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# Therapeutic prospects for p73 and p63: Rising from the shadow of p53

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## Abstract

The p53 protein family consists of three transcription factors: p53, p63, and p73. These proteins share significant structural and functional similarities and each has unique biological functions as well. Although the role of p53 in cellular stress is extensively studied, many questions remain about p63 and p73. In this review we summarize current data on functional interactions within the p53 family, their regulation and roles in response to genotoxic stress. We also discuss significance of p73 and p63 for cancer therapy and outline novel approaches in development of therapeutic drugs that specifically target the p53 family.

### Keywords

apoptosis; E2F1; ZEB1; RETRA; iASPP; YAP1; drug resistance

## 1. Introduction

For almost three decades, p53 has been receiving significant attention from the scientific community. This interest originates from its dominant role in tumor suppression paraphrased as "the guardian of the genome" (Lane, 1992). p53 is also known to be the most mutated gene in human cancer. In fact, inactivation of p53 is strongly associated with an increased susceptibility to tumors in laboratory animals and humans. At the cellular level, p53 plays a role in the regulation of multiple intracellular processes including apoptosis, cell cycle progression, cellular senescence, autophagy, differentiation, and repair from genotoxic damage. Perhaps the best studied of these functions is the involvement of p53 in apoptosis. Many molecular events upstream and downstream of p53 have been intensively investigated, and it is evident that p53 integrates stress signals and transmits them through a large number of effectors including ones involved in apoptosis and cell cycle regulation (Maddika et al., 2007; Yu, 2006). Anti-cancer treatment takes advantage of the p53 role in the regulation of cell fate, an inherent biological function that plays an important role in maintaining the integrity

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of multicellular organisms. From an evolutionary perspective p53 activity has to be tightly regulated and backed up by other protein(s) if one fails for any reason.

p53 is the founding member of the protein family, which also includes p63 and p73. These proteins share considerable structural and functional similarity with p53 and exhibit unique properties as well. Both p63 and p73 can significantly affect p53 activity.

In this review, we outline current data on the specific roles of p73 and p63 in the modulation of therapeutic response in human tumors. We also discuss how functional interactions within the p53 family affect anti-cancer therapy.

## 2. p73 and p63: structural and functional aspects

p73 and p63 are both homologs of p53, the genes for which *TP73* and *TP63* are localized at the 1p36.32 and 3q28 chromosomal regions, respectively. According to the current paradigm, the p73/p63 subfamily arose from an ancestral gene during the early evolution of Metazoa (Melino et al., 2002; Ou et al., 2007). Extensive homology exists in the transactivation, DNA-binding, and oligomerization domains of p73/p63 and p53 (Figure 1). The highest similarity is found in the DNA-binding domain, in which p63 and p73 share approximately 60% aminoacid identity with p53 (Melino et al., 2003). Evolutionally, this domain is the most conserved, suggesting that regulation of transcription plays a pivotal role in an array of functions attributed to the p53 family. Less similarity is found in the oligomerization domain (~30%) perhaps explaining why p63 and p73 cannot hetero-oligomerize with wild-type p53. In contrast, the oligomerization domains of p63 and p73 share greater homology (~70%) and are able to form homo- as well as heterotetramers. However, hetero-oligomerization between p63 and p73 appears to be less efficient than homo-oligomerization of either protein (Davison et al., 1999).

The *TP73* and *TP63* genes encode an additional region at the COOH-terminus that is not found in p53. It combines sterile alpha motif (SAM) and transcription inhibitory domains (TID). The COOH-terminus of p73/p63 has been implicated in lipid-membrane binding and repression of transcription (Barrera et al., 2003; Ghioni et al., 2002; Sauer et al., 2008). These functions appear to be biologically important, as mutations in the p63 SAM and TID domains are associated with several inherited human syndromes (Rinne et al., 2007). The mechanism through which the C-terminus of p63 represses transcription is not completely understood; however, it is proposed that the intramolecular interaction of the C-terminus with the transactivation domain masks residues that are important for transactivation (Serber et al., 2002). Another mechanism was described for p73. The C-terminus of p73 suppresses the ability of p73 to interact with the transcription co-activators p300/CBP that are required for the initiation of transcription (Liu and Chen, 2005). Recently, it was reported that the inhibitory C-terminal portion of TAp73α can be proteolytically cleaved by HtrA2 serine protease following apoptotic stimuli (Marabese et al., 2008). This generates a smaller TAp73 protein with an increased transactivation activity.

Similar to *TP53*, the *TP73* and *TP63* genes each have two promoters (P1 and P2), and their transcripts undergo extensive splicing at the NH2- and COOH termini (Figure 1). Splice variants that retain the N-terminal transactivation domain are named TA isoforms. Studies have thus far identified nine TAp73 (α, β, γ, δ, ε, θ, φ, η and η1) and three TAp63 (α, β and γ) transcripts produced by splicing at the C-terminus (De Laurenzi et al., 1998;Ishimoto et al., 2002;Kaghad et al., 1997;Scaruffi et al., 2000;Ueda et al., 1999;Yang et al., 1998;Zaika et al., 1999). The transactivation and apoptotic potential of TAp73 and TAp63 vary greatly depending on the isoform. However, they are generally considered to be weaker than the corresponding activity of p53. The TAp73β, TAp73γ and TAp63γ variants possess the most “p53-like” properties. However, it should be noted that a majority of p63 and p73 studies focus on a few

isoforms of p63 and p73, specifically α, β, and γ. Little is known about the functions of other isoforms.

Additional level of complexity is generated by $NH_2$-terminal splicing, alternative intragenic promoter usage, and translation from the internal ribosome entry site producing at least six other p73 isoforms (ΔNp73α, ΔNp73β, ΔN'p73α, ΔN'p73β, Ex2Delp73, and Ex2/3Delp73) and three other p63 isoforms (ΔNp63α, ΔNp63β and ΔNp63γ) (Sayan et al., 2007; Stiewe et al., 2002b; Yang et al., 1998; Yang et al., 2000). These p73/p63 variants are named ΔN (or ΔTA) because they lack the transactivation domain at the N-terminus. This results in inhibitory, dominant-negative properties towards p53/TAp63/TAp73.

A number of activities attributed to p53 are shared by TAp73 or TAp63. These include induction of apoptosis, cell cycle arrest and cellular senescence. This similarity can be explained, at least in part, by transactivation of an overlapping set of target genes. TAp73 can induce transcription of p53-inducible genes such as p21/Waf1, GADD45, 14-3-3σ, Bax, PUMA, NOXA, CD95/FAS, PIG3, and p53AIP1 (for review see (Harms et al., 2004)). TAp63 can perform a similar repertoire of functions activating promoters of p21/Waf1, 14-3-3ó, Bax, PUMA, NOXA, CD95/FAS, PERP, and APAF-1 genes (Perez and Pietenpol, 2007). Genome-wide high throughput analyses confirmed an overlap of the transcription profiles of p53, p73 and p63, though unique targets were identified as well (Fontemaggi et al., 2002; Perez et al., 2007). Despite identification of specific p73 and p63 targets, it appears that p73 and p63-mediated apoptosis occurs mostly through the activation of a set of genes that were originally found to be activated by p53. Analyses using reporter and gel-shift assays found that TAp73 and TAp63 interact with p53-responsive elements. Binding of endogenous TAp73 and TAp63 to a number of p53-regulated promoters was also found using chromatin immunoprecipitation (Perez et al., 2007; Tomkova et al., 2006, Costanzo, 2002).

In contrast, ΔN isoforms of p63 and p73 are potent dominant-negative inhibitors of TAp73, TAp63, and p53. The net effect of these interactions in a given context appears to be dependent on the TA/ΔN expression ratio. Based on the fact that ΔNp73 and TAp73 isoforms share the same DNA binding and tetramerization domains, two non-mutually exclusive mechanisms of ΔN transdominance have been suggested: promoter competition and heterocomplex formation (Nakagawa et al., 2002; Stiewe et al., 2002a; Zaika et al., 2002). In the promoter competition mechanism, the suggestion is that ΔN competes off TA isoforms (including p53) from their target gene promoters, thus preventing efficient transcription. In the heterocomplex formation mechanism, ΔN isoforms would inhibit TA by forming transcriptionally inactive hetero-oligomeric complexes.

p53 and TAp73 can regulate the levels of ΔNp73 by directly activating transcription from the second (P2) promoter of the p73 gene (Grob et al., 2001; Kartasheva et al., 2002; Nakagawa et al., 2002). This creates a feedback mechanism that controls p53 and TAp73 transcriptional activities. For ΔNp63, both positive and negative regulation by p53 were reported (Harmes et al., 2003; Lanza et al., 2006).

A recent characterization of the dominant negative p53 isoforms that can control p53 activity further confirmed that principal regulatory mechanisms are similar for all family members. However, it also raises a question of functional differences between ΔN isoforms. Current data suggest that functions of ΔN isoforms are not limited to regulation of TA isoforms. ΔNp73 and ΔNp63 exert their own transcriptional programs that are attributed to additional transactivation domains that are distinct from that of TA isoforms (Dohn et al., 2001; Kartasheva et al., 2003; Liu et al., 2004; Tanaka et al., 2004; Vilgelm et al., 2008; Wu et al., 2003; Wu et al., 2005). They are also involved in apoptosis and cell cycle regulation independent of the TA isoforms.

The p73/p63 isoforms have a tissue-specific pattern of expression (Ishimoto et al., 2002; Stiewe et al., 2002a; Yang et al., 1998; Zaika et al., 1999). This expression pattern can be substantially altered in tumors. It appears that cancer tissues tend to exhibit a more complex pattern of the isoform expression (Stiewe et al., 2004; Zaika et al., 1999). For instance, the tumor-associated variant of p73 mRNA, ΔN'p73, has only been detected in tumors but not in normal tissue (Stiewe et al., 2004). A large number of studies have demonstrated that ΔNp73 and ΔNp63 isoforms are specifically over-expressed in tumor tissues. Up-regulation of TA isoforms was also reported in tumors (Figure 2). Notably, this occurs in the absence of mutations in the *TP73* or *TP63* genes (Zaika and El-Rifai, 2006).

## 3. Regulation of p73 and p63 activities in response to genotoxic stress

p53 is controlled at multiple levels including regulation of the protein stability, activity, and subcellular localization. Compared to p53, the regulation of p73 and p63 activities is less understood but is likely to be as complex. The primary focus of this review is p73, as less data are available for p63 (Figure 3).

### 3.1. Posttranslational modifications

The first insights into molecular mechanisms of p73 regulation have been provided by studies that found phosphorylation of p73 by non-receptor tyrosine kinase c-Abl following DNA damage (Agami et al., 1999; Gong et al., 1999; Yuan et al., 1999). This interaction is critical for activation of p73, as inhibition of c-Abl activity leads to a decrease of p73-induced transcription and apoptosis. c-Abl binds to p73 through the SH3 (Src homology 3) domain of c-Abl and the proline-rich PxxP motif of p73. p73 is then phosphorylated on tyrosine residues at positions 99, 121, and 240 (Agami et al., 1999; Gong et al., 1999; Tsai and Yuan, 2003; Yuan et al., 1999). It was suggested that this multisite phosphorylation generates an additional binding motif required for interaction with the SH2 domain of c-Abl and stabilization of p73 protein (Tsai and Yuan, 2003). TAp73α, TAp73β, ΔNp73α, and ΔNp73β have been reported to be stabilized by c-Abl, but most likely other isoforms are affected given that they also contain the c-Abl interaction motif (Tsai and Yuan, 2003). Functional significance of ΔNp73 stabilization remains unclear, although it may play anti-apoptotic and oncogenic roles. Interestingly, p73 can also be stabilized by BCR-Abl oncoprotein that produced by chromosomal translocation t(9;22) in leukemias (Sanchez-Arevalo Lobo et al., 2005). The consequence of this translocation is the production of chimeric protein with deregulated c-Abl activity.

The activation of c-Abl tyrosine kinase by ionizing radiation (IR) requires a functional serine-protein kinase ATM (Ataxia Telangiectasia Mutated) (Baskaran et al., 1997). As a result, p73 phosphorylation is attenuated in ATM-deficient cells (Yuan et al., 1999). Checkpoint kinases Chk2 and Chk1, downstream effectors of ATM/ATR, are also implicated in the p73 activation after treatment with genotoxic drugs. These proteins stabilize the E2F1 transcription factor, which activates the p73 gene transcription (Urist et al., 2004). Additionally, Chk1, but not Chk2, also directly phosphorylates p73 at Ser47, thereby augmenting its transcriptional and apoptotic function (Gonzalez et al., 2003). Recently, an additional mechanism was suggested by Yoshida *et al*. The authors suggest that in response to cisplatin treatment, ATM mediates the nuclear accumulation of IKK-α protein, a component of the NFκB signalosome, which in turn activates the p73 pro-apoptotic function, likely by direct phosphorylation at the N-terminal region of p73 (Furuya et al., 2007; Yoshida et al., 2007).

Other stress-activated pathways, specifically JNK/SAPK and p38 MAPK, have been shown to be involved in regulation of p73 following DNA damage (Sanchez-Prieto et al., 2002; Toh et al., 2004). Although the JNK pathway can be activated by c-Abl, p73 up-regulation by JNK occurs in a c-Abl-independent manner (Toh et al., 2004). It is mediated by the c-Jun

transcription factor, which inhibits proteasome-mediated degradation of p73 and stabilizes p73 protein. A recent study refined this conclusion and suggested that c-Jun up-regulates YAP protein (see below) in turn stabilizing p73 (Danovi et al., 2008). JNK also directly phosphorylates TAp73 at several serine and threonine residues located within both the N- and C-termini after treatment with cisplatin. This leads to TAp73 protein stabilization, increased p73-mediated transcriptional activity and apoptosis. *In vitro*, all JNK isoforms (JNK1, JNK2, and JNK3) are able to phosphorylate TAp73 (Jones et al., 2007). Interestingly, ΔNp73 binds to JNK kinase and inhibits its activity implying that JNK may mediate regulation of TAp73 activity by ΔNp73 (Lee et al., 2004).

In contrast to JNK, p38 is required for activation and stabilization of p73 protein by c-Abl and involves direct phosphorylation of p73 on threonine residues (Sanchez-Prieto et al., 2002). This phosphorylation event is a prerequisite for the binding of p73 to PML (promyelocytic leukemia protein). PML recruits p73 protein into PML-nuclear bodies (PML-NB), inhibits its degradation and enhances the p73 transcriptional activity (Bernassola et al., 2004). Similar to p73, interaction with PML increases protein stability and transcriptional activity of p63 isoforms (Bernassola et al., 2005).

The augmented activity of c-Abl and p38 MAP kinase stimulates binding of Pin1, a peptidyl-prolyl isomerase that catalyzes cis-trans isomerization of Ser/Thr-Pro phosphorylated peptides. Conformational change induced by Pin1 enhances p73 acetylation mediated by p300/CBP (Mantovani et al., 2004 50). p300/CBP are chromatin modifying acetyltransferases that also acetylate a plethora of transcription factors. TAp73α acetylation sites were mapped to three lysine residues at positions 321, 327 and 331. Mutations of these lysines to arginines impair p73 acetylation and render this mutant defective in growth suppression and apoptosis (Costanzo et al., 2002). In context of DNA-damage response, p300/CBP-mediated acetylation has been shown to increase protein stability and enhance transcriptional activity of both p53 and p73. Interestingly, another acetyltransferase PCAF can also contribute to the p73 acetylation and transcriptional activation (Zhao et al., 2003). Currently, it is not clear how these acetyltransferases interplay in the regulation of the p53 family.

Besides p38, PML and Pin1 mentioned earlier, p300-mediated acetylation of p73 is potentiated by YAP (YAP1, YAP65) protein. YAP1 (Yes-associated protein 1) was originally identified as a binding partner of Src family tyrosine kinase Yes. The interaction between p73 and YAP involves the WW domain of YAP and the PPXY motif in TAp73. YAP functions as a transcriptional coactivator of p73, which also affects p73 protein accumulation in response to DNA damage. Silencing of YAP with siRNA impairs p73 stabilization in cells exposed to cisplatin (Strano et al., 2005). A potential mechanism of the p73 protein stabilization was recently suggested by Levy et al (Levy et al., 2007). The authors have found that YAP1 competes with Itch for binding to p73 at the PPXY motif. Since Itch functions as an E3 ubiquitin ligase involved in degradation of p73, YAP prevents ubiquitination and subsequent degradation of the p73 protein. Binding to YAP is isoform-specific. p73α, p73β and p63α interact with YAP, but p73γ and p53 do not (Basu et al., 2003; Strano et al., 2001). The latter is especially interesting because it positions YAP as a transcription cofactor that can differentiate p73 from p53. YAP promotes p73-dependent apoptosis through selective activation of pro-apoptotic target genes (Strano et al., 2005). This specificity is regulated by phosphorylation of YAP by c-Abl. In its phosphorylated state, YAP is preferentially recruited to promoters of pro-apoptotic genes and facilitates their activation by p73 (Levy et al., 2008). To exert its function, YAP requires PML and has to be localized to the PML-NBs in the nucleus. YAP localization is controlled by protein kinase B/Akt phosphorylation and binding to 14-3-3 (Basu et al., 2003).

Similar to YAP, interaction of p73 and p63 with members of the ASPP family, ASPP1 and ASPP2 (p53BP2), stimulate the transactivation of pro-apoptotic genes such as Bax, PIG3, and

PUMA, but not MDM2 or p21 (Bergamaschi et al., 2004). At the present time, the mechanism of this selectivity remains to be fully investigated. Interestingly, ASPP protein physically binds to YAP (Espanel and Sudol, 2001). However, this interaction was not fully investigated in the context of p73 and YAP binding. The third member of the ASPP family, iASPP, which has inhibitory and proto-oncogenic properties, has recently been shown to interact with p63 and p73 as well (Robinson et al., 2008). Disruption of iASPP interaction with TAp73 using a small peptide induces p73-dependent transcription and apoptosis in cancer cells (Bell et al., 2007).

Among other serine/threonine kinases, protein kinase Cδ (PKCδ) and its active catalytic fragment PKCδCF were shown to phosphorylate TAp73β at position Ser-289. PKCδCF stabilizes and activates p73-dependent transcription. Although c-Abl contributes to phosphorylation and activation of PKCδ in response to DNA damage, the role of c-Abl in the PKCδ-induced up-regulation of p73 remains to be seen (Ren et al., 2002).

Stress-induced phosphorylation of p73 by the aforementioned protein kinases leads to its transcriptional activation. However, inhibitory phosphorylation has been described as well. The Src family kinase, HCK, c-Src, PKA, polo-like kinase 1 (Plk1), and cyclin-dependent kinases directly phosphorylate p73 and inhibit its activity (Gaiddon et al., 2003; Hanamoto et al., 2005; Koida et al., 2008; Paliwal et al., 2007). c-Src can also inhibit p73 indirectly by phosphorylation of WWOX protein, resulting in increased binding of WWOX to p73 and suppression of p73 transcription (Aqeilan et al., 2004).

### 3.2. Regulation of protein degradation

Regulation of protein stability and degradation is an important mechanism that keeps p53 in check. A classic example is the p53-MDM2-MDMX regulatory mechanism that controls activity and stability of p53. p73 and p63 degradation pathways are less characterized but appear to be as intricate. Since the regulation of p73 and p63 protein degradation has recently been reviewed (Melino et al., 2006; Ozaki et al., 2005; Watson and Irwin, 2006; Müller et al., 2006), we will only highlight key components. p63 and p73 are degraded in a proteasome-dependent manner as steady-state levels of the endogenous proteins increase in the presence of proteasome inhibitors (Balint et al., 1999; Bernassola et al., 2004; Lee and La Thangue, 1999; Okada et al., 2002). Pathways leading to the degradation of p63 and p73 appear to be different from that of p53. Even though p73 can physically interact with MDM2, this does not result in its ubiquitination or degradation (Balint et al., 1999; Dobbelstein et al., 1999; Zeng et al., 1999). Instead, MDM2 over-expression inhibits p73 transcriptional activity and enhances its stability. Disruption of this interaction with the small molecule inhibitor, Nutlin-3, leads to activation of p73 (Kitagawa et al., 2008; Lau et al., 2008). Several E3 ligases have been shown to ubiquitinate p73 and p63 proteins as a prerequisite for protein degradation. HECT domain containing ubiquitin ligase NEDD4, which facilitates ΔNp63 degradation (Bakkers et al., 2005), and HECT ubiquitin E3 ligase Itch, which targets TA and ΔN isoforms of p73 and p63, have been characterized so far (Rossi et al., 2006a; Rossi et al., 2005; Rossi et al., 2006b). p73 protein stability is also regulated by ubiquitin-independent mechanisms. Interaction with NQO1 (NADH quinine oxidoreductase 1) or UFD2a (U-box type E3/E4 ligase) regulates the proteasomal degradation of TAp73 in a ubiquitin-independent manner. Both enzymes affect the levels of p73 during DNA damage (Asher et al., 2005; Hosoda et al., 2005). Proteolytic degradation of p73 by calcium-dependent protease calpain I has also been reported (Munarriz et al., 2005).

The degradation of p73 and p63 proteins is isoform-dependent. For instance, Itch-mediated regulation of p73 protein stability is selective for isoforms containing the C-terminal proline-rich motif, such as the α- and β-variants, while δ and γ are not affected. Stability of p73 and p63 isoforms was found to vary depending on their transcription properties. Transcriptionally active (TA) isoforms are generally less stable than ΔN variants suggesting that the downstream

transcriptional target(s) of TA isoforms may function as negative regulators of protein stability (Petitjean et al., 2008; Slade et al., 2004; Wu et al., 2004). To date, the effectors that act in a negative autoregulatory feedback loop analogous to p53-MDM2 have not been identified.

DNA damage stabilizes the TA isoforms (Maisse et al., 2004). It correlates with down-regulation of Itch, implicating Itch-mediated regulation of p73 in response to DNA damage (Rossi et al., 2005). Consistent with this, the down-regulation of Itch by siRNA augments apoptosis upon treatment with chemotherapeutic drugs (Hansen et al., 2007). In contrast to TAp73 and TAp63, ΔNp63α is destabilized by genotoxic stress in a RACK1 (receptor for activated C-kinase 1) and stratifin (14-3-3σ)-dependent manner (Fomenkov et al., 2004). This down-regulation correlates with phosphorylation of ΔNp63 at Ser66/68 and Ser361 (Westfall et al., 2005).

### 3.3. Regulation of transcription

Since the initial discovery of p73, mechanisms involved in regulation of p73 gene transcription have also been explored. In response to DNA damage, E2F1-driven transcription is an important component involved in the up-regulation of p73 mRNA; this is in contrast to p53, which is primarily regulated at a post-translational level. E2F1 is a member of the E2F family of transcription factors implicated in cell-cycle regulation and apoptosis. The *TP73* gene contains several functional E2F-binding sites within its promoter, allowing direct regulation of p73 expression (Ding et al., 1999; Stiewe and Putzer, 2000). DNA-damage stimulates E2F1 binding to the p73 promoter and activates p73 gene transcription (Irwin et al., 2000; Pediconi et al., 2003; Stiewe and Putzer, 2000). Phosphorylation of E2F1 by Chk1/2 protein kinases and acetylation by the histone acetyltransferase PCAF promote activation of p73 {Urist, 2004).

At the p73 promoter level, E2F1 is regulated by transcription repressor c-EBPα and activator YY1 (Marabese, 2003; Wu, 2008). Following DNA damage, c-EBPα is exported from the nucleus that allows E2F1 to fully operate on its binding site within the p73 promoter (Marabese et al., 2003). An additional negative regulatory region that may affect E2F1-dependent transcription is located in the first intron of the *TP73* gene immediately upstream of exon 2 (Fontemaggi et al., 2001). It is occupied by the transcriptional repressor ZEB1(δEF1), which inhibits expression of TAp73 and ΔNp73. This regulatory region is frequently deleted in colon tumors suggesting that it may play a role in tumorigenesis (Dominguez et al., 2006). ZEB1 also inhibits expression of ΔNp63 by binding to the P2 promoter of the *TP63* gene (Fontemaggi et al., 2005).

## 4. p73 and p63 in anti-cancer therapy

The accumulating evidence converges on the view that p73 plays a significant role in curative anti-cancer therapy. Similar to p53, activated p73 mediates a cellular response to radio- and chemotherapy, including γ-irradiation and treatment with cisplatin, doxorubicin, camptothecin, etoposide, bleomycin, mitoxantrone, taxol, and the cytosine analogues gemcitabine, Ara-C and T-ara-C (Agami et al., 1999; Costanzo et al., 2002; Gong et al., 1999; Lin et al., 2004; Müller et al., 2005; Müller et al., 2006; Thottassery et al., 2006; Vayssade et al., 2005; Yuan et al., 1999). TAp73 is regulated on both transcriptional and post-translational levels following cellular stress. The exact mechanism appears to be dependent on the nature of the stress-inducing agent and cellular context. For instance, cisplatin, which several groups have found to activate and stabilize the p73 protein, does not induce p73 mRNA. Doxorubicin and taxol can induce p73 mRNA and protein at the same time. In contrast, γ-irradiation increases the p73 protein activity without affecting the protein or mRNA levels (Agami et al., 1999; Hamer et al., 2001). However, other studies reported accumulation of p73 following γ-irradiation (Dai et al., 2007; Lin et al., 2004). It is worth noting that p73 protein accumulation can be transient and depend on a treatment regiment (Irwin et al., 2003; Lin et al., 2004).

TAp63 isoforms are also induced by various genotoxic agents, although their effect appears to be weaker than that of p53 and TAp73. Using a tet-off inducible system, Diez *et al.* found that apoptosis following TAp63α or TAp63γ up-regulation was weaker compared to that of p53 or TAp73α (Dietz et al., 2002). UV-C irradiation or treatment with actinomycin D, bleomycin, doxorubicin, and etoposide leads to the activation of transcription and accumulation of the ectopic p63γ protein in stably transfected cells. However, in these experiments the p63γ increase was attributed to cell differentiation rather than apoptosis (Katoh et al., 2000). Both doxorubicin and etoposide induced endogenous TAp63 mRNA and protein in mouse hepatocytes and human hepatocellular carcinoma cell lines, which was accompanied by cell cycle arrest and up-regulation of p21/Waf1, PIG3, 14-3-3σ, and GADD45 (Petitjean et al., 2005). Similarly, in the hepatoma cell line Hep3B, treatment with bleomycin, doxorubicin, or mitoxantrone led to apoptosis via up-regulation of endogenous TAp63α (Gressner et al., 2005). Adenovirus-mediated transduction of TAp63γ is an efficient inducer of cell-cycle arrest and apoptosis, particularly when combined with adriamycin or X-ray irradiation (Sasaki et al., 2001).

Inhibition of p73 with a dominant-negative mutant or a small-inhibitory RNA (siRNA) suppresses apoptosis and induces chemoresistance in cancer cells, irrespective of the p53 status (Irwin et al., 2003; Thottassery et al., 2006; Vayssade et al., 2005). Similarly, TAp63 can be a determinant of chemotherapeutic efficacy in some cell types as inhibition of endogenous TAp63 with siRNA leads to a decreased chemosensitivity (Gressner et al., 2005). Combined loss of p73 and p63 results in the failure of mouse embryonic fibroblasts containing p53 to undergo apoptosis in response to DNA damage (Flores et al., 2002). Although this is not a universal phenomenon for all cell types (Senoo et al., 2004), it suggests that the p53 family members cooperate in the cellular response to genotoxic stress. The mechanism of this cooperativity remains largely unknown. However, several studies suggest multiple functional interactions within the p53 family. Using sequential ChIP assay, Cui *et al.* found that TAp73 and p53 simultaneously bind to the shared regulatory site within α-fetoprotein (AFP) gene promoter and cooperate in chromatin modification and inhibition of the gene transcription (Cui et al., 2005). Moreover, p73 functionally cooperates with p53 in the transcription regulation of p53 and p73 genes. It was reported that both p73 and p53 bind to the response element within the p53 gene promoter and activate expression of the p53 gene (Wang and El-Deiry, 2006) and vise-versa, p53 and TAp73 can bind to the TAp73 gene promoter and induce p73 gene transcription (Chen et al., 2001; Wang et al., 2007). Although the cross talk between TAp73 and p53 through these amplification loops was not investigated in detail, these interactions may potentially contribute to DNA damage response. p73 can also affect subcellular localization of the p53 protein. In neuroblastoma cells, which express wild-type but non-functional cytoplasmic p53, adenovirus-mediated expression of TAp73 led to nuclear translocation of endogenous p53, up-regulation of p21, and induction of growth arrest (Goldschneider et al., 2004). TAp73 has an effect not only on p53, but also on p63 isoforms. Down-regulation of p73 with siRNA resulted in reduced DNA binding of TAp63γ and ΔNp63γ to the p21 promoter probe in mouse keratinocytes. Reconstitution of TAp73 expression restored DNA-binding capabilities of the p63 isoforms (Johnson et al., 2007). ΔN isoforms of p73 and p63 as well as mutant p53 are also involved in cross regulation. Upon treatment with doxorubicin, ΔNp73 and mutant p53 associate with the ΔNp63 promoter and induce the ΔNp63 transcription through proximal CCAAT boxes. This may have an anti-apoptotic effect and induce chemoresistance (Lanza et al., 2006). In addition, mutant p53 can affect activities of TAp73 and TAp63. It has been shown that certain tumor-derived p53 mutants (R175H, R248W, Y220C, R249S, R283H, and D281G) can physically associate and inhibit activation of TAp73 and/or TAp63 (Di Como et al., 1999; Gaiddon et al., 2001; Strano et al., 2002). Taken collectively, these data suggest highly dynamic interactions between members of the p53 family.

Another aspect of these interactions relates to TA and ΔN isoforms. Several studies showed that dominant-negative ΔN isoforms suppress the transcriptionally active counterparts, and this induces chemoresistance. *In vitro*, the inhibitory effect of ΔN isoforms has been demonstrated for all p53 family members. Less data are available for primary tumors, although accumulating evidence suggests that a dominant-negative mechanism may contribute to efficacy of chemotherapy. In carcinomas of ovary and childhood acute lymphoblastic leukemia, increased expression of dominant-negative p73 isoforms correlates with resistance to conventional chemotherapy (Concin et al., 2005; Meier et al., 2006). Inhibitory interactions between ΔNp63 and TAp73 have also been shown to affect response to platinum-based chemotherapy in a subset of breast and head and neck tumors (Leong et al., 2007; Rocco et al., 2006). Ellisen's group at Harvard Medical School found that apoptosis is uniquely dependent on TAp73 activity in these tumors. Upon treatment with cisplatin, TAp73 undergoes c-Abl–dependent phosphorylation, which promotes dissociation of the ΔNp63α/TAp73 protein complex, activation of TAp73-dependent transcription and apoptosis (Leong et al., 2007; Rocco et al., 2006). Several studies, however, show that biological and therapeutic significance of TA-ΔN interactions might be complex. For example, ΔNp73β inhibits p53-dependent apoptosis in primary sympathetic neurons (Pozniak et al., 2000) but when over-expressed in cancer cell lines ΔNp73β induces cell cycle arrest and apoptosis (Liu et al., 2004). Similarly, several studies showed that the inhibitory effect of ΔNp73α on p53 and TAp73 leads to chemoresistance in tumor cells (Meier et al., 2006; Müller et al., 2005; Zaika et al., 2002), although another study did not find this phenomenon (Sabatino et al., 2007). In head and neck tumors, high ΔNp63 protein levels correlate with a favorable response to platinum-based therapy (Leong et al., 2007; Zangen et al., 2005). It suggests that the dominant negative concept can not explain all complexity of interactions attributed to ΔN isoforms. These interactions are determined by cellular context and should be analyzed through a prism of multiple interactions within and beyond the p53 family.

Do the aforementioned experiments imply that p73 and p63 are potential cancer therapeutic targets? Several facts speak in favor. In contrast to p53, both p73 and p63 are rarely mutated in cancer cells and therefore, could potentially be activated. Up-regulation of p73 and p63 in tumor tissues (Zaika and El-Rifai, 2006) can also be advantageous as it may contribute to drug specificity. Recently, the first steps were made along a difficult road to the development of drugs that specifically target the p53 protein family.

i) Bell et al. demonstrated that a small peptide molecule that targets interaction of TAp73 with the inhibitory protein iASPP is capable inducing apoptosis selectively in tumor cells. Furthermore, this peptide was found to be effective in suppression of tumor growth in a xenograft mouse model when it was delivered through intravenous injection of DNA-polypropylenimine dendrimeric complexes (Bell et al., 2007).

ii) Targeting of interactions that inhibit p73 was also demonstrated in another recent study (Kravchenko et al., 2008). The authors describe a small molecule, RETRA, which releases p73 from the blocking complex with mutant p53. Treatment of mutant p53-expressing cancer cells with RETRA results in a substantial activation of a set of p53-regulated genes and specific suppression of mutant p53-bearing tumor cells *in vitro* and in mouse xenografts. Notably, RETRA does not affect normal cells.

iii) Another useful approach is a chemical library screen for small molecules that activate p53 response in p53-deficient cancer cells. Such compounds were characterized as being able to induce expression of p53 target genes and apoptosis by increasing the p73 expression (Wang et al., 2006).

## 5. Conclusion

Current analysis shows that not only p53 but also p73 and p63 contribute to the effectiveness of anti-cancer therapy. So called "p53-independent apoptosis and drug response" is mediated at least in part by the other members of the p53 family. All of these proteins are involved in complex network interactions that determine cell fate following apoptotic signals and may have profound effects on chemotherapeutic drug sensitivity in primary tumors. However, several critical issues have to be addressed before the therapeutic potential of compounds that target p73 and p63 can be fully appreciated. Many outstanding questions remain about the roles of p73 and p63 in human tumorigenesis as accumulated data suggest that these proteins play both tumor suppressor and oncogenic roles (Zaika and El-Rifai, 2006). Studies of molecular mechanisms that underlie biological interactions between p53 and other family members as well as interactions of multiple p53, p63 and p73 isoforms will also help to advance the drug development. Future studies hold a key to therapeutic benefits.

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**Figure 1. Gene architectures of the human *TP73* and *TP63***

The *TP73* and *TP63* genes encode a transactivation (TAD), DNA-binding (DBD), oligomerization (OD), sterile alpha motif (SAM), and transcription inhibitory domains (TID), and have two promoters (P1 and P2). The P1 promoters produce transactivation-competent full-length proteins (TA) while the P2 promoters produce TAD-deficient proteins (ΔN) with dominant-negative functions. In addition, alternative splicing at the COOH- and NH2- termini generate multiple isoforms of p73 and p63.

**Figure 2. Immunochistochemical staining for p73 in colon tumor (original magnification; x50)**
Strong expression of p73 protein is present in the nuclei of cancer cells. Antibody used for this analysis (Santa-Cruz, H-79) primarily recognizes TAp73 isoform. Inset depicts magnified view of the cancer cells positive for p73 (x200).

**Figure 3. Regulation of p73 protein (see text for details)**