η, & θ).

Tumor suppression by p53 family members

p53 can be found biallelically mutated or deleted in over 50% of human tumors, and there are p53 mutation databases containing a wealth of information in this regard (Olivier *et al.*, 2002). Germline mutations in p53 are found in Li Fraumeni syndrome, a tumor-prone disorder predisposing affected individuals to tumors of the brain, breast, bone and adrenal cortex (Malkin *et al.*, 1992). Finally, the p53-knockout mouse is predisposed to early onset tumors, chiefly thymic lymphoma, sarcoma, and testicular tumors (Donehower *et al.*, 1992).

p63 and p73 map to two regions within the human genome that are often altered and deleted in cancers, respectively (Kaelin, 1999). This observation, combined with the observation that p63 and p73 mimic p53 action in tissue culture when over-expressed, suggests that p63 and p73 have tumor suppressive properties analogous to p53. However, characterization of these proteins as *bona fide* tumor suppressors has not been straightforward. Studies aimed at identifying mutations within the *p63* and *p73* gene in human cancers have demonstrated that, while p53 is mutated in 50% of all human cancers, p63 and p73 mutations occur rarely (Irwin and Kaelin, 2001; Melino *et al.*, 2003; Deyoung *et al.*, 2007). Rather, altered expression of p63

and p73 isoforms is more commonly observed (Muller *et al.*, 2006). For example, p63 is upregulated in 80% of head and neck squamous cell carcinomas (HNSCCs), with ΔNp63 being the predominant isoform overexpressed (Weber *et al.*, 2002; Sniezek *et al.*, 2004; DeYoung *et al.*, 2006; Rocco *et al.*, 2006). ΔNp63 overexpression is observed in over 60 % of bladder carcinomas (Park *et al.*, 2000), while loss of TAp63 expression occurs in bladder cancer and is associated with metastasis and poor prognosis (Urist *et al.*, 2002; Koga *et al.*, 2003). Similar to p63, altered expression of p73 occurs in a multitude of different cancers (Muller *et al.*, 2006). Recent studies analyzing p73 isoform expression indicate that both TAp73 and ΔNp73 isoforms are upregulated in ovarian cancer and rhabdomyosarcomas (Concin *et al.*, 2004; Cam *et al.*, 2006), while exclusive upregulation of ΔNp73 isoforms can be observed in gliomas as well as carcinomas of the breast and colon (Dominguez *et al.*, 2006; Wager *et al.*, 2006). Down-regulation of TAp73 is found in lymphoblastic leukemias and Burkitt's lymphoma as a result of p73 P1 promoter methylation (Corn *et al.*, 1999; Kawano *et al.*, 1999). The general, but by no means definitive, consensus appears to be that either methylation-mediated silencing of the entire gene, or upregulation of the dominant-negative ΔN variant, predominates in human tumors for p53 family members. How upregulation of specific ΔN variants occurs in human tumors is not presently understood.

Research on the predisposition of p63 and p73 knockout mice to tumor development has been conflicting and has added to the complexity of defining p63 and p73 as tumor suppressor proteins. p73 knockout mice do not develop spontaneous tumors (Yang *et al.*, 2000) and heterozygous p63 mice are not tumor prone, nor do they develop tumors at an accelerated rate upon exposure to chemical carcinogens (Keyes *et al.*, 2006). In fact, p63 deficiency was found to induce a cellular senescence program, increased aging, and shortened life span (Keyes *et al.*, 2008). In contrast to these observations is a study examining tumor development in aging heterozygous p63 and p73 knockout mice. This study demonstrated that p63+/− and p73+/− mice develop spontaneous tumors, similar to p53+/− mice, with a median survival time of 10 months for p53+/−, 15 months for p63+/−, and 14 months for p73+/− (Flores *et al.*, 2005). These latter data offer the firmest evidence that p63 and p73 have tumor suppressive properties analogous to p53.

Further evidence that p63 and p73 have tumor suppressor function comes from four independent observations: 1.) siRNAs specific for p63 and p73 enhance the transformation potential of p53 −/− mouse embryo fibroblasts (Lang *et al.*, 2004); 2.) Both p63 and p73 can mediate chemo-sensitivity independent of p53 status by induction of apoptosis (Irwin *et al.*, 2000; Bergamaschi *et al.*, 2003a; Irwin *et al.*, 2003; Lang *et al.*, 2004; Gressner *et al.*, 2005); 3.) Within tumor cells mutant p53 directly binds to both p63 and p73 via the DNA binding domain, rendering p63 and p73 impaired in their ability to induce growth suppression and apoptosis (Di Come *et al.*, 1999; Marin *et al.*, 2000; Gaiddon *et al.*, 2001; Strano *et al.*, 2002); and 4.) Silencing mutant p53 expression by siRNA-mediated knockdown sensitizes cells to anticancer agents (Bergamaschi *et al.*, 2003a). These observations indicate that inactivation of p63 and p73 confers a growth advantage to tumor cells that have mutant forms of p53. Binding of mutant p53 to p73 is influenced by a polymorphism at codon 72, which encodes either arginine or proline. p53 mutants associated with the arginine polymorphic variant bind and inactivate p73 most efficiently (Marin *et al.*, 2000; Bergamaschi *et al.*, 2003a). Consistent with this observation, these p53 mutants demonstrate a less favorable response to chemotherapy (Bergamaschi *et al.*, 2003a). Likewise, in osteosarcoma cells from p53R172H (equivalent to human hotspot mutant R175H) knock-in mice, mutant p53 co-immunoprecipitates and functionally inactivates p63 as well as p73, resulting in enhanced cellular transformation (Lang *et al.*, 2004). Collectively, these observations suggest that p63 and p73 may complement, and in some situations may substitute for, the actions of p53. However, which effects p63 and p73 exert on cell proliferation and death may ultimately

depend on the cellular context and genetic background, since p63 and p73 may not be required for the development and apoptosis of all cell types (Senoo *et al.*, 2004).

Apoptosis by p53

Transcription-dependent apoptosis

The best understood activity of p53 is as a transcription factor that binds to the promoters and introns of target genes, and recruits the basal transcriptional machinery to activate expression of that gene. In 1992, a paper that searched for the consensus binding site for p53 using an unbiased affinity binding approach revealed that p53 bound best to two tandem repeats of the consensus 5'-Pu-Pu-Pu-C-(A/T)-(T/A)-G-Pyr-Pyr-Pyr-3', where Pu is purine, and Pyr is pyrimidine, separated by 0–13 bases (el-Deiry *et al.*, 1992). Since that time, literally hundreds of p53 target genes have been identified, and each contain one or more consensus sites in their 5' promoter regions, introns, and in some cases, exons. These include $p53^{AIP1}$, *Apaf-1, BAX, Caspase-1, Caspase-6, cathepsin D, DINP1, DR4, DR5, Fas, IGF-BP3, NOXA, p85, PERP, PIDD, PIG3, PTEN, PUMA, Scotin, EI24/PIG8*, as well as others (for review see Riley *et al.*, 2008). Once bounds to the promoter regions of these genes, p53 recruits general transcription factors as well as histone acetyltransferases (HATs), such as CBP, p300 and PCAF. These HATs acetylate lysine residues of histones, thereby increasing the accessibility of chromatin to the transcriptional apparatus.

Two p53 target genes deserve specific mention as being particularly important in the apoptotic armamentarium of p53: these are *PUMA* and *NOXA*, BH-3 only members of the BCL-2 family. Mice lacking either *PUMA* or *NOXA*, or mice doubly-knocked out for both genes (*PUMA/NOXA* double knockout) develop normally and are not tumor-prone (Jeffers *et al.*, 2003; Villunger *et al.*, 2003; Michalak *et al.*, 2008). The importance of *PUMA* to p53-dependent apoptosis is underscored by the fact that thymocytes and cells from the developing nervous system from the *PUMA* knockout mice are almost completely impaired for p53-dependent apoptosis, to levels equivalent to the p53 knock-out mouse (Jeffers *et al.*, 2003). In contrast, apoptosis in other cell types of the *PUMA* knockout mouse is only partially impaired, and in these cells NOXA appears to contribute. For example, in mouse embryo fibroblasts, both PUMA and NOXA proteins play critical roles, as MEFs from the *PUMA/NOXA* double knock-out are most protected from cell death by etoposide, to levels similar to p53 knockout MEFs (Michalak *et al.*, 2008). Finally, there are other cell types in the mouse where even the *PUMA/NOXA* double knockout mouse is not protected from apoptosis as well as the p53 knockout mouse; this includes mature T and B cells, which are partially but not completely protected by *PUMA/NOXA* loss (Michalak *et al.*, 2008). These data firmly implicate PUMA and NOXA proteins as critical players in the p53 apoptotic program, at least in these cell types, but also suggest that the overall importance of individual p53 target genes in apoptosis is likely to be cell context and/or stress-specific.

Transcription-independent apoptosis by p53

Several studies, which have examined p53-dependent apoptosis in the presence of inhibitors of RNA and protein synthesis, indicate that p53 induces apoptosis not only by transcription-dependent, but also by transcription-independent mechanisms (Caelles *et al.*, 1994; Wagner *et al.*, 1994; Yan *et al.*, 1997; Gao and Tsuchida, 1999). Moreover, deletion or mutation of the p53 transactivation domain, which is critical to the induction of p53 apoptotic target genes, does not eliminate the ability of p53 to induce apoptosis (Haupt *et al.*, 1995). Likewise, a p53 deletion mutant containing only the first 214 N-terminal amino acid residues induces apoptosis and suppresses the transformation of rat embryo fibroblasts by several oncogenes, but it does not transactivate p53 target genes (Chen *et al.*, 1996; Haupt *et al.*, 1997).

Compelling evidence has accumulated over the last ten years indicating that p53 can directly activate components of the apoptotic machinery and that this involves translocation of p53 to the mitochondria. More specifically, addition of recombinant p53 to nuclear free cytosolic extract of irradiated cells leads to cytochrome C release, caspase activation, and apoptosis induction (Ding *et al.*, 1998; Ding *et al.*, 2000; Schuler *et al.*, 2000). Therefore, activation of p53 in enucleated cytoplasts is sufficient to induce hallmarks of apoptosis. Immunofluorescence, electron microscopy and cell fractionation studies have demonstrated that in response to genotoxic stress, including DNA damage, hypoxia, and activated oncogenes, p53 rapidly translocates to the mitochondria (Marchenko *et al.*, 2000; Mihara *et al.*, 2003; Sansome *et al.*, 2001; Nemajerova *et al.*, 2005). Mitochondrial translocation of p53 precedes loss of mitochondrial membrane potential and caspase activation, suggesting that p53 may trigger mechanisms at the mitochondria that ultimately lead to induction of apoptosis (Marchenko *et al.*, 2000). Indeed, mitochondrial translocation of p53 in irradiated mouse tissues triggers an early wave of caspase activation, which occurs long before the transcriptional program of p53 is activated (Erster *et al.*, 2004). Notably, specific targeting of p53 to the mitochondria by fusion of the p53 N-terminus to the mitochondrial import leader peptide from ornithine transcarbamylase is sufficient to induce apoptosis, suppress colony formation, and to allow p53 to function effectively as a tumor suppressor *in vivo* (Marchenko *et al.*, 2000; Dumont *et al.*, 2003; Talos *et al.*, 2005; Palacios *et al.*, 2006).

The importance of p53 mitochondrial translocation in apoptosis is underscored by several observations. First, in cells or tissues that undergo cell cycle arrest (and not apoptosis), p53 mitochondrial translocation does not occur following exposure to genotoxic stress (Marchenko *et al.*, 2000; Erster *et al.*, 2004). Second, studies on the apoptotic potential of the proline and arginine p53 codon 72 polymorphic variants revealed that the proline codon 72 polymorphic variant, which is markedly impaired for apoptosis induction compared to the arginine codon 72 variant, does not translocate to the mitochondria as efficiently as the arginine variant (Dumont *et al.*, 2003). In a mouse model for B cell lymphoma, p53 targeted exclusively to mitochondria is efficiently tumor suppressive in vivo (Talos *et al.*, 2005). In contrast, an artificial p53 chimeric mutant that is capable of robustly transactivating pro-apoptotic p53 target genes is capable of inducing senescence, but incapable of inducing apoptosis in knock-in mouse embryo fibroblasts (Johnson *et al.*, 2008). Moreover, the compound pifithrin μ, which selectively blocks p53's mitochondrial function but leaves intact its transcriptional function, can completely block p53-mediated apoptosis of irradiated thymocytes (Strom *et al.*, 2006). The apoptotic defect in thymocytes from the *PUMA* knockout mouse, once heralded as the single most important piece of evidence regarding p53's transcriptional role in cell death, can now clearly be ascribed to a defect in both the transcriptional, and the direct mitochondrial, pathways of p53 (Chipuk *et al.*, 2005). More recently the transcription-independent mitochondrial pathway of p53-mediated apoptosis has been shown to be particularly important for the apoptosis of embryonic stem cells. Specifically, reactive oxygen species, which efficiently induce apoptosis in embryonic stem cells, mediate apoptosis by inducing mitochondrial trafficking of p53, in a manner that is regulated by the histone deacetylase SIRT1 (Han *et al.*, 2008).

Several pieces of evidence demonstrate that p53 regulates transcription-independent apoptosis at the mitochondria by regulating the activity of BCL-2 family members. Proteins of this family are key regulators of the intrinsic pathway of apoptosis (Cory and Adams, 2002). The main mechanism by which BCL-2 family members initiate or prevent apoptosis is by controlling release of cytochrome C and other pro-apoptotic factors from the mitochondria. The BCL-2 family of proteins is broadly divided into two groups that contain pro-apoptotic and anti-apoptotic members. Pro-apoptotic proteins within this family include BAK and BAX and the BH3-only proteins BID, BIM, BAD, NOXA, and PUMA. BCL-2, BCL-xl, and MCL-1 are anti-apoptotic family members, which bind to pro-apoptotic BCL-2 family members and

thereby impair the apoptotic activity of these proteins. Among the pro-apoptotic family members, BH3-only proteins function as sensors of apoptotic signaling, and the key role of these proteins is to activate the main pro-apoptotic effector proteins BAK and BAX (Wei *et al.*, 2001). BH3-only proteins are divided into "activator" and "de-repressor" proteins (Letai *et al.*, 2002; Kuwana *et al.*, 2005). In particular, a subset of BH3-only proteins directly activates BAK and BAX by inducing a conformational change in these proteins, allowing BAK and BAX to form homo-oligomers. These oligomers are thought to serve as channels within the outer mitochondrial membrane through which apoptotic factors are released. The second set of BH3-only proteins indirectly activates (derepresses) BAX and BAK by binding to BCL-2 and/or BCL-xl and thereby preventing these proteins from binding to and inhibiting the action of activator BH3-only proteins and the effecter proteins BAK and BAX.

Upon translocation to the mitochondria p53 seems to function analogous to BH3-only members. Specifically, p53 binds to pro- and anti-apoptotic BCL-2 family proteins and modulates their function. p53 physically interacts with the effector protein BAK, causes release of BAK from the inhibitory protein MCL-1 and induces a conformational change in BAK which results in oligomerization of this protein and subsequent cytochrome C release from mitochondria (Leu *et al.*, 2004). Conserved residues within the DNA binding domain, located in the H2 α–helix and the L1 and L3 loop mediate BAK oligomerization (Pietsch *et al.*, 2008). Significantly, tumor-derived mutations in the DNA binding domain of p53 render this protein unable to induce cytochrome C release and apoptosis (Pietsch *et al.*, 2008). Although a physical interaction between BAX and p53 cannot be detected, p53 also induces BAX oligomerization and cytochrome C release (Chipuk *et al.*, 2004). Specifically, p53 releases pro-apoptotic BAX and the BH3-only protein Bid from anti-apoptotic BCL-xl, and allows BAX to oligomerize (Chipuk *et al.*, 2004).

In addition to interacting with pro-apoptotic BCL-2 family members, p53 also binds and inhibits the anti-apoptotic family members BCL-2 and BCL-xl (Mihara *et al.*, 2003; Tomita *et al.*, 2006). Molecular modeling studies predicted that the p53 DNA binding domain contacts BCL-xl via a domain encompassing the L3 loop (aa 239–248) and two flanking regions ranging from amino acid 135–141 and amino acid 173–187. NMR studies confirmed the involvement of these residues and pointed to additional residues within the H2 α–helix as important for p53-BCL-xl interaction (Petros *et al.*, 2004). More recent NMR studies revealed that p53 utilizes very similar residues within the DNA binding domain to interact with BCL-2 (Tomita *et al.*, 2006). Collectively, data from different laboratories indicate that p53 uses conserved residues within the DNA binding domain to contact BAK, BCL-2 and BCL-xl, although recent data suggest that slightly different contact regions of the p53 DNA binding domain are involved (Sot *et al.*, 2007). One consistency in all of the combined studies, however, has been that tumor-derived mutants of p53 uniformly fail to induce transcription-independent cell death (Tomita *et al.*, 2006; Pietsch et al 2008). This observation was unexpected since many of these mutations were thought to block only the DNA binding and transcriptional functions of p53.

Although most research with respect to p53 transcription-independent apoptosis has heavily focused on BCL-2 family members, it should be noted that p53 has transcription independent apoptotic functions unrelated to the BCL-2 family. For example, in the presence of inhibitors of RNA and protein synthesis, p53 causes the cell surface redistribution of the cytoplasmic death receptor Fas (CD95) by transport from the Golgi complex (Bennet *et al.*, 1998). Notably, cells that contain inactivating mutations in Fas and Fas ligand have reduced p53-induced apoptosis compared to wild type cells.

Post-translational modifications that influence p53-mediated apoptosis

When cells encounter genotoxic stress, the levels of p53 protein rapidly increase. This correlates with a decrease in MDM2 catalyzed poly-ubiquitylation and an increase in a variety of other post-translational modifications (PTMs) including acetylation, phosphorylation, methylation, poly(ADP-ribosyl)ation, neddylation, sumoylation, and non-proteolytic monoubiquitylation (Bode and Dong, 2004; Toledo and Wahl, 2006; Olsson *et al.*, 2007). These post-translational modifications are catalyzed by a large number of enzymes and they contribute to the activation of p53 through a variety of mechanisms. For example, p53 is phosphorylated on at least 18 different residues by over a dozen different kinases (Olsson *et al.*, 2007). Most modifications are correlated with a generic increase in the ability of p53 to induce both cell cycle arrest and apoptosis. However, a small subset of these modifications are specifically linked to the apoptotic function of p53. Exploiting pathways that specifically enhance p53-mediated apoptosis has long been a goal of cancer therapeutics. Some of the better-understood examples from this subset will be discussed below.

Acetylation of Lysines 320 and 373 by PCAF and p300/CBP, respectively

The HATs p300, CBP and PCAF not only acetylate lysine residues in histones, they also acetylate p53 (Gu & Roeder, 1997; Liu *et al.*, 1999; Sakaguchi *et al.*, 1998). Interestingly, there is support for the premise that the specific HAT that gets recruited to p53 influences the outcome of p53 induction (growth arrest or apoptosis). More specifically, PCAF is the HAT that acetylates lysine 320 of p53 (Sakaguchi *et al.*, 1998). Acetylation of this residue is often associated with cell cycle arrest, as mutating this residue to an unacetylatable arginine disrupts p53-mediated cell growth arrest but enhances p53-directed apoptosis (Barlev *et al.*, 2001; Chao *et al.*, 2006; Liu *et al.*, 1999; Sakaguchi *et al.*, 1998). Furthermore, acetylation at this residue allows for p53 to bind only at certain “high affinity” p53 binding sites, such as *p21/waf1* (Di Stefano *et al.*, 2005; Knights *et al.*, 2006). In contrast, acetylation of lysine 373 of p53, catalyzed by p300 and/or CBP, leads to increased phosphorylation of N-terminal residues of p53, and markedly increased ability of this protein to transactivate lower affinity binding sites, such as those found at the pro-apoptotic target genes PIG3, Bax, and $p53^{AIP1}$ (Knights *et al.*, 2006). These results are most consistent with a model whereby acetylation of lysines 320 and 373 acts as a sensor system that enables p53 to choose between growth arrest and cell death, and to coordinate gene expression patterns appropriately following DNA damage.

Serine 46 phosphorylation

Among the PTMs specifically linked to apoptosis is phosphorylation of p53 at serine 46 (Ser^{46}). Enhanced apoptosis occurs as a result of this phosphorylated form of p53 selectively activating pro-apoptotic target gene transcription (Oda *et al.*, 2000; Rinaldo *et al.*, 2007; Taira *et al.*, 2007). For example, phosphorylation of p53-Ser^{46} is necessary for p53 to activate the transcription of the pro-apoptotic target gene $p53^{AIP}$ (Oda *et al.*, 2000). Hints about the biochemical mechanism by which Ser^{46} phosphorylation might enhance the transcription of pro-apoptotic targets have come from studies of the prolyl isomerase PIN1. PIN1 binds proteins containing a motif that consists of a phosphorylated-serine or -threonine residue that is followed by a proline residue (Shaw, 2007). Ser^{46} within p53 is followed by a proline, and recently it was shown that binding of PIN1 to p53 following Ser^{46} phosphorylation promotes the dissociation of the apoptosis inhibitor iASPP from p53 (Mantovani *et al.*, 2007). (The role of ASPP family proteins in p53-mediated apoptosis will be discussed below.) The isomerization of p53 by PIN1 has also been shown to facilitate the binding and acetylation of p53 by the acetyltransferase p300 (Mantovani *et al.*, 2007).

There are two caveats regarding the importance of Ser^{46} phosphorylation in apoptosis. First, Ser^{46} is not conserved in mice. That being said, a large portion of the mouse p53 locus

(corresponding to amino acids residues 33–332) can be replaced with the corresponding human p53 DNA sequence by homologous recombination (Hupki, Humanized p53 knock-in mouse) (Luo *et al*., 2001). This mouse/human chimera of p53 completely recapitulates the function of mouse p53, and Serine 46 is phosphorylated in mouse cells containing this protein (Feng *et al.*, 2006). Further, mutation of Serine 46 to an alanine in the Hupki mouse (Hupki^{S46A}) can inhibit p53-mediated apoptosis, though this inhibition is somewhat more modest than that seen in human cells (Feng *et al.*, 2006). Second, the interaction with PIN1 cannot explain the totality of the effect of Ser^{46} phosphorylation on p53 function, because loss of PIN1 also reduces the ability of p53 to bind to the promoters of the non-apoptotic target genes *p21* and *hMDM2* (Zheng *et al*., 2002). Additional studies are clearly required before we have a better understanding of how Ser^{46} phosphorylation selectively regulates the ability of p53 to selectively transactivate pro-apoptotic genes and induce apoptosis.

Lysine 120 acetylation

Recent studies showing that the MYST family members TIP60 and hMOF acetylate p53 in the DNA binding domain at lysine 120 (Lys^{120}) have provided additional insight regarding how p53 makes the decision between cell cycle arrest and apoptosis. These studies showed that mutation of Lys^{120} to a non-acetylatable arginine residue (K120R) diminishes the ability of p53 to induce apoptosis but does not affect p53-mediated cell cycle arrest (Sykes *et al.*, 2006; Tang *et al.*, 2006). Unlike most other amino acids in p53 that are substrates for PTMs, K120 is mutated in human cancer. These mutations, while rare, occasionally convert K120 to an arginine, thus conserving most of the biophysical properties at this site but eliminating acetylation. Significantly, loss of Lys^{120} acetylation prevents p53 from up-regulating the expression of target genes important for apoptosis, but not growth arrest. Additionally, chromatin immunoprecipitation studies indicate that the acetylated form at Lys^{120} appears to be selectively recruited to the promoters of pro-apoptotic p53 target genes, as opposed to those involved in cell cycle arrest (Sykes *et al.*, 2006; Tang *et al.*, 2006).

While S46 phosphorylation and K120 acetylation appear to enhance apoptosis by allowing p53 to selectively enhance the transcription of pro-apoptotic targets, other PTMs that modulate the apoptotic potential of p53 influence the transcription-independent apoptosis pathway.

Mono- versus poly-ubiquitylation

The addition of poly-ubiquitin chains to a protein commonly results in proteasome-mediated destruction. On the contrary, mono-ubiquitylation often acts as a protein modification that affects protein function without altering protein stability (Welchman *et al.*, 2005). Consistent with this premise, while p53 poly-ubiquitylation acts as a protein destruction signal, mono-ubiquitylation of p53 promotes nuclear export and mitochondrial localization (Li *et al.*, 2003; Marchenko *et al.*, 2007). Interestingly, once targeted to the mitochondria by monoubiquitylation, the ubiquitin moiety must be removed before p53 can trigger transcription-independent apoptosis (Marchenko *et al*., 2007). At present it is unclear which residues of p53 must be ubiquitylated in order to target this protein to mitochondria. One group has found that the E3-ligase E4F1 catalyzes ubiquitylation of p53 at Lys$^{319,\ 320\ \&\ 321}$ (Le Cam *et al.*, 2006), and that ubiquitylation of these residues is associated with chromatin-bound p53, and specifically directs a p53-transcriptional program that promotes cell-cycle arrest and not apoptosis. Therefore, while it remains unclear what site(s) are mono-ubiquitylated in order to target p53 to the mitochondria, those residues targeted by E4F1 appear unlikely to be candidates.

While attractive, models that evoke selective PTM patterns to explain the ability of p53 to induce cell cycle arrest versus apoptosis tend to be plagued by considerable complexity. For example, lysine 320 of p53 (K320) is not only subject to acetylation, but can also be

ubiquitylated or neddylated. Interestingly, for the Lys^{320} equivalent in mice, Lys^{317}, mutation to an arginine leads to enhanced p53-mediated transcription of pro-apoptotic target genes, such as *PIDD, PUMA*, and *NOXA* (Chao *et al.*, 2006). Correlating with enhanced target gene transcription, an increase in p53-mediated apoptosis in thymocytes, transformed MEFs, and intestinal crypt cells exposed to DNA damaging agents was also observed. These observations are consistent with a loss of E4F1-catalyzed ubiquitylation of human p53 at Lys^{320}, which promotes p53 cell cycle arrest (Le Cam *et al.*, 2006). Furthermore, FBXO11-mediated neddylation of human p53 at Lys^{320} represses p53 activity and therefore the observations made in $p53^{K317R}$ mice are also representative of a loss of neddylation (Abida *et al.*, 2007). As discussed above, p53 transcriptional activity and growth arrest function is enhanced by acetylation at Lys320 by the acetyltransferase PCAF (Liu *et al.*, 1999; Barlev *et al.*, 2001). Thus, the increased apoptotic activity of mouse $p53^{K317R}$ may be explained by impaired acetylation, neddylation, or ubiquitylation at this residue. In addition to these caveats, it should be noted that PTMs on p53 may affect p53 function in a context-dependent manner, depending on tissue specificity and type of cellular stress.

Protein-protein interactions that influence p53-mediated apoptosis

Recent years have witnessed the identification of a number of p53-interacting proteins that direct either cell cycle arrest or apoptosis. These findings occur on a backdrop in which dozens and perhaps hundreds of partners for p53 have been identified. Here we will focus on the examples of Hzf, MUC1, and the Brn-3, ASPP and p63/p73 families.

Hematopoietic Zinc Finger protein

Hzf (Hematopoietic Zinc Finger) is a gene that is up-regulated in hematopoietic progenitor cells and is also a direct transcriptional target of p53 (Das *et al.*, 2007). In mouse embryonic fibroblasts lacking Hzf (Hzf−/− MEFs), p53 cannot bind to the promoters and activate transcription of some growth arrest specific genes, such as *p21* and *14-3-3* σ (Das *et al.*, 2007). However, p53 displays enhanced promoter binding and transcription of the pro-apoptotic p53 target genes *BAX, PUMA, NOXA* and *PERP* in Hzf−/− MEFs. Furthermore, ectopic expression of Hzf enhances *p21* and *14-3-3*σ transcription in a p53-dependent manner but diminishes the ability of p53 to induce expression of *BAX, PUMA, NOXA* and *PERP* (Das *et al.*, 2007). A model has thus been developed in which interaction between p53 and Hzf blocks apoptosis by inhibiting the transcription of the pro-apoptotic targets of p53.

MUC1

The trans-membrane protein, MUC1 (Mucin1) is another co-factor that selectively promotes p53-mediated cell cycle arrest, thereby inhibiting apoptosis (Wei *et al.*, 2005). Upon proteolytic cleavage from the plasma membrane, the cytoplasmic domain of MUC1 (MUC1-CD) is targeted to the nucleus and mitochondria. In the nucleus, MUC1-CD binds and co-localizes with p53 at the *p21/waf1* and *BAX* promoters. Depletion of MUC1 from human cells leads to an increase in p53-dependent *BAX* transcription and a decrease in *p21/waf1* transcription (Wei *et al.*, 2005). Furthermore, MUC1 attenuates *BAX* transcription by preventing loading of the pre21 initiation complex. Conversely, MUC1 cooperates with p53 to recruit transcriptional co-activators to the *p21/waf1* promoter.

Brn3 family

The transcription factors Brn-3a and Brn-3b are important for proper neuronal development (Gan *et al.*, 1996; Xiang *et al.*, 1996). Although Brn-3a and Brn-3b share a domain of homology (POU domain) they have opposite effects on cell survival. Brn-3a supports cell survival while Brn-3b promotes cell death. Brn-3a can promote cell survival by inhibiting p53-mediated transcription of the pro-apoptotic genes *BAX* and *NOXA* (Hudson *et al.*, 2005). Consistent with

this, neurons derived from Brn-3a knockout mice display enhanced apoptosis. In contrast, Brn-3b −/− neurons are defective for *BAX* expression and p53-induced apoptosis, indicating that Brn-3b is important for p53 apoptotic activities in neuronal cells (Budhram-Mahadeo *et al.*, 2006).

ASPP family

Some co-factors for p53 can specifically promote p53-mediated apoptosis. For example, three members of the ASPP (ankyrin repeats, SH3 domain, polyproline-rich domains) family of proteins ASPP1, ASPP2, and iASPP also influence the pro-apoptotic activity of p53. iASPP binds to the proline-rich and DNA-binding domains of p53 and inhibits the ability of p53 to induce transcription of *BAX*, as well as p53-dependent apoptosis (Bergamaschi *et al.*, 2003b). Alternatively, ectopic expression of either ASPP1 or ASPP2 enhances p53-dependent apoptosis, and depletion of ASPP1 and/or ASPP2 suppresses the ability of p53 to induce apoptosis (Samuels-Lev *et al.*, 2001). Mechanistically, ASPP1 and ASPP2 disrupt iASPP binding to p53, which results in increased p53 binding and transcription from the *BAX* promoter (Samuels-Lev *et al.*, 2001; Bergamaschi *et al.*, 2003b).

It is worth noting that the activity of p53 partners that specifically inhibit or enhance apoptosis may also be influenced by the selective PTMs discussed above. It is attractive to speculate that a given PTM might facilitate the binding of a specific partner whose activity is coupled to inhibiting or enhancing apoptosis.

p63 and apoptosis

Similar to p53, p63 protein levels increase upon treatment of cells with DNA damaging agents (Katoh *et al.*, 2000; Okada *et al.*, 2002; Bergamaschi *et al.*, 2004; Petitjean *et al.*, 2008). Increased p63 protein levels are not the result of transcriptional activation of the p63 gene, but most likely are due to post-translational modifications of this protein (Katoh *et al.*, 2000; Okada *et al.*, 2002). In addition, p63α, which contains an internal inhibitory domain in its C-terminal region, is cleaved by caspases at a non-classical caspase cleavage consensus sequence centered on amino acid 458, which enhances the apoptotic activity of this protein (Sayan *et al.*, 2007).

p63-dependent apoptosis appears to be primarily regulated by transcriptional mechanisms and no transcription independent mechanisms have been identified to date. All p63 isoforms transcriptionally activate exogenously transfected promoters and endogenous mRNA or protein of pro-apoptotic p53 target genes (Katoh *et al.*, 2000; Helton *et al.*, 2008). p63 target genes include the death receptors *CD-95*, *TNF-R and TRAIL-R*, the pro-apoptotic Bc-2 family member *BAX*, the oxidoreductase *PIG3*, the tetraspan membrane protein *PERP*, the nuclear and mitochondrial localized apoptosis inducer *RAD9*, the apoptotic protease *APAF1*, and *caspase-3*, *-8*, and *-9* (Gressner *et al.*, 2005; Ihrie *et al.*, 2005; Helton *et al.*, 2008). When ectopically expressed in cells p63 isoforms induce apoptosis, although not as robustly as p53 or p73 (Dohn *et al.*, 2001; Dietz *et al.*, 2002). Evidence suggests that the transactivation domain (aa 1–59) and the proline rich domain (aa 67–127) of p63 are required for transactivation activity. Notably, deletion of the proline rich domain does not affect the ability of p63 to suppress growth, but does abolish apoptosis induction by p63 (Helton *et al.*, 2008).

The regulatory mechanisms that drive p63-dependent apoptosis are not as well understood. A few post-translational modifications and protein interaction partners have been identified, and while there is some evidence that these affect p63 transactivation activity, it is not clear that these specifically influence the apoptosis function of this protein. Phosphorylation of p63 by as yet unknown proteins may have a role in regulating intracellular p63 protein levels. For example, treatment of cells with okadaic acid, a serine threonine phosphatase inhibitor, results in elevation of p63 protein levels, suggesting that phosphorylation by serine and threonine

specific kinases may stabilize p63 protein (Okada *et al.*, 2002). Additionally, upon treatment of cells with DNA damaging agents, p63α, p63β, and p63γ are phosphorylated on serine residues located between amino acids 160–162 (Petitjean *et al.*, 2008). Thus far ASPP1 and 2 are the only p63 interacting proteins that specifically direct p63 activity towards apoptotic functions versus cell cycle regulatory functions (Bergamaschi *et al.*, 2004). Both ASPP1 and 2 interact with p63, enhance transactivation of the *BAX* and *PIG3* promoters, and increase the apoptotic activity of p63, while transactivation of *p21/waf1* or *MDM2* by p63 are not affected when ASPP1 and 2 are overexpressed.

p73 and apoptosis

p73 is activated in response to a variety of genotoxic agents, including DNA damaging agents and the oncogenes E1A and myc (Zaika *et al.*, 2001). Once activated p73, like p53, can regulate induction of apoptosis and cell cycle arrest. p73-dependent apoptosis seems to be primarily regulated by its ability to transcriptionally activate pro-apoptotic p53 target genes. These include the BCL2 family members *BAX*, *PUMA*, *NOXA*, *BAD* and *BIK*, the oxidoreductase *PIG3*, the tetraspan membrane protein *PERP*, the death receptors *CD95*, *TNFR1*, *TRAIL-R1* and *TRAIL-R2*, the mitochondrial membrane protein $p53^{AIP1}$, as well as the *caspases-3*, *-6* and *-8* (Melino *et al.*, 2002; Muller *et al.*, 2005).

Evidence for a transcription independent role of p73 in apoptosis induction has been controversial. One report has described cleavage of p73 by caspase 3 and caspase 8 in response to DNA damaging agents and TRAIL (TNF-related apoptosis inducing factor), along with localization of both full length and caspase-cleaved p73 to the mitochondria. Importantly, a transactivation deficient p73 mutant was found to enhance TRAIL-induced apoptosis, and addition of recombinant p73 to purified mitochondria resulted in cytochrome C release (Sayan *et al.*, 2008). These findings contrast with an earlier publication that reported that, although p73 induces BAX translocation from the cytosol to the mitochondria, p73 does not itself localize to the mitochondria, and translocation of BAX is not a direct effect of interaction with p73 (Melino *et al.*, 2004). More research is clearly needed in this area to clarify this issue.

p73 post-translational modifications and protein-protein interactions

Induction of apoptosis by p73 in response to DNA damaging agents is intimately linked to its transcriptional activation by p53, along with post-translational modifications and interaction with transcriptional co-activators. In response to DNA damaging agents and oxidative stress p73 is transcriptionally activated by p53 (Chen *et al.*, 2001; Wang *et al.*, 2007). Additionally, DNA damage activates E2F1, which in turn transactivates p73 (Pediconi *et al.*, 2003) and induces p73 to upregulate its own expression (Chen *et al.*, 2001). Several post-translational modifications, including ubiquitylation, acetylation, phosphorylation, in addition to transcriptional co-activator recruitment, have been identified that regulate the apoptotic activity of p73. Similar to p53, HATs, Pin1, and ASPP family members all modify p73's apoptotic function, and these are discussed briefly below. Unique to p73 are the apoptotic regulators c-Abl and YAP (Yes-associated protein).

c-Abl

The first kinase identified to phosphorylate p73 and enhance the transcriptional and apoptotic activity of this protein is the tyrosine kinase c-Abl (Gong *et al.*, 1999; Agami *et al.*, 1999; Yuan *et al.*, 1999). c-Abl overexpression stabilizes p73 (Gong *et al.*, 1999; Sanchez-Prieto *et al.*, 2002), and p73 and c-Abl physically interact with each other via the SH3 domain of c-Abl and a PXXP motif of p73 located in the linker region between the DNA binding domain and the oligomerization domain (Agami *et al.*, 1999; Yuan *et al.*, 1999). DNA damage induces phosphorylation of p73 at tyrosine 99 (Agami *et al.*, 1999; Yuan *et al.*, 1999) in a c-Abl

dependent manner, and p73 proteins mutated at tyrosine 99 are impaired in mediating an apoptotic response upon ionizing radiation treatment. In addition to c-Abl, p38MAPK phosphorylates p73 on threonine residues, and the enhanced transcriptional function of p73 by c-Abl is further enhanced by p38MAPK (Sanchez-Prieto *et al.*, 2002).

YAP

Interaction with the Yes-associated protein (YAP) appears to be uniquely important for p73 function, as it is critical to p73 transactivation of apoptotic target genes and induction of apoptotic cell death (Strano *et al.*, 2001). Physical interaction between YAP and p73 is mediated by the WW domain of YAP and a PPXY motif within p73 that serves as a target sequence for WW domains (Strano *et al.*, 2001). Mutation of tyrosine 487 within the PPXY motif of p73 abolishes binding of p73 to YAP (Strano *et al.*, 2001). YAP-p73 interaction prolongs p73 half-life and prevents proteasomal degradation of p73, by blocking binding of the E3 ubiquitin-like protein ligase Itch to p73 (Levy *et al.*, 2007). Interestingly, YAP appears to function in integrating converging signaling modules to potentiate or diminish p73 transcriptional and apoptotic activity; ultimately, association of p73 with YAP appears to determine whether 73 exerts apoptotic or cell cycle regulatory properties. Ectopic expression of YAP together with p73 increases BAX protein levels within cells and augments p73-mediated transactivation of a *BAX* and $p53^{AIP1}$ promoter luciferase reporter construct, but not a *p21/waf1* promoter-driven luciferase reporter (Strano *et al.*, 2001; Strano *et al.*, 2005). Concomitantly, p73 and YAP co-expression potentiate p73-mediated apoptosis (Strano *et al.*, 2005), while siRNA-mediated silencing of YAP attenuates DNA damage induced p73 dependent apoptosis (Strano *et al.*, 2005; Basu *et al.*, 2003; Levy *et al.*, 2007). YAP also enhances acetylation of p73 by p300 and association between YAP and p73 is required for p300 recruitment to the p73 pro-apoptotic target gene $p53^{AIP1}$ (Strano *et al.*, 2005).

Intracellular YAP protein levels are regulated by the c-Abl tyrosine kinase (Levy *et al.*, 2008). YAP and c-Abl physically interact and phosphorylation of YAP on tyrosine 357 by c-Abl occurs in a DNA damage dependent manner resulting in stabilization of the YAP protein. Notably, phosphorylation of YAP by c-Abl promotes interaction with p73 and association with the promoter region of pro-apoptotic proteins, but not cell cycle regulatory proteins (Strano *et al.*, 2005; Levy *et al.*, 2008). In contrast to the ability of c-Abl to promote stabilization and activation of YAP, Akt phosphorylates YAP on serine 127 and promotes cytoplasmic localization of YAP due to enhanced interaction of YAP with the cytosolic 14-3-3 scaffold proteins (Basu *et al.*, 2003). Hence, active Akt impairs YAP from associating with p73, leading to attenuation of p73's transactivation and apoptotic activity (Basu *et al.*, 2003; Strano *et al.*, 2005).

p300 and CBP

The CH1 domain (amino acid 350 to 450) of the transcriptional coactivators p300 and CREB-binding protein (CBP) interacts with the N-terminus of p73 (Zeng *et al.*, 2000). p300 acetylates p73 on lysines 321, 327, and 331 and transactivation or induction of apoptosis by p73 is impaired in p300 or CBP deficient cells, suggesting an important role of both of these proteins in p73-dependent apoptosis (Zeng *et al.*, 2000; Costanzo *et al.*, 2002). The importance of acetylation in controlling the apoptotic activity of p73 is underscored by the fact that a p73 protein mutated at lysine residues 321, 327, and 331 is able to transactivate p21 and induce cell cycle arrest, but demonstrates a reduced apoptotic response and an inability to suppress colony formation (Costanzo *et al.*, 2002). These data suggest that, as in the case for p53, acetylation of p73 fine-tunes its apoptotic function. Consistent with this premise, whereas wild type p73 is recruited to the promoter of the pro-apoptotic gene, $p53^{AIP1}$, a non-acetylatable mutant of p73 does not bind the $p53^{AIP1}$ promoter and is impaired in transactivating a $p53^{AIP1}$ promoter driven luciferase reporter gene construct (Costanzo *et al.*, 2002).

Pin1

Pin1, a peptidyl-prolyl cis/trans isomerase, stabilizes the p73 protein and enhances the transactivation and apoptotic activity of p73 (Mantovani *et al.*, 2004). Pin1 and p73 interact in a c-Abl dependent manner and interaction requires phosphorylation of p73 on tyrosine 99. Additional phosphorylation by the p38MAPK seems to further enhance p73-Pin1 interaction. Pin1 augments binding of p73 to p300 and stimulates subsequent acetylation of p73 by p300. Similarly to YAP, Pin1 may be an essential factor in determining transactivation of genes encoding pro-apoptotic proteins by p73. Specifically, siRNA mediated downregulation of PIN1 abrogates the increases in the protein levels of PIG3 and BAX by p73 upon DNA damage while transactivation of the *p21/waf1* gene is unaffected.

ASPP family

Apoptosis stimulating proteins of p53 1 and 2 (ASPP1 and ASPP2) affect p73 dependent apoptosis very similarly to p53 dependent apoptosis. ASPP1 and -2 both bind to p73 and augment p73 transactivation of *BAX* and *PIG3* promoter driven luciferase reporter gene constructs, but fail to stimulate p73 transactivation of a *p21/waf1* and *MDM2* promoter driven luciferase reporter gene construct. Consistent with these findings, ASPP1 and -2 enhance the apoptotic activity of p73 (Bergamaschi *et al.*, 2004).

Cooperation and antagonism between p53 family members in apoptosis induction and tumorigenesis

The majority of research with respect to p53 family members has focused on defining the individual activities of p53, p63, and p73 in apoptosis induction and have demonstrated that p73 and to a lesser extent p63 share similar activities with p53. A few research studies that examined the interplay between p53 family members in apoptosis induction and tumorigenesis have unveiled very interesting cooperative as well as antagonistic interactions between these family members. For example, both MEFs and neuronal cells derived from mice lacking *p63* and *p73* genes (p63−/−; p73−/−) are resistant to p53-mediated apoptosis (Flores *et al.* 2002). This defect results from the inability of p53 to bind to the promoters and activate transcription of the p53 pro-apoptotic target genes *BAX*, *NOXA*, and *PERP* in the absence of p63 and p73 expression. Notably, although p63−/−; p73−/− MEFs are resistant to apoptosis they are still competent for p53-induced growth arrest (Flores *et al.* 2002). Moreover, mice heterozygous for p53 and p63, or p53 and p73, display a more aggressive cancer phenotype (higher tumor burden and metastasis) compared to heterozygous p53 mice (Flores *et al.*, 2005) again pointing to an important cooperative activity of p63 and p73 with p53. Further support for this notion comes from the observation that p73 is transcriptionally regulated by p53 under oxidative stress conditions and functions as an irreplaceable downstream target of p53 in controlling the cellular defense against stress (Wang *et al.*, 2007).

While p53 family members cooperate with each other in apoptosis induction, members within this family can also negatively regulate each other's functions. The transactivation domain-truncated isoforms of p53, p63, and p73 act in antagonizing the activity of full-length p53 and TA forms of p63 and p73. For example, ectopic expression of Δ133p53 impairs p53 apoptotic activity (Bourdon *et al.*, 2005). Similar to p53 and p63, ΔNp73 overexpression down-regulates the transactivation activity of p53 and p73 and attenuates the apoptotic activity of p53 and p73 (Grob *et al.*, 2001; Kartasheva *et al.*, 2002), likely by direct competition for promoter binding (Stiewe *et al.*, 2002). Interestingly, although ΔNp63 has been found to exert dominant negative effects over p53 and p63 (Yang *et al.*, 1998; Westfall *et al.*, 2003) two independent reports have demonstrated that ΔNp63 isoforms can also transactivate gene expression (Dohn *et al.*, 2001; Wu *et al.*, 2003).

Future Directions

Several of the most informative advances in the area of p53 family members and apoptosis have been made very recently. These include the identification of a role for p53 at the mitochondria and in transcription-independent apoptosis, the identification of apoptosis-specific partner proteins such as the ASPP family, and the characterization of PTMs that may help direct p53 toward apoptosis rather than induction of cell cycle arrest, such as Lysine 373 acetylation, Lysine 120 acetylation, and Serine 46 phosphorylation. How these PTMs and interacting proteins cooperate or compete with each other remains to be determined.

Given the prevalence of p53 pathway mutations in human cancer, strategies for rescuing the apoptotic activity of p53 and its family members have been long sought after. Recent studies in mice have now formally proven that restoration of p53 function in tumor cells lacking p53 can restore tumor suppression (Martins *et al.*, 2006). A complete understanding of the role played by p53 in apoptosis in response to genotoxic stress is also of clinical importance because many traditional chemotherapeutic strategies rely on the induction of DNA damage and the subsequent activation of apoptosis. It is perhaps most remarkable that with over 45,000 publications related to p53, we are only now beginning to understand how this critical protein makes the decision between growth arrest and apoptosis. Among the central unresolved questions related to p53-mediated apoptosis that we envision are the following: How do different combinatorial patterns of PTMs influence p53 binding partner interactions, and how do these dictate the ability of p53 to induce apoptosis? At present, the relevance of many of these PTMs has been assessed individually, or perhaps in combination with one or a few others. Related to this, how do the p53 family members p63 and p73 contribute to apoptosis and what role do they play in human cancer? What is the link between p53-mediated apoptosis and its role in regulating autophagy and DNA repair? How are recent links between p53 and miRNA important in apoptosis?

Finally, the relative contributions of transcription-dependent and –independent pathways to apoptosis induction by p53 need to be resolved. There are now over sixty peer-reviewed manuscripts in the literature that report detection of p53 at mitochondria in apoptotic cells. Despite the breadth and depth of this literature, there remains skepticism in the field regarding this pathway. Certainly, the importance of the direct mitochondrial pathway of p53 will not be solidified until a mutant of p53 that can transactivate gene expression normally, but fails to traffic to mitochondria (or vice versa) is identified and characterized for its apoptotic function. However, this requirement exists on the backdrop of a protein for which there are few (or no) mutations that completely abrogate only one function: even the well-described 22/23 QS mutant of p53, once believed to be transcriptionally inactive, is now known to possess some transcriptional function, along with apoptotic ability (Johnson *et al.* 2005). Perhaps further analysis of the ancestral p53 family members can be a guide. For example, while it is known that *C. elegans* p53 can bind and oligomerize BAK (Pietsch *et al.*, 2008), it is not clear if *C. elegans* p53 (or even *Drosophila* p53, for that matter) can localize to mitochondria, or interact with the BCL-2 family member CED-9. Such studies may help clarify the importance of transcription-independent apoptosis by p53. The very recent identification of the transcription-independent pathway makes it clear that we as a field need to be open minded and perhaps think more broadly about potential functions for p53.

References

Abida WM, Nikolaev A, Zhao W, Zhang W, Gu W. J Biol Chem 2007;282:1797–1804. [PubMed: 17098746]

Agami R, Blandino G, Oren M, Shaul Y. Interaction of c-Abl and p73alpha and their collaboration to induce apoptosis. Nature 1999;399:809–813. [PubMed: 10391250]

Bargonetti J, Manfredi JJ. Multiple roles of the tumor suppressor p53. Curr Opin Oncol 2002;14:86–91. [PubMed: 11790986]

Barlev NA, Liu L, Chehab NH, Mansfield K, Harris KG, Halazonetis TD, Berger SL. Mol Cell 2001;8:1243–1254. [PubMed: 11779500]

Basu S, Totty NF, Irwin MS, Sudol M, Downward J. Akt phosphorylates the Yes-associated protein, YAP, to induce interaction with 14-3-3 and attenuation of p73-mediated apoptosis. Mol Cell 2003;11:11–23. [PubMed: 12535517]

Bennett M, Macdonald K, Chan SW, Luzio JP, Simari R, Weissberg P. Cell surface trafficking of Fas: a rapid mechanism of p53-mediated apoptosis. Science 1998;282:290–293. [PubMed: 9765154]

Bergamaschi D, Gasco M, Hiller L, Sullivan A, Syed N, Trigiante G, et al. p53 polymorphism influences response in cancer chemotherapy via modulation of p73-dependent apoptosis. Cancer Cell 2003a; 3:387–402. [PubMed: 12726864]

Bergamaschi D, Samuels Y, O'Neil NJ, Trigiante G, Crook T, Hsieh JK, et al. iASPP oncoprotein is a key inhibitor of p53 conserved from worm to human. Nat Genet 2003b;33:162–167. [PubMed: 12524540]

Bergamaschi D, Samuels Y, Jin B, Duraisingham S, Crook T, Lu X. ASPP1 and ASPP2: common activators of p53 family members. Mol Cell Biol 2004;24:1341–1350. [PubMed: 14729977]

Bode AM, Dong Z. Nat Rev Cancer 2004;4:793–805. [PubMed: 15510160]

Bourdon JC, Fernandes K, Murray-Zmijewski F, Liu G, Diot A, Xirodimas DP, et al. p53 isoforms can regulate p53 transcriptional activity. Genes Dev 2005;19:2122–2137. [PubMed: 16131611]

Budhram-Mahadeo VS, Bowen S, Lee S, Perez-Sanchez C, Ensor E, Morris PJ, Latchman DS. Nucleic Acids Res 2006;34:6640–6652. [PubMed: 17145718]

Caelles C, Helmberg A, Karin M. p53-dependent apoptosis in the absence of transcriptional activation of p53-target genes. Nature 1994;370:220–223. [PubMed: 8028670]

Cam H, Griesmann H, Beitzinger M, Hofmann L, Beinoraviciute-Kellner R, Sauer M, et al. p53 family members in myogenic differentiation and rhabdomyosarcoma development. Cancer Cell 2006;10:281–293. [PubMed: 17045206]

Chao C, Wu Z, Mazur SJ, Borges H, Rossi M, Lin T, Wang JY, Anderson CW, Appella E, Xu Y. Mol Cell Biol 2006;26:6859–6869. [PubMed: 16943427]

Chen X, Ko LJ, Jayaraman L, Prives C. p53 levels, functional domains, and DNA damage determine the extent of the apoptotic response of tumor cells. Genes Dev 1996;10:2438–2451. [PubMed: 8843196]

Chen X, Zheng Y, Zhu J, Jiang J, Wang J. p73 is transcriptionally regulated by DNA damage, p53, and p73. Oncogene 2001;20:769–774. [PubMed: 11314010]

Chipuk JE, Kuwana T, Bouchier-Hayes L, Droin NM, Newmeyer DD, Schuler M, et al. Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. Science 2004;303:1010–1014. [PubMed: 14963330]

Chipuk JE, Bouchier-Hayes L, Kuwana T, Newmeyer DD, Green DR. PUMA couples the nuclear and cytoplasmic proapoptotic function of p53. Science 2005;309:1732–1735. [PubMed: 16151013]

Concin N, Becker K, Slade N, Erster S, Mueller-Holzner E, Ulmer H, et al. Transdominant DeltaTAp73 isoforms are frequently up-regulated in ovarian cancer. Evidence for their role as epigenetic p53 inhibitors in vivo. Cancer Res 2004;64:2449–2460. [PubMed: 15059898]

Corn PG, Kuerbitz SJ, van Noesel MM, Esteller M, Compitello N, Baylin SB, et al. Transcriptional silencing of the p73 gene in acute lymphoblastic leukemia and Burkitt's lymphoma is associated with 5' CpG island methylation. Cancer Res 1999;59:3352–3356. [PubMed: 10416592]

Cory S, Adams JM. The Bcl2 family: regulators of the cellular life-or-death switch. Nat Rev Cancer 2002;2:647–656. [PubMed: 12209154]

Costanzo A, Merlo P, Pediconi N, Fulco M, Sartorelli V, Cole PA, et al. DNA damage-dependent acetylation of p73 dictates the selective activation of apoptotic target genes. Mol Cell 2002;9:175–186. [PubMed: 11804596]

Das S, Raj L, Zhao B, Kimura Y, Bernstein A, Aaronson SA, Lee SW. Cell 2007;130:624–637. [PubMed: 17719541]

Deissler H, Kafka A, Schuster E, Sauer G, Kreienberg R, Zeillinger R. Oncol Rep 2004;11:1281–1286. [PubMed: 15138567]

DeYoung MP, Johannessen CM, Leong CO, Faquin W, Rocco JW, Ellisen LW. Tumor-specific p73 up-regulation mediates p63 dependence in squamous cell carcinoma. Cancer Res 2006;66:9362–9368. [PubMed: 17018588]

Deyoung MP, Ellisen LW. p63 and p73 in human cancer: defining the network. Oncogene 2007;26:5169–5183. [PubMed: 17334395]

Di Como CJ, Gaiddon C, Prives C. p73 function is inhibited by tumor-derived p53 mutants in mammalian cells. Mol Cell Biol 1999;19:1438–1449. [PubMed: 9891077]

Dietz S, Rother K, Bamberger C, Schmale H, Mössner J, Engeland K. Differential regulation of transcription and induction of programmed cell death by human p53-family members p63 and p73. FEBS Lett 2002;525:93–99. [PubMed: 12163168]

Ding HF, McGill G, Rowan S, Schmaltz C, Shimamura A, Fisher DE. Oncogene-dependent regulation of caspase activation by p53 protein in a cell-free system. J Biol Chem 1998;273:28378–28383. [PubMed: 9774464]

Ding HF, Lin YL, McGill G, Juo P, Zhu H, Blenis J, et al. Essential role for caspase-8 in transcription-independent apoptosis triggered by p53. J Biol Chem 2000;275:38905–38911. [PubMed: 10988287]

Di Stefano V, Soddu S, Sacchi A, D'Orazi G. Oncogene 2005;24:5431–5442. [PubMed: 15897882]

Dohn M, Zhang S, Chen X. p63alpha and DeltaNp63alpha can induce cell cycle arrest and apoptosis and differentially regulate p53 target genes. Oncogene 2001;20:3193–3205. [PubMed: 11423969]

Domínguez G, García JM, Peña C, Silva J, García V, Martínez L, et al. DeltaTAp73 upregulation correlates with poor prognosis in human tumors: putative in vivo network involving p73 isoforms, p53, and E2F-1. J Clin Oncol 2006;24:805–815. [PubMed: 16380414]

Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery CA Jr, Butel JS, et al. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature 1992;356:215–221. [PubMed: 1552940]

Dumont P, Leu JI, Della Pietra AC 3rd, George DL, Murphy M. The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. Nat Genet 2003;33:357–365. [PubMed: 12567188]

el-Deiry WS, Kern SE, Pietenpol JA, Kinzler KW, Vogelstein B. Definition of a consensus binding site for p53. Nat Genet 1992;1:45–49. [PubMed: 1301998]

Erster S, Mihara M, Kim RH, Petrenko O, Moll UM. In vivo mitochondrial p53 translocation triggers a rapid first wave of cell death in response to DNA damage that can precede p53 target gene activation. Mol Cell Biol 2004;24:6728–6741. [PubMed: 15254240]

Feng L, Hollstein M, Xu Y. Cell Cycle 2006;5:2812–2819. [PubMed: 17172844]

Flores ER, Tsai KY, Crowley D, Sengupta S, Yang A, McKeon F, et al. p63 and p73 are required for p53-dependent apoptosis in response to DNA damage. Nature 2002;416:560–564. [PubMed: 11932750]

Flores ER, Sengupta S, Miller JB, Newman JJ, Bronson R, Crowley D, et al. Tumor predisposition in mice mutant for p63 and p73: evidence for broader tumor suppressor functions for the p53 family. Cancer Cell 2005;7:363–373. [PubMed: 15837625]

Fridman JS, Lowe SW. Control of apoptosis by p53. Oncogene 2003;22:9030–9040. [PubMed: 14663481]

Gaiddon C, Lokshin M, Ahn J, Zhang T, Prives C. A subset of tumor-derived mutant forms of p53 down-regulate p63 and p73 through a direct interaction with the p53 core domain. Mol Cell Biol 2001;21:1874–1887. [PubMed: 11238924]

Gan L, Xiang M, Zhou L, Wagner DS, Klein WH, Nathans J. Proc Natl Acad Sci U S A 1996;93:3920–3925. [PubMed: 8632990]

Gao C, Tsuchida N. Activation of caspases in p53-induced transactivation-independent apoptosis. Jpn J Cancer Res 1999;90:180–187. [PubMed: 10189888]

Gong JG, Costanzo A, Yang HQ, Melino G, Kaelin WG Jr, Levrero M, et al. The tyrosine kinase c-Abl regulates p73 in apoptotic response to cisplatin-induced DNA damage. Nature 1999;399:806–809. [PubMed: 10391249]

Gressner O, Schilling T, Lorenz K, Schulze Schleithoff E, Koch A, Schulze-Bergkamen H, et al. TAp63alpha induces apoptosis by activating signaling via death receptors and mitochondria. EMBO J 2005;24:2458–2471. [PubMed: 15944736]

Grob TJ, Novak U, Maisse C, Barcaroli D, Lüthi AU, Pirnia F, et al. Human delta Np73 regulates a dominant negative feedback loop for TAp73 and p53. Cell Death Differ 2001;8:1213–1223. [PubMed: 11753569]

Gu W, Roeder RG. Cell 1997;90:595–606. [PubMed: 9288740]

Han MK, Song EK, Guo Y, Ou X, Mantel C, Broxmeyer HE. SIRT1 regulates apoptosis and Nanog expression in mouse embryonic stem cells by controlling p53 subcellular localization. Cell Stem Cell 2008;2:241–251. [PubMed: 18371449]

Hashimoto T, Tokuchi Y, Hayashi M, Kobayashi Y, Nishida K, Hayashi S, Ishikawa Y, Tsuchiya S, Nakagawa K, Hayashi J, Tsuchiya E. Cancer Res 1999;59:5572–5577. [PubMed: 10554037]

Haupt Y, Rowan S, Shaulian E, Vousden KH, Oren M. Induction of apoptosis in HeLa cells by trans-activation-deficient p53. Genes Dev 1995;9:2170–2183. [PubMed: 7657168]

Haupt Y, Rowan S, Shaulian E, Kazaz A, Vousden K, Oren M. p53 mediated apoptosis in HeLa cells: transcription dependent and independent mechanisms. Leukemia 1997;11:337–339. [PubMed: 9209383]

Haupt Y, Maya R, Kazaz A, Oren M. Nature 1997;387:296–299. [PubMed: 9153395]

Hayes VM, Dirven CM, Dam A, Verlind E, Molenaar WM, Mooij JJ, Hofstra RM, Buys CH. Brain Pathol 1999;9:463–467. [PubMed: 10416986]

Helton ES, Zhang J, Chen X. The proline-rich domain in p63 is necessary for the transcriptional and apoptosis-inducing activities of TAp63. Oncogene 2008;27:2843–2850. [PubMed: 18037962]

Hudson CD, Morris PJ, Latchman DS, Budhram-Mahadeo VS. J Biol Chem 2005;280:11851–11858. [PubMed: 15598651]

Ihrie RA, Marques MR, Nguyen BT, Horner JS, Papazoglu C, Bronson RT, et al. Perp is a p63-regulated gene essential for epithelial integrity. Cell 2005;120:843–856. [PubMed: 15797384]

Irwin M, Marin MC, Phillips AC, Seelan RS, Smith DI, Liu W, et al. Role for the p53 homologue p73 in E2F-1-induced apoptosis. Nature 2000;407:645–648. [PubMed: 11034215]

Irwin MS, Kaelin WG. p53 family update: p73 and p63 develop their own identities. Cell Growth Differ 2001;12:337–349. [PubMed: 11457731]

Irwin MS, Kondo K, Marin MC, Cheng LS, Hahn WC, Kaelin WG Jr. Chemosensitivity linked to p73 function. Cancer Cell 2003;3:403–410. [PubMed: 12726865]

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;4:321–328. [PubMed: 14585359]

Johnson TM, Hammond EM, Giaccia A, Attardi LD. The p53QS transactivation-deficient mutant shows stress-specific apoptotic activity and induces embryonic lethality. Nat Genet 2005;37:145–152. [PubMed: 15654339]

Johnson TM, Meade K, Pathak N, Marques MR, Attardi LD. Knockin mice expressing a chimeric p53 protein reveal mechanistic differences in how p53 triggers apoptosis and senescence. Proc Natl Acad Sci U S A 2008;105:1215–1220. [PubMed: 18216268]

Kaelin WG Jr. The p53 gene family. Oncogene 1999;18:7701–7705. [PubMed: 10618710]

Kaghad M, Bonnet H, Yang A, Creancier L, Biscan JC, Valent A, et al. Monoallelically expressed gene related to p53 at 1p36, a region frequently deleted in neuroblastoma and other human cancers. Cell 1997;90:809–819. [PubMed: 9288759]

Kartasheva NN, Contente A, Lenz-Stöppler C, Roth J, Dobbelstein M. p53 induces the expression of its antagonist p73 Delta N, establishing an autoregulatory feedback loop. Oncogene 2002;21:4715–4727. [PubMed: 12101410]

Katoh I, Aisaki KI, Kurata SI, Ikawa S, Ikawa Y. p51A (TAp63gamma), a p53 homolog, accumulates in response to DNA damage for cell regulation. Oncogene 2000;19:3126–3130. [PubMed: 10871867]

Kawano S, Miller CW, Gombart AF, Bartram CR, Matsuo Y, Asou H, et al. Loss of p73 gene expression in leukemias/lymphomas due to hypermethylation. Blood 1999;94:1113–1120. [PubMed: 10419905]

Keyes WM, Vogel H, Koster MI, Guo X, Qi Y, Petherbridge KM, et al. p63 heterozygous mutant mice are not prone to spontaneous or chemically induced tumors. Proc Natl Acad Sci U S A 2006;103:8435–8440. [PubMed: 16714381]

Keyes WM, Wu Y, Vogel H, Guo X, Lowe SW, Mills AA. p63 deficiency activates a program of cellular senescence and leads to accelerated aging. Genes Dev 2008;19:1986–1999. [PubMed: 16107615]

Knights CD, Catania J, Di Giovanni S, Muratoglu S, Perez R, Swartzbeck A, et al. Distinct p53 acetylation cassettes differentially influence gene-expression patterns and cell fate. J Cell Biol 2006;173:533–544. [PubMed: 16717128]

Koga F, Kawakami S, Fujii Y, Saito K, Ohtsuka Y, Iwai A, et al. Impaired p63 expression associates with poor prognosis and uroplakin III expression in invasive urothelial carcinoma of the bladder. Clin Cancer Res 2003;9:5501–5507. [PubMed: 14654529]

Kubbutat MH, Jones SN, Vousden KH. Nature 1997;387:299–303. [PubMed: 9153396]

Kuwana T, Bouchier-Hayes L, Chipuk JE, Bonzon C, Sullivan BA, Green DR, et al. BH3 domains of BH3-only proteins differentially regulate Bax-mediated mitochondrial membrane permeabilization both directly and indirectly. Mol Cell 2005;17:525–535. [PubMed: 15721256]

Lang GA, Iwakuma T, Suh YA, Liu G, Rao VA, Parant JM, et al. Gain of function of a p53 hot spot mutation in a mouse model of Li-Fraumeni syndrome. Cell 2004;119:861–872. [PubMed: 15607981]

Le Cam L, Linares LK, Paul C, Julien E, Lacroix M, Hatchi E, Triboulet R, Bossis G, Shmueli A, Rodriguez MS, Coux O, Sardet C. Cell 2006;127:775–788. [PubMed: 17110336]

Leitao MM, Soslow RA, Baergen RN, Olvera N, Arroyo C, Boyd J. Gynecol Oncol 2004;93:301–306. [PubMed: 15099937]

Letai A, Bassik MC, Walensky LD, Sorcinelli MD, Weiler S, Korsmeyer SJ. Distinct BH3 domains either sensitize or activate mitochondrial apoptosis, serving as prototype cancer therapeutics. Cancer Cell 2002;2:183–192. [PubMed: 12242151]

Leu JI, Dumont P, Hafey M, Murphy ME, George DL. Mitochondrial p53 activates Bak and causes disruption of a Bak-Mcl1 complex. Nat Cell Biol 2004;6:443–450. [PubMed: 15077116]

Levine AJ. p53, the cellular gatekeeper for growth and division. Cell 1997;88:323–331. [PubMed: 9039259]

Levy D, Adamovich Y, Reuven N, Shaul Y. The Yes-associated protein 1 stabilizes p73 by preventing Itch-mediated ubiquitination of p73. Cell Death Differ 2007;14:743–751. [PubMed: 17110958]

Levy D, Adamovich Y, Reuven N, Shaul Y. Yap1 phosphorylation by c-Abl is a critical step in selective activation of proapoptotic genes in response to DNA damage. Mol Cell 2008;29:350–361. [PubMed: 18280240]

Li M, Brooks CL, Wu-Baer F, Chen D, Baer R, Gu W. Science 2003;302:1972–1975. [PubMed: 14671306]

Liu L, Scolnick DM, Trievel RC, Zhang HB, Marmorstein R, Halazonetis TD, Berger SL. Mol Cell Biol 1999;19:1202–1209. [PubMed: 9891054]

Luo JL, Yang Q, Tong WM, Hergenhahn M, Wang ZQ, Hollstein M. Oncogene 2001;20:320–328. [PubMed: 11313961]

Malkin D, Jolly KW, Barbier N, Look AT, Friend SH, Gebhardt MC, et al. Germline mutations of the p53 tumor-suppressor gene in children and young adults with second malignant neoplasms. N Engl J Med 1992;326:1309–1315. [PubMed: 1565144]

Mantovani F, Piazza S, Gostissa M, Strano S, Zacchi P, Mantovani R, et al. Pin1 links the activities of c-Abl and p300 in regulating p73 function. Mol Cell 2004;14:625–636. [PubMed: 15175157]

Mantovani F, Tocco F, Girardini J, Smith P, Gasco M, Lu X, Crook T, Sal GD. Nat Struct Mol Biol 2007;14:912–920. [PubMed: 17906639]

Marchenko ND, Zaika A, Moll UM. Death signal-induced localization of p53 protein to mitochondria. A potential role in apoptotic signaling. J Biol Chem 2000;275:16202–16212. [PubMed: 10821866]

Marchenko ND, Wolff S, Erster S, Becker K, Moll UM. Monoubiquitylation promotes mitochondrial p53 translocation. EMBO J 2007;26:923–934. [PubMed: 17268548]

Marin MC, Jost CA, Brooks LA, Irwin MS, O'Nions J, Tidy JA, et al. A common polymorphism acts as an intragenic modifier of mutant p53 behaviour. Nat Genet 2000;25:47–54. [PubMed: 10802655]

Martins CP, Brown-Swigart L, Evan GI. Modeling the therapeutic efficacy of p53 restoration in tumors. Cell 2006;127:1323–1334. [PubMed: 17182091]

Melino G, De Laurenzi V, Vousden KH. p73: Friend or foe in tumorigenesis. Nat Rev Cancer 2002;2:605–615. [PubMed: 12154353]

Melino G, Lu X, Gasco M, Crook T, Knight RA. Functional regulation of p73 and p63: development and cancer. Trends Biochem Sci 2003;28:663–670. [PubMed: 14659698]

Melino G, Bernassola F, Ranalli M, Yee K, Zong WX, Corazzari M, et al. p73 Induces apoptosis via PUMA transactivation and Bax mitochondrial translocation. J Biol Chem 2004;279:8076–8083. [PubMed: 14634023]

Meyers FJ, Chi SG, Fishman JR, deVere White RW, Gumerlock PH. J Natl Cancer Inst 1993;85:1856–1858. [PubMed: 7693956]

Michalak EM, Villunger A, Adams JM, Strasser A. In several cell types tumour suppressor p53 induces apoptosis largely via Puma but Noxa can contribute. Cell Death Differ 2008;15:1019–1029. [PubMed: 18259198]

Mihara M, Erster S, Zaika A, Petrenko O, Chittenden T, Pancoska P, Moll UM. p53 has a direct apoptogenic role at the mitochondria. Mol Cell 2003;11:577–590. [PubMed: 12667443]

Mills AA, Zheng B, Wang XJ, Vogel H, Roop DR, Bradley A. p63 is a p53 homologue required for limb and epidermal morphogenesis. Nature 1999;398:708–713. [PubMed: 10227293]

Müller M, Schilling T, Sayan AE, Kairat A, Lorenz K, Schulze-Bergkamen H, et al. TAp73/Delta Np73 influences apoptotic response, chemosensitivity and prognosis in hepatocellular carcinoma. Cell Death Differ 2005;12:1564–1577. [PubMed: 16195739]

Müller M, Schleithoff ES, Stremmel W, Melino G, Krammer PH, Schilling T. One, two, three--p53, p63, p73 and chemosensitivity. Drug Resist Updat 2006;9:288–306. [PubMed: 17287142]

Murray-Zmijewski F, Lane DP, Bourdon JC. p53/p63/p73 isoforms: an orchestra of isoforms to harmonise cell differentiation and response to stress. Cell Death Differ 2006;13:962–972. [PubMed: 16601753]

Nemajerova A, Wolff S, Petrenko O, Moll UM. Viral and cellular oncogenes induce rapid mitochondrial translocation of p53 in primary epithelial and endothelial cells early in apoptosis. FEBS Lett 2005;579:6079–6083. [PubMed: 16226255]

Oda K, Arakawa H, Tanaka T, Matsuda K, Tanikawa C, Mori T, Nishimori H, Tamai K, Tokino T, Nakamura Y, Taya Y. Cell 2000;102:849–862. [PubMed: 11030628]

Okada Y, Osada M, Kurata S, Sato S, Aisaki K, Kageyama Y, et al. p53 gene family p51(p63)-encoded, secondary transactivator p51B(TAp63alpha) occurs without forming an immunoprecipitable complex with MDM2, but responds to genotoxic stress by accumulation. Exp Cell Res 2002;276:194–200. [PubMed: 12027449]

Olivier M, Eeles R, Hollstein M, Khan MA, Harris CC, Hainaut P. The IARC TP53 database: new online mutation analysis and recommendations to users. Hum Mutat 2002;19:607–614. [PubMed: 12007217]

Olsson A, Manzl C, Strasser A, Villunger A. Cell Death Differ 2007;14:1561–1575. [PubMed: 17627286]

Osada M, Ohba M, Kawahara C, Ishioka C, Kanamaru R, Katoh I, et al. Cloning and functional analysis of human p51, which structurally and functionally resembles p53. Nat Med 1998;4:839–843. [PubMed: 9662378]

Palacios G, Moll UM. Mitochondrially targeted wild-type p53 suppresses growth of mutant p53 lymphomas in vivo. Oncogene 2006;25:6133–6139. [PubMed: 16682948]

Park BJ, Lee SJ, Kim JI, Lee SJ, Lee CH, Chang SG, et al. Frequent alteration of p63 expression in human primary bladder carcinomas. Cancer Res 2000;60:3370–3374. [PubMed: 10910040]

Pediconi N, Ianari A, Costanzo A, Belloni L, Gallo R, Cimino L, et al. Differential regulation of E2F1 apoptotic target genes in response to DNA damage. Nat Cell Biol 2003;5:552–558. [PubMed: 12766778]

Petitjean A, Ruptier C, Tribollet V, Hautefeuille A, Chardon F, Cavard C, et al. Properties of the six isoforms of p63: p53-like regulation in response to genotoxic stress and cross talk with DeltaNp73. Carcinogenesis 2008;29:273–281. [PubMed: 18048390]

Petros AM, Gunasekera A, Xu N, Olejniczak ET, Fesik SW. Defining the p53 DNA-binding domain/Bcl-x(L)-binding interface using NMR. FEBS Lett 2004;559:171–174. [PubMed: 14960327]

Pietsch EC, Perchiniak E, Canutescu AA, Wang G, Dunbrack RL, Murphy ME. Oligomerization of bak by p53 utilizes conserved residues of the p53 DNA binding domain. J Biol Chem. 2008in press

Riley T, Sontag E, Chen P, Levine A. Transcriptional control of human p53-regulated genes. Nat Rev Mol Cell Biol 2008;9:402–412. [PubMed: 18431400]

Rinaldo C, Prodosmo A, Mancini F, Iacovelli S, Sacchi A, Moretti F, Soddu S. Mol Cell 2007;25:739–750. [PubMed: 17349959]

Rocco JW, Leong CO, Kuperwasser N, DeYoung MP, Ellisen LW. p63 mediates survival in squamous cell carcinoma by suppression of p73-dependent apoptosis. Cancer Cell 2006;9:45–56. [PubMed: 16413471]

Sakaguchi K, Herrera JE, Saito S, Miki T, Bustin M, Vassilev A, Anderson CW, Appella E. Genes Dev 1998;12:2831–2841. [PubMed: 9744860]

Samuels-Lev Y, O'Connor DJ, Bergamaschi D, Trigiante G, Hsieh JK, Zhong S, Campargue I, Naumovski L, Crook T, Lu X. Mol Cell 2001;8:781–794. [PubMed: 11684014]

Sanchez-Prieto R, Sanchez-Arevalo VJ, Servitja JM, Gutkind JS. Regulation of p73 by c-Abl through the p38 MAP kinase pathway. Oncogene 2002;21:974–979. [PubMed: 11840343]

Sansome C, Zaika A, Marchenko ND, Moll UM. Hypoxia death stimulus induces translocation of p53 protein to mitochondria. Detection by immunofluorescence on whole cells. FEBS Lett 2001;488:110–115. [PubMed: 11163756]

Sayan BS, Sayan AE, Yang AL, Aqeilan RI, Candi E, Cohen GM, et al. Cleavage of the transactivation-inhibitory domain of p63 by caspases enhances apoptosis. Proc Natl Acad Sci 2007;104:10871–10876. [PubMed: 17581882]

Sayan AE, Sayan BS, Gogvadze V, Dinsdale D, Nyman U, Hansen TM, et al. p73 and caspase-cleaved p73 fragments localize to mitochondria and augment TRAIL-induced apoptosis. Oncogene. 2008in press

Schmale H, Bamberger C. A novel protein with strong homology to the tumor suppressor p53. Oncogene 1997;15:1363–1367. [PubMed: 9315105]

Schuler M, Bossy-Wetzel E, Goldstein JC, Fitzgerald P, Green DR. p53 induces apoptosis by caspase activation through mitochondrial cytochrome c release. J Biol Chem 2000;275:7337–7342. [PubMed: 10702305]

Senoo M, Manis JP, Alt FW, McKeon F. p63 and p73 are not required for the development and p53-dependent apoptosis of T cells. Cancer Cell 2004;6:85–89. [PubMed: 15261144]

Shaw PE. EMBO Rep 2007;8:40–45. [PubMed: 17203101]

Sniezek JC, Matheny KE, Westfall MD, Pietenpol JA. Dominant negative p63 isoform expression in head and neck squamous cell carcinoma. Laryngoscope 2004;114:2063–2072. [PubMed: 15564824]

Sot B, Freund SM, Fersht AR. Comparative biophysical characterization of p53 with the pro-apoptotic BAK and the anti-apoptotic BCL-xl. J Biol Chem 2007;282:29193–29200. [PubMed: 17699158]

Stiewe T, Theseling CC, Pützer BM. Transactivation-deficient Delta TA-p73 inhibits p53 by direct competition for DNA binding: implications for tumorigenesis. J Biol Chem 2002;277:14177–14185. [PubMed: 11844800]

Strano S, Munarriz E, Rossi M, Castagnoli L, Shaul Y, Sacchi A, et al. Physical interaction with Yes-associated protein enhances p73 transcriptional activity. J Biol Chem 2001;276:15164–15173. [PubMed: 11278685]

Strano S, Fontemaggi G, Costanzo A, Rizzo MG, Monti O, Baccarini A, et al. Physical interaction with human tumor-derived p53 mutants inhibits p63 activities. J Biol Chem 2002;277:18817–18826. [PubMed: 11893750]

Strano S, Monti O, Pediconi N, Baccarini A, Fontemaggi G, Lapi E, et al. The transcriptional coactivator Yes-associated protein drives p73 gene-target specificity in response to DNA Damage. Mol Cell 2005;18:447–459. [PubMed: 15893728]

Strom E, Sathe S, Komarov PG, Chernova OB, Pavlovska I, Shyshynova I, et al. Small-molecule inhibitor of p53 binding to mitochondria protects mice from gamma radiation. Nat Chem Biol 2006;2:474–479. [PubMed: 16862141]

Sykes SM, Mellert HS, Holbert MA, Li K, Marmorstein R, Lane WS, et al. Acetylation of the p53 DNA-binding domain regulates apoptosis induction. Mol Cell 2006;24:841–851. [PubMed: 17189187]

Taira N, Nihira K, Yamaguchi T, Miki Y, Yoshida K. Mol Cell 2007;25:725–738. [PubMed: 17349958]

Talos F, Petrenko O, Mena P, Moll UM. Mitochondrially targeted p53 has tumor suppressor activities in vivo. Cancer Res 2005;65:9971–9981. [PubMed: 16267022]

Tang Y, Luo J, Zhang W, Gu W. Tip60-dependent acetylation of p53 modulates the decision between cell-cycle arrest and apoptosis. Mol Cell 2006;24:827–839. [PubMed: 17189186]

Toledo F, Wahl GM. Nat Rev Cancer 2006;6:909–923. [PubMed: 17128209]

Trink B, Okami K, Wu L, Sriuranpong V, Jen J, Sidransky D. A new human p53 homologue. Nat Med 1998;4:747–748. [PubMed: 9662346]

Tomita Y, Marchenko N, Erster S, Nemajerova A, Dehner A, Klein C, et al. WT p53, but not tumor-derived mutants, bind to Bcl2 via the DNA binding domain and induce mitochondrial permeabilization. J Biol Chem 2006;281:8600–8606. [PubMed: 16443602]

Urist MJ, Di Como CJ, Lu ML, Charytonowicz E, Verbel D, Crum CP, et al. Loss of p63 expression is associated with tumor progression in bladder cancer. Am J Pathol 2002;161:1199–1206. [PubMed: 12368193]

Villunger A, Michalak EM, Coultas L, Müllauer F, Böck G, Ausserlechner MJ, et al. p53- and drug-induced apoptotic responses mediated by BH3-only proteins puma and noxa. Science 2003;302:1036–1038. [PubMed: 14500851]

Vousden KH, Lu X. Live or let die: the cell's response to p53. Nat Rev Cancer 2002;2:594–604. [PubMed: 12154352]

Wager M, Guilhot J, Blanc JL, Ferrand S, Milin S, Bataille B, et al. Prognostic value of increase in transcript levels of Tp73 DeltaEx2-3 isoforms in low-grade glioma patients. Br J Cancer 2006;95:1062–1069. [PubMed: 17047653]

Wagner AJ, Kokontis JM, Hay N. Myc-mediated apoptosis requires wild-type p53 in a manner independent of cell cycle arrest and the ability of p53 to induce p21waf1/cip1. Genes Dev 1994;8:2817–2830. [PubMed: 7995520]

Wang J, Liu YX, Hande MP, Wong AC, Jin YJ, Yin Y. TAp73 is a downstream target of p53 in controlling the cellular defense against stress. J Biol Chem 2007;282:29152–29162. [PubMed: 17693405]

Weber A, Bellmann U, Bootz F, Wittekind C, Tannapfel A. Expression of p53 and its homologues in primary and recurrent squamous cell carcinomas of the head and neck. Int J Cancer 2002;99:22–28. [PubMed: 11948487]

Wei MC, Zong WX, Cheng EH, Lindsten T, Panoutsakopoulou V, Ross AJ, et al. Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. Science 2001;292:727–730. [PubMed: 11326099]

Wei X, Xu H, Kufe D. Cancer Cell 2005;7:167–178. [PubMed: 15710329]

Welchman RL, Gordon C, Mayer RJ. Nat Rev Mol Cell Biol 2005;6:599–609. [PubMed: 16064136]

Westfall MD, Mays DJ, Sniezek JC, Pietenpol JA. The Delta Np63 alpha phosphoprotein binds the p21 and 14-3-3 sigma promoters in vivo and has transcriptional repressor activity that is reduced by Hay-Wells syndrome-derived mutations. Mol Cell Biol 2003;23:2264–2276. [PubMed: 12640112]

Wu G, Nomoto S, Hoque MO, Dracheva T, Osada M, Lee CC, et al. DeltaNp63alpha and TAp63alpha regulate transcription of genes with distinct biological functions in cancer and development. Cancer Res 2003;63:2351–2357. [PubMed: 12750249]

Yan Y, Shay JW, Wright WE, Mumby MC. Inhibition of protein phosphatase activity induces p53-dependent apoptosis in the absence of p53 transactivation. J Biol Chem 1997;272:15220–15226. [PubMed: 9182545]

Yang A, Kaghad M, Wang Y, Gillett E, Fleming MD, Dötsch V, et al. p63, a p53 homolog at 3q27–29, encodes multiple products with transactivating, death-inducing, and dominant-negative activities. Mol Cell 1998;2:305–316. [PubMed: 9774969]

Yang A, Walker N, Bronson R, Kaghad M, Oosterwegel M, Bonnin J, et al. p73-deficient mice have neurological, pheromonal and inflammatory defects but lack spontaneous tumours. Nature 2000;404:99–103. [PubMed: 10716451]

Yuan ZM, Shioya H, Ishiko T, Sun X, Gu J, Huang YY, et al. p73 is regulated by tyrosine kinase c-Abl in the apoptotic response to DNA damage. Nature 1999;399:814–817. [PubMed: 10391251]

Xiang M, Gan L, Zhou L, Klein WH, Nathans J. Proc Natl Acad Sci U S A 1996;93:11950–11955. [PubMed: 8876243]

Zaika A, Irwin M, Sansome C, Moll UM. Oncogenes induce and activate endogenous p73 protein. J Biol Chem 2001;276:11310–11316. [PubMed: 11115495]

Zeng X, Li X, Miller A, Yuan Z, Yuan W, Kwok RP, et al. The N-terminal domain of p73 interacts with the CH1 domain of p300/CREB binding protein and mediates transcriptional activation and apoptosis. Mol Cell Biol 2000;201299-12310

Zeng X, Zhu Y, Lu H. NBP is the p53 homolog p63. Carcinogenesis 2001;22:215–219. [PubMed: 11181441]

Zheng H, You H, Zhou XZ, Murray SA, Uchida T, Wulf G, Gu L, Tang X, Lu KP, Xiao ZX. Nature 2002;419:849–853. [PubMed: 12397361]

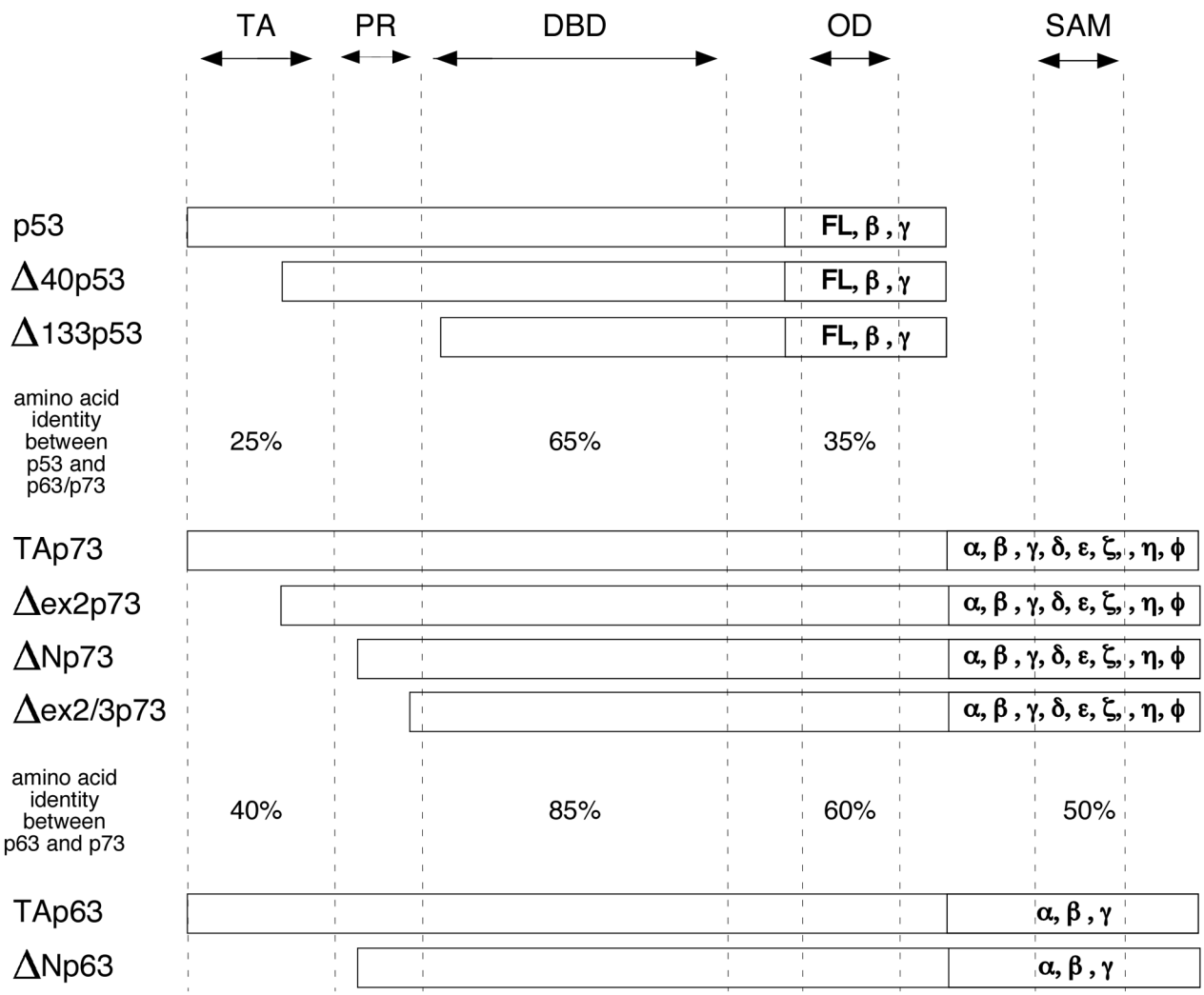

Figure 1. p53 family member isoforms

Schematic presentation of p53, p63, and p73 isoforms. Approximate location of the transactivation (TA) domain, proline rich domain (PR), DNA binding domain (DBD), oligomerization domain (OD) and the sterile alpha motif (SAM) are indicated. The amino acid identity between the TA, DBD, and OD of p53 and p63/p73 as well as between p63 and p73 is denoted. Full length (FL) p53 or TAp63 and TAp73 protein are transcribed from a promoter located in the non-coding region of exon 1 (P1 promoter) of the p53, p63, and p73 gene. Δ133p53, ΔNp63, and ΔNp73 isoforms are generated by transcription from a promoter (P2 promoter) located in intron 3 of the p63 and p73 gene or intron 4 of the p53 gene. Further ΔN variants are generated by alternative splicing of the N-terminus (Δ40p53, Δex2p73, Δex2/3p73). Alternative splicing of the C-terminal region yields additional variants for p53 (FL, β, & γ), p63 (α, β, & γ), and p73(α, β, γ, δ, ε, ζ, η, & θ). p53 β and γ lack the oligomerization domain.

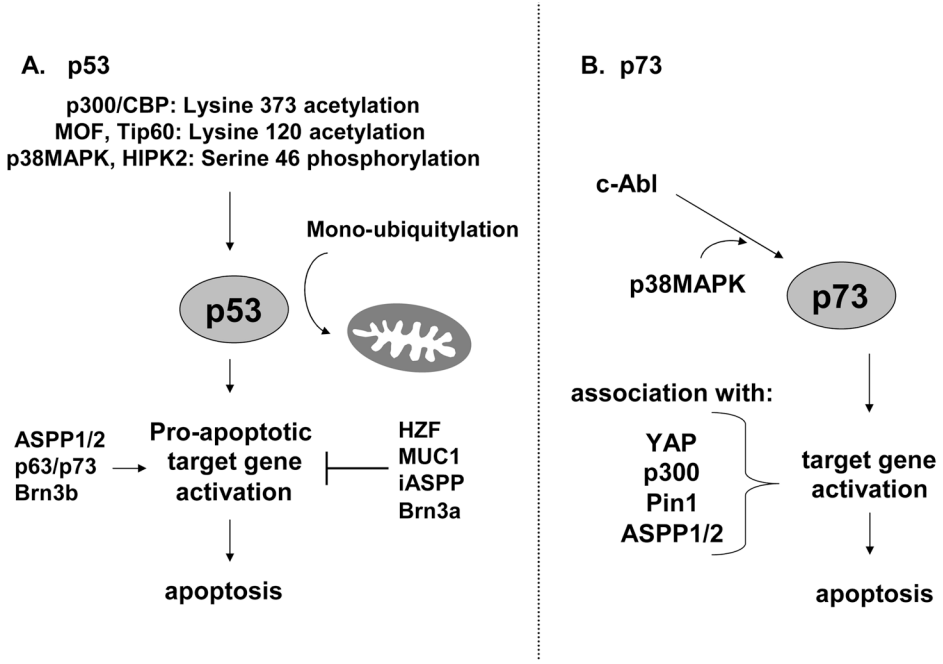

Figure 2. Upstream mediators of p53 and p73-dependent cell death