Oncogene (2007) 26, 5169–5183

www.nature.com/onc

REVIEW

# p63 and p73 in human cancer: defining the network

MP DeYoung and LW Ellisen

*Massachusetts General Hospital Cancer Center and Harvard Medical School, Boston, MA, USA*

**The *p53*-related genes *p63* and *p73* exhibit significant structural homology to p53; however, they do not function as classical tumor suppressors and are rarely mutated in human cancers. Both p63 and p73 exhibit tissue-specific roles in normal development and a complex contribution to tumorigenesis that is due to their expression as multiple protein isoforms. The predominant p63/p73 isoforms expressed both in normal development and in many tumors lack the conserved transactivation (TA) domain; these isoforms instead exhibit a truncated N-terminus (ΔN) and function at least in part as transcriptional repressors. p63 and p73 isoforms are regulated through both transcriptional and post-translational mechanisms, and they in turn regulate diverse cellular functions including proliferation, survival and differentiation. The net effect of p63/p73 expression in a given context depends on the ratio of TA/ΔN isoforms expressed, on physical interaction between p63 and p73 isoforms, and on functional interactions with p53 at the promoters of specific downstream target genes. These multifaceted interactions occur in diverse ways in tumor-specific contexts, demonstrating a functional 'p53 family network' in human tumorigenesis. Understanding the regulation and mechanistic contributions of p63 and p73 in human cancers may ultimately provide new therapeutic opportunities for a variety of these diseases.**
*Oncogene* (2007) **26,** 5169–5183; doi:10.1038/sj.onc.1210337; published online 5 March 2007

**Keywords:** p53; p63; p73; apoptosis; tumorigenesis; squamous cell carcinoma (SCC)

## Introduction

The p53 tumor suppressor functions as a key nodal point that integrates upstream signals and directs the response to DNA damage, oncogenic stress and other cellular stress conditions (Vogelstein *et al*., 2000; Vousden, 2000). The tumor-suppressive property of p53 is most closely linked to its ability to transactivate a diverse variety of downstream target genes involved in the regulation of cell-cycle progression, apoptosis, DNA repair and other functions (Vogelstein *et al*., 2000). Most human tumors exhibit inactivation of *p53*, either through direct mutation or deletion of *p53* itself, or through disruption of regulatory pathways essential for p53 function.

The identification of the two *p53*-related genes, *p63* and *p73*, initially provoked speculation that all three genes might play an analogous role in human tumors (Yang and McKeon, 2000; Melino *et al*., 2003; Moll and Slade, 2004). In particular, the striking homology among the family members within both their DNA-binding domain (DBD) and oligomerization domain (OD) suggested that these genes might regulate transcription of a common subset of target genes, by binding to common promoters as either homo- or hetero-tetrameric complexes. Work in the ensuing years, however, has revealed a much more complex picture of the contribution of *p63* and *p73* to human cancer. In contrast to *p53*, *p63* and *p73* have essential roles in normal development, they are most commonly expressed as N-terminally truncated isoforms, and they are rarely mutated in human tumors. In addition, the expression and function of p63 and p73 are regulated through distinct mechanisms that are only beginning to be understood. Nevertheless, a substantial body of evidence supports a contribution of p63 and p73 in many human tumors through both p53-dependent and p53-independent pathways.

## Gene structure of functional domains of the p53 family transcription factors

Although *p63* and *p73* are the more recently identified *p53* family members, a *p63*/*p73*-like gene is considered to be the ancestral gene of the family (Yang *et al*., 2002). The proteins encoded by these two genes are more structurally similar to each other than to p53; however, all three family members possess several conserved protein domains (Figure 1). These include an N-terminal transactivation (TA) domain, a central DBD, and an OD (Yang and McKeon, 2000). The highest degree of homology among the three members is observed within the DBD (>60% amino-acid identity between p53 and both p63 and p73, and ~85% amino-acid identity between p63 and p73), including conservation of all essential DNA contact residues (De Laurenzi and Melino, 2000). These structural similarities allow for both physical and functional interactions among all three family members that are relevant in human cancer.

Correspondence: Dr LW Ellisen, MGH Cancer Center, GRJ-904, 55 Fruit Street, Boston, MA 02114, USA.
E-mail: ellisen@helix.mgh.harvard.edu
Received 17 January 2007; accepted 17 January 2007; published online 5 March 2007

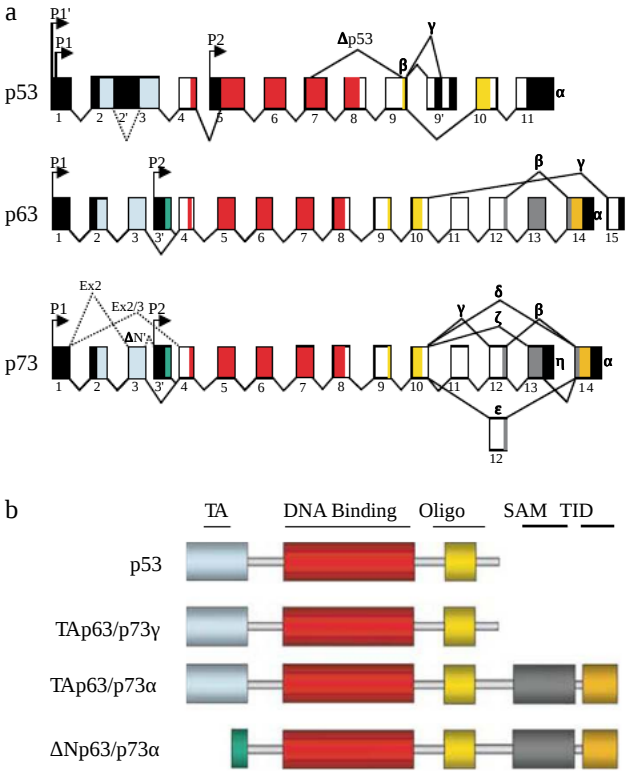


**Figure 1** Structure and expression of p53 family members. (**a**) Structure of p53, p63 and p73 transcription units. Numbered boxes indicate exons, and black shading denotes untranslated sequences. The approximate regions encoding the transactivation (TA) domain (light blue), DN-specific region (green), DNA-binding domain (red), oligomerization domain (yellow), sterile alpha motif (SAM, grey), and transactivational inhibitory domain (TID, orange) are indicated. Distinct transcription start sites are indicated by arrows. N-terminal alternative splicing for p53 and p73 are indicated by dotted lines, and C-terminal splicing events for all p53 family members are indicated by solid lines and Greek letter designation. (**b**) Protein domains of p53 family members. All three family members share a homologous DNA-binding domain and oligomerization domain (oligo). The TA domain is shared by p53, TAp63, and TAp73 isoforms. TAp63g/TAp73g isoforms most closely resemble p53. N-terminally truncated DN isoforms possess unique N-terminal sequences. Alpha isoforms of p63 and p73 possess a C-terminal SAM domain followed by a transactivational inhibitory domain (TID). Other isoforms of p53, p63 and p73 are not shown.

## Alternative promoter usage produces functionally distinct classes of p53, p63 and p73 isoforms

All three members of the *p53* gene family give rise to multiple protein products resulting from both alternative promoter usage and alternative mRNA splicing (Figure 1). Transcription of both *p63* and *p73* can occur from two distinct promoters: one upstream of exon 1 (P1) and another located within intron 3 (P2). Isoforms transcribed from P1 contain an N-terminal acidic TA domain (TAp63 and TAp73), which is highly homologous to the TA domain of full-length p53, whereas genes transcribed from P2 lack the N-terminal TA domain (DNp63 and DNp73) (Kaghad *et al*., 1997; Yang *et al*., 1998). In addition, $5^0$ exon splicing within the P1 transcripts of *p73* gives rise to additional mRNA isoforms whose protein products also lack the TA domain ($DN^0$p73, Ex2Delp73 and Ex2/3Delp73) (Fillippovich *et al*., 2001; Ishimoto *et al*., 2002; Stiewe and Putzer, 2002). Recently, it has been reported that *p53*, which for many years was thought to encode a single protein product, also produces multiple isoforms through the use of two promoters and alternative mRNA splicing (Bourdon *et al*., 2005). When overexpressed, TAp63 and TAp73 proteins are capable of transactivating distinct but overlapping subsets of known p53-regulated genes involved in cell-cycle arrest and apoptosis (Zhu *et al*., 1998; Dohn *et al*., 2001; Melino *et al*., 2002). Both TAp63 and TAp73 also regulate independent sets of genes that are not transcriptional targets of p53 (Barbieri and Pietenpol, 2006; Harms *et al*., 2004). In contrast, DNp63 and DNp73 proteins have been shown to function in part as dominant-negative inhibitors of the p53 family, leading to the hypothesis that these isoforms may exhibit proto-oncogenic function. Inhibition by DN isoforms occurs both through direct competition for DNA-binding sites and through formation hetero-oligomeric complexes with TAp63/TAp73, and less strongly with p53 (Yang *et al*., 1998; Grob *et al*., 2001; Stiewe *et al*., 2002; Chan *et al*., 2004). Recent studies by several groups have demonstrated that DNp63 is also capable of activating transcription through a cooperative effect of a DNp63-specific, N-terminal TA domain and a C-terminal proline-rich region (Ghioni *et al*., 2002; Helton *et al*., 2006). Although no analogous C-terminal domain has been identified in DNp73 isoforms, one study suggests that these isoforms may also contain an N-terminal TA domain (Liu *et al*., 2004). Like TAp63, DNp63 activates a group of genes that includes but is not restricted to genes regulated by p53 (Dohn *et al*., 2001).

## C-terminal mRNA splicing generates additional structural and functional diversity

C-terminal splicing of both p63 and p73 transcripts generates a variety of TA and DN isoforms whose distinct functional contributions are only beginning to be understood (Figure 1). To date, at least seven C-terminal isotypes have been identified for p73; three have been identified for p63, and two for p53 (Moll and Slade, 2004; Bourdon *et al*., 2005). The TAp63a and TAp73a isoforms are the largest proteins in each family. Internal alternative exon splicing generates the additional isoforms of p73, whereas both internal splicing and alternative $3^0$ exon usage generate p63 C-terminal isoforms. In addition, splicing-associated frameshifts yield unique C-terminal sequences for some p63 and p73 isoforms (Courtois *et al*., 2004; Moll and Slade, 2004).

These distinct C-termini are thought to modulate the ability of the respective TA isoforms to transactivate gene expression. The TAp63g and TAp73g isoforms most closely resemble full-length p53. In overexpression studies, TAp63g has been shown to be as potent as p53

in inducing target gene expression and apoptosis, whereas the most potent transactivating p73 isoform appears to be TAp73b (Kaghad *et al*., 1997; Yang *et al*., 1998). Both p63a and p73a isoforms also contain a protein–protein interaction domain known as the sterile alpha motif (SAM) (Thanos and Bowie, 1999). The SAM is a globular domain composed of four a-helices and a small $3_{10}$-helix. Although this motif is often found to mediate homodimerization within developmentally regulated proteins, in p53 family members the SAM domain is thought to be monomeric (Chi *et al*., 1999b). An additional post-SAM region known as the transactivational inhibitory domain (TID) has been identified in p63a and p73a isoforms (Serber *et al*., 2002). This region ($\sim$70 amino acids) has been proposed to inhibit the transcriptional activity of both TAp63a and TAp73a through inter- or intra-molecular association with the TA domain (Serber *et al*., 2002). Indeed, both TAp63a and TAp73a isoforms have decreased potency in transactivation and apoptosis induction compared to other TA isoforms, and deletion of this region restores transactivation potency of both TAp73a and TAp63a (De Laurenzi *et al*., 1998; Yang *et al*., 1998; Serber *et al*., 2002). The presence of this domain within DNp63a and DNp73a isoforms may allow trans-repression of associated TA isoforms, thereby explaining the potent inhibitory effect of DNp63a and DNp73a isoforms when bound to DNA as hetero-tetramers composed of both DNa and TAg isoforms (Serber *et al*., 2002).

## Tissue-specific roles for p63 and p73 in normal development

p63 is expressed in a highly restricted pattern during embryonic development. Its expression is first detectable within the primitive ectoderm, which gives rise to the epidermis as well as epithelial appendages including the mammary gland, prostate, teeth and sweat glands. p63 is also expressed in the apical ectodermal ridge, a specialized cluster of ectodermal cells required for inductive events during limb formation (Mills *et al*., 1999; Yang *et al*., 1999). Remarkably, p63 is essential for the development of most tissues in which it is expressed, as *p63*-null mice exhibit profound developmental abnormalities of the skin, limbs, mammary, prostate and other epithelial tissues (Mills *et al*., 1999; Yang *et al*., 1999). This ectodermal phenotype is recapitulated in humans who inherit a variety of heterozygous mutations in *p63* that are thought to function as dominant-negative or potentially dominant gain-of-function alleles (van Bokhoven and Brunner, 2002).

Little overlap is observed in the embryonic expression patterns of p63 and p73, consistent with the distinct developmental phenotype associated with *p73* nullizygosity. Expression of p73 is most prominent within discrete structures of the developing brain, and within the sympathetic superior cervical ganglia. *p73*-null animals demonstrate hippocampal dysgenesis, hydrocephalus owing to probable hypersecretion from the choroid plexus, and chronic infection and inflammation likely owing to hypersecretion of mucous from the respiratory mucosa (Pozniak *et al*., 2000; Yang *et al*., 2000). These mice also exhibit abnormal social and reproductive behavior attributed to failed development of the vomeronasal organ that is essential for detection of pheromonal signaling (Yang *et al*., 2000).

The contribution of individual p63 and p73 isoforms to their respective developmental phenotypes remains to be firmly established. Although mRNA for both TAp63 and TAp73 are detectable during embryogenesis, the predominant isoforms of both *p63* and *p73* expressed during development are the N-terminally truncated DN isoforms (Yang *et al*., 1998, 2000; Pozniak *et al*., 2000). As discussed below, these isoforms are also the predominant forms expressed in many human cancers, and they are therefore likely to contribute importantly to p63- and p73-dependent functions in tumorigenesis. Nevertheless, the fundamental mechanisms of endogenous DNp63 and DNp73 isoforms in development and tumorigenesis are poorly understood. Among the most central questions in this regard are (1) whether these isoforms function exclusively as repressors and to what degree they may have important roles as transcriptional activators; (2) whether their repressive function is directed toward their respective TA isoforms, toward another family member, or independently of the other family members; and (3) whether these isoforms perform analogous roles in normal development and tumorigenesis.

## Expression and mutation of p63 and p73 in human cancer

### D*Np63*a *is commonly overexpressed in squamous epithelial malignancies*

Both *p63* and *p73* were initially hypothesized to function as tumor suppressors based on their homology to *p53*. However, mutation of either of these genes in human cancer is quite rare (o1%) (Hagiwara *et al*., 1999; Sunahara *et al*., 1999; Melino *et al*., 2002). In fact, the *p63* gene maps to chromosome 3q27–28, a region frequently amplified in squamous cell carcinomas (Bjorkqvist *et al*., 1998; Hibi *et al*., 2000; Massion *et al*., 2003). Although some controversy exists as to whether *p63* is the targeted gene driving amplification of this locus, several groups have reported increased wild-type p63 mRNA levels that correlate with an increase in *p63* gene copy number in squamous cell carcinomas of the lung and head and neck (Table 1) (Hibi *et al*., 2000; Tonon *et al*., 2005). In other cases, overexpression of p63 appears to involve mechanisms independent of genomic amplification (Redon *et al*., 2001). In any event, numerous studies have demonstrated p63 overexpression in up to 80% of primary head and neck squamous cell carcinomas (HNSCCs), and p63 overexpression is also commonly observed in other squamous epithelial cancers, including lung, nasopharyngeal, esophageal and cervical cancers (Wang *et al*., 2001;

**Table 1** Expression levels of p63 and p73 in cancer tissues (tumor vs normal)

| *Cancer type* | *TAp63* | D*Np63* | *TAp73* | D*Np73*[a] | *Correlation with p53*[b] | *References*[c] |
|---|---|---|---|---|---|---|
| SCC[d] | rare | mm | mm | NC | p63 (variable) | A–D |
| Cervical | NC | mm | m | m | ND | E, F |
| Breast | m[g] | m[g] | m | m | p73 (variable) | G–I |
| Bladder[e] | NC | mm | m | ND | No (p63) | J, K |
| Neuroblastoma | ND | ND | m | m | ND | L, M |
| Glioma | ND | ND | mm | mm | ND | N, O |
| Ependymoma | ND | ND | mm[g] | mm[g] | ND | N |
| Colon | NC | Neg | m | m | p73 | I, P |
| Gastric | m[g] | m[g] | mm | ND | No (p63 & p73) | Q |
| Ovary | ND | ND | m | mm | p73 | R |
| Hepatocellular | Neg | Neg | m | m | No (p73) | S, T |
| Cholangiocarcinoma | mm[g] | mm[g] | m | ND | ND | T |
| CML | mm | Rare | mm[g] | mm[g] | ND | U, V |
| AML | ND | ND | mm | mm | ND | W |
| CLL | ND | ND | mm | mm | ND | X |
| Lymphoma[f] | mm | Neg | mm | mm | No (p73) | Y, Z |

m, upregulation (T > N) in p50% of specimens; mm, upregulation in > 50% of specimens. Abbreviations: SCC, squamous-cell carcinoma; CML, chronic myeloid leukemia; AML, acute myeloid leukemia; CLL, chronic lymphoid leukemia; NC, no change in expression between tumor and normal tissues; ND, not determined; Neg, no expression detected in tumor and normal tissues. [a]Refers to p73 isoforms that lack the TA domain (DNp73, $DN^0$p73, Ex2Delp73, and Ex2/3Delp73). [b]Correlation between wild type p53 status and overexpression of the indicated p53 family member. Variable results between independent studies are noted. [c]Select references are representative of the existing literature. References: A, Choi *et al*. (2002); B, Massion *et al*. (2003); C, Cui *et al*. (2005); D, Tokuchi *et al*. (1999); E, Wang *et al*. (2001); F, Liu *et al*. (2006); G, Ribeiro-Silva *et al*. (2005); H, Dominguez *et al*. (2001); I, Dominguez *et al*. (2006); J, Park *et al*. (2000); K, Chi *et al*. (1999a); L, Kovalev *et al*. (1998); M, Douc-Rasy *et al*. (2002); N, Kamiya and Nakazato (2002); O, Wager *et al*. (2006); P, Nylander *et al*. (2002); Q, Tannapfel *et al*. (2001); R, Concin *et al*. (2004); S, Muller *et al*. (2005); T, Ramalho *et al*. (2006); U, Yamaguchi *et al*. (2001); V, Peters *et al*. (1999); W, Rizzo *et al*. (2004); X, Leupin *et al*. (2004); Y, Zamo *et al*. (2005); Z, Cuadros *et al*. (2006). [d]Includes head and neck, lung, nasopharyngeal, and esophageal SCCs. [e]Loss of p63 and/or p73 expression occurs in advanced stages and correlates with poor prognosis. [f]Loss of p73 expression in a subset of specimens due to P1 promoter methylation. [g]Analyses did not discriminate between TA and DN isoforms.

Hu *et al*., 2002; Weber *et al*., 2002; Massion *et al*., 2003; Sniezek *et al*., 2004). Reports have varied as to the frequency of p63 expression in invasive breast carcinomas, with studies ranging from 0 to 30% (Wang *et al*., 2002; Reis-Filho *et al*., 2003; Koker and Kleer, 2004; Ribeiro-Silva *et al*., 2005). It now seems clear, however, that p63 is expressed in at least a subset of breast tumors that are known to exhibit a basal epithelial phenotype (Perou *et al*., 2000).

Although early studies of p63 expression used techniques that do not discriminate among different isoforms, several recent reports have used quantitative isoform-specific reverse transcription-polymerase chain reaction (RT-PCR) coupled with immunoblot analysis to demonstrate that DNp63a is the predominant p63 isoform expressed in squamous cell carcinomas (Table 1). Using such an approach, we recently found that TAp63 overexpression is rare in primary HNSCC tumors and tumor-derived cell lines, and that DNp63 mRNA expression was at least 100-fold more abundant than TAp63 mRNA expression in all cases (Deyoung *et al*., 2006; Rocco *et al*., 2006). These findings are consistent with the inability of many investigators to detect TAp63 protein isoforms by immunoblot analysis in either primary keratinocytes or HNSCC cells. One exception to the consistent overexpression of p63 observed in tumor cells is in bladder cancer. Whereas one study showed that the majority of invasive carcinomas of the bladder overexpress DNp63, others found that a subset of such cancers demonstrate loss of p63 expression (Park *et al*., 2000; Urist *et al*., 2002; Koga *et al*., 2003). In the latter cases, loss of p63 expression was associated with progression to invasion and metastasis and correlated with a poor prognosis. On the other hand, overexpression of DNp63a in squamous cell carcinomas of both the lung and head/neck has been shown to be a favorable indicator of response to therapy and overall clinical outcome (Massion *et al*., 2003; Zangen *et al*., 2005). One possible model to reconcile these findings would be that DNp63a contributes to the early stages of tumorigenesis while maintaining epithelial cell fate (Green *et al*., 2003; Troung *et al*., 2006). In contrast, loss of p63 may mark tumors that have accumulated additional genetic events and have acquired mesenchymal properties, both of which are correlated with a refractory clinical behavior (Barbieri *et al*., 2006).

### *Overexpression of multiple p73 isoforms is observed in diverse human cancers*

Like *p63*, *p73* was initially hypothesized to function as a classical tumor suppressor gene. This supposition was based both on its homology to *p53* and its cytogenetic locus, 1p36.33, which is commonly deleted in a variety of cancers (Kaghad *et al*., 1997). Subsequent studies have clearly demonstrated that *p73* is not the target of deletion at this site. Furthermore, extensive mutational analysis has failed to demonstrate a significant frequency of *p73* mutation in human tumors (o1.0%) (Melino *et al*., 2002; Moll and Slade, 2004). Studies of multiple tumor types have demonstrated that *p73* is overexpressed, rather than mutated or deleted in human cancer. Overexpression of p73 mRNA and/or protein

relative to the respective normal tissues has been demonstrated in a large variety of tumor types, including neuroblastoma, glioma, breast, lung, colon, stomach, ovarian, bladder, liver, HNSCC, cholangiocellular carcinoma, ependymoma, chronic myelogenous leukemia and acute myelogenous leukemia (Table 1) (Moll and Slade, 2004). Many of these studies measured p73 mRNA using semiquantitative approaches and did not examine expression of multiple isoforms. Recent work, however, has provided more comprehensive expression analysis of p73 isoforms. These include TAp73 produced by the P1 promoter and DNp73 produced by the P2 promoter. Additional P1-transcribed N-terminal splice variants exist ($DN^0$p73, Ex2-Delp73 and Ex2/3Delp73) and are all predicted to encode proteins lacking the TA domain (Stiewe *et al*., 2002; Zaika *et al*., 2002; Concin *et al*., 2004). One recent study compared isoform-specific p73 expression levels in a large panel of ovarian tumors. $DN^0$p73 and TAp73 mRNA were found to be co-upregulated in a substantial fraction of tumor samples compared to normal tissues (Concin *et al*., 2004). Similarly, in primary rhabdomyosarcomas and tumor-derived cell lines, DNp73 and TAp73 protein isoforms are co-upregulated compared to normal muscle (Cam *et al*., 2006). Consistent with an important function for truncated p73 isoforms, overexpression of the N-terminal splice variants, but not TAp73 mRNA, was shown to convey a poor prognosis in low-grade gliomas, and was associated with advanced stage in breast and colon carcinomas (Dominguez *et al*., 2006; Wager *et al*., 2006). Of note, methylation-induced silencing of the P1 (TAp73) promoter has been found in lymphoblastic leukemias and Burkitt's lymphoma (Corn *et al*., 1999; Kawano *et al*., 1999). Together with functional data discussed below, these findings imply that either DNp73/DNp63 overexpression or TAp73 promoter silencing may be required to inactivate a tumor-suppressive property of TAp73. In agreement with this view are our recent findings in squamous cell carcinomas. Although TAp73 isoforms were found to be dramatically overexpressed (18–30-fold) in HNSCC-derived cell lines and primary tumors compared to normal basal epithelia, DNp63a was also overexpressed in these tumors and was physically associated with TAp73, thereby inhibiting p73-dependent proapoptotic activity (Deyoung *et al*., 2006; Rocco *et al*., 2006).

## Mechanisms of p63 and p73 of potential relevance to tumorigenesis

### *Cooperation and competition occur among p53 family isoforms*

A complex picture emerges when trying to define the precise contribution of either p63 or p73 to tumor development. As demonstrated above, a major part of this complexity stems from the expression of both DN and TA isoforms of p63 and p73 in many human tumors. Studies described below support the view that TAp63/TAp73 isoforms, like p53, exhibit tumor-suppressive properties, and that upregulation of DNp63/DNp73 isoforms is a common mechanism of their inactivation during tumorigenesis. Indeed, important physical and functional interactions among family members have now been demonstrated in tumor-specific contexts. These diverse interactions are mediated through two general mechanisms. First, cooperation and competition at the conserved p53 family binding sites within promoters of particular shared target genes are likely to regulate interactions among all three family members (Flores *et al*., 2002; Stiewe *et al*., 2002; Yang *et al*., 2006). Second, direct physical interaction of isoforms is known to alter the function of the tetrameric complex required for DNA-binding and transcriptional regulation (Chan *et al*., 2004). In ectopic expression studies, heteromeric complexes have been demonstrated between different isoforms of the same gene, between TAp63 and DNp73 isoforms, and between TAp73 and DNp63 isoforms (Chan *et al*., 2004). In each case, the respective DN isoforms function as potent inhibitors of transactivation by the respective TA isoforms. Consistent with these findings, endogenous complexes have also been demonstrated between different p73 isoforms, and between DNp63 and TAp73 (Deyoung *et al*., 2006; Rocco *et al*., 2006). Wild-type p53 binds p63 and p73 with much lower affinity than p63 and p73 bind one another (Davison *et al*., 1999). Despite the absence of a strong physical interaction, however, it seems highly plausible that in some tumor contexts both DNp63 and DNp73 serve to inhibit the function of p53 through promoter competition or other indirect mechanisms (Stiewe *et al*., 2002).

### *Proliferation and differentiation are regulated by p63 and p73 in tumor cells*

In normal basal epithelial cells, DNp63a promotes proliferation through regulation of shared p53 target genes such as $p21^{Cip1}$, and likely through other pathways as well (Patturajan *et al*., 2002; Westfall *et al*., 2003). Whether these effects depend entirely on the ability of p63 to functionally repress p53 is unclear, as studies have reached different conclusions using different strategies to ablate both p63 and p53 in human and mouse cells (Keyes *et al*., 2005; Deyoung *et al*., 2006; Troung *et al*., 2006). Nevertheless, the ability of DNp63a to enhance proliferation and suppress cellular senescence during development suggests a possible contribution of p63 early in tumorigenesis (Keyes *et al*., 2005). In addition to promoting proliferation, the expression of DNp63a is closely linked to the undifferentiated state in basal epithelial cells, and DNp63a expression is rapidly downregulated in normal cells during the course of differentiation (Parsa *et al*., 1999; Green *et al*., 2003). Although the mechanisms by which p63 may influence differentiation remain largely speculative, some evidence has been provided that TAp63 may directly control the expression of p53-independent genes required for keratinocyte differentiation, including loricrin and involucrin (De Laurenzi *et al*., 2000). p63 isoforms could conceivably also regulate differentiation through

p53-independent effects on adhesive signaling, Notch activation, and regulation of Rb phosphorylation (Cam *et al*., 2006; Carroll *et al*., 2006; Nguyen *et al*., 2006).

Despite their remarkably similar structures, p63 and p73 appear to regulate largely non-overlapping sets of cell-cycle regulatory genes. Although overexpression of TAp73 isoforms leads to induction of p63-regulated cell-cycle inhibitors, it is unclear whether most of these genes are direct targets of endogenous p73 (Harms *et al*., 2004). However, the cyclin-dependent kinase inhibitor $p57^{Kip2}$ is a well-established target gene of p73 (Blint *et al*., 2002). Recent evidence has linked regulation of $p57^{Kip2}$ to a differentiation effect mediated by DNp73. Thus DNp73, which is overexpressed in rhabdomyosarcoma cells, was shown to block both cell-cycle exit and differentiation of murine myoblasts (Cam *et al*., 2006). These effects were shown to be owing to the ability of DNp73 to suppress $p57^{Kip2}$ expression, resulting in pRb inactivation and a consequent block in myogenic differentiation (Cam *et al*., 2006). Notably, both DNp73-dependent repression of $p57^{Kip2}$ and DNp63a-mediated repression of $p21^{Cip1}$ are at least in part owing to direct promoter binding rather than physical interaction with p53 or TAp63/73 isoforms. This example demonstrates that effects of DN isoforms in tumors are not mediated exclusively through a trans-repression effect on TA isoforms. As described below, however, repression through physical interaction with other family members appears to be an additional important mechanism by which DNp63 and DNp73 contribute to tumorigenesis.

*Mouse models point to apoptosis regulation as a tumor-suppressive role for p63 and p73*

Both p63 and p73 isoforms contribute to the regulation of cell survival and apoptosis in human tumors (Moll and Slade, 2004). Indeed, their ability to regulate apoptosis is clearly a major mechanism by which these genes contribute to human tumorigenesis (Rocco *et al*., 2006). Initial studies in mice demonstrated the pro-apoptotic activity of endogenous p63 and p73. Germline deletion of *p63* or *p73* yielded mouse embryo fibroblasts (MEFs) that were less sensitive to DNA damage-induced apoptosis than wild-type cells when transformed by adenoviral E1A protein (Flores *et al*., 2002). This phenotype was recapitulated in the embryonic mouse brain *in vivo*. Defective apoptosis observed in these mice is likely to reflect loss of the respective proapoptotic TA isoforms, which are the predominant isoforms induced in these cells following DNA damage. In agreement with this notion, reconstitution of TAp63 or TAp73, but not DNp63 or DNp73 isoforms into the respective null MEFs rescued apoptosis following doxorubicin treatment to some degree. Subsequent studies in mice with heterozygous deletion of *p63* or *p73* further supported the hypothesis that inactivation of these genes leads to a tumor-prone phenotype, potentially by disabling apoptosis (Flores *et al*., 2005). Both *p63* and *p73* heterozygous mice developed spontaneous tumors characterized in most cases by loss of the respective wild-type alleles (Flores *et al*., 2005). Although these studies seemed to point to a clear role for these genes as tumor suppressors, their lack of mutation in human tumors fails to support this notion. In addition, mice harboring a distinct targeted *p63*-null allele did not develop tumors (Keyes *et al*., 2006). In fact, these animals demonstrated a decreased incidence of tumors and accelerated features of aging. This phenotype was reminiscent of that induced following germline expression of a hypermorphic mutant p53 (Tyner *et al*., 2002), and was seemingly the opposite of the phenotype expected following loss of a tumor suppressor. These disparate results might be attributable to differences in the p63 gene-targeting approach or to other factors (Mills, 2006).

*Multiple mechanisms lead to tumor-specific inactivation of proapoptotic TAp73*

Whether p63 and p73 promote or inhibit human tumorigenesis may depend on the predominant isoform(s) expressed in a given tissue. Most studies concur that TAp73 isoforms exhibit proapoptotic activity and potentially other suppressor properties in tumor cells. For example, several studies have demonstrated that TAp73 is specifically activated and is an important mediator of apoptosis following chemotherapy-induced DNA damage (Gong *et al*., 1999; Yuan *et al*., 1999; Irwin *et al*., 2003). As discussed below, the ability of certain mutant p53 species to specifically inactivate TAp73 provides additional evidence for their anti-tumorigenic properties. Furthermore, p73 is silenced in a number of tumor types through methylation, as described above. Finally, overexpression of DNp63 and DNp73 isoforms appears to be an important and common mechanism for inhibiting the proapoptotic activity of TAp73 in human tumors. Indeed, DNp73 isoforms have been demonstrated to suppress apoptosis induced by expression of either E1A or c-Myc in primary fibroblasts (Petrenko *et al*., 2003). This effect seems at least in part to be owing to transdominant inhibition of p53, and potentially TAp73 and TAp63. Similarly, the DNp63a isoform functions as a survival factor in certain tumors including squamous cell carcinomas, at least in part through its ability to suppress TAp73, which is highly upregulated in these same tumors (Rocco *et al*., 2006).

In addition to expression of DNp63/p73 isoforms, another important mechanism of TAp73 inhibition in tumor cells is through expression of particular p53 mutant proteins. In contrast to the weak binding of wild-type p53, significant binding has been reported between p63/p73 and certain p53 mutants harboring mutations within the DBD (Di Como *et al*., 1999; Gaiddon *et al*., 2001). Rather than binding through the OD, however, interaction with these mutant p53 proteins and p63/p73 occurs through the p53 DBD and the OD of p63/p73 (Gaiddon *et al*., 2001). Furthermore, this interaction is regulated by a common Arg/Pro polymorphism at codon 72 of *p53*. Cancer-associated mutants of p53 that harbor the 72R

polymorphism interact more strongly and are therefore more effective inhibitors than those expressing 72P (Marin *et al*., 2000). This interaction has been shown to inhibit the function of p63/p73 in part through down-regulation of their protein expression. The presence of such mutant p53 forms has been shown to inhibit the ability of p73 to mediate apoptosis during the DNA damage response, thereby promoting chemotherapy resistance (Bergamaschi *et al*., 2003). In a mouse model, germline knock-in of the p53R175H DBD mutation enhanced cellular transformation through an inhibitory effect on p63 and p73 (Lang *et al*., 2004). In human head and neck cancers, p53 mutations in the context of 72R have been correlated with an inferior clinical outcome (Bergamaschi *et al*., 2003). In addition to providing a new twist on the mechanisms of mutant p53, these data provide further support for the contention that inhibiting certain functions of p63 and p73 may be important to promote both tumorigenesis and resistance to therapy. Conceivably, these observations might also explain at least in part why p53 is so commonly targeted for mutation rather than degradation in tumors. Finally, these findings suggest that mutant p53 could in some cases be an attractive therapeutic target, because eliminating its inhibitory function might activate a tumor-suppressive function of p63 and/or p73.

### *A p53/p63/p73 functional network contributes to squamous carcinogenesis*

These numerous and diverse potential functional interactions among the three p53 family members are manifest in distinct, tumor-specific contexts. One example that illustrates this principle is HNSCC (Figure 2). As previously noted, these tumors commonly overexpress DNp63a relative to normal basal epithelial cells. Initially it was speculated that this p63 isoform might inhibit p53 function, thereby abrogating the requirement for *p53* mutation in these tumors. Although an initial study supported this possibility (Hibi *et al*., 2000), subsequent reports have not demonstrated a consistent correlation between p63 overexpression and wild-type *p53* status in these tumors (Hibi *et al*., 2000; Choi *et al*., 2002; Sniezek *et al*., 2004). Instead, recent studies by our group and others have suggested that DNp63a functions as an essential repressor of TAp73, rather than p53 in these tumors. Knockdown of DNp63a by RNA interference (RNAi) induces cell death that is p53-independent but is instead mediated by TAp73. In HNSCC cells, DNp63a binds TAp73, thereby blocking its ability to transactivate proapoptotic bcl-2 family members and to induce cell death (Rocco *et al*., 2006). Consistent with this model, tumor-derived cell lines that lacked DNp63a expression instead exhibited high-level bcl-2 expression, which was sufficient to block TAp73-dependent apoptosis in these cells. Notably, the DNp63a-dependent survival pathway appears tumor-specific, as TAp73 is not highly expressed in primary basal epithelia (Carroll *et al*., 2006). The absence of significant TAp73 expression in normal primary cells may explain why p63 inhibition in these cells triggers senescence rather than the high levels of cell death observed in tumor cells (Deyoung *et al*., 2006). In an ironic twist, the requirement for DNp63a to suppress TAp73-dependent apoptosis in HNSCC cells may in fact explain why a correlation has been observed in some studies between *p53* mutation and absence of DNp63a overexpression in these tumors. As mentioned above, it is predicted that certain p53 mutant proteins that also contain the 72R polymorphism are able to functionally inhibit TAp73. Therefore, tumors that express these particular mutant p53 proteins may not require DNp63a upregulation to inhibit TAp73 (Figure 2). As a result, low DNp63a expression might be predicted in tumors that express this subset of p53 mutant proteins. Although confirmation of such an association awaits further studies, collectively these data demonstrate the existence of a functional p53 family network relevant to human cancer.

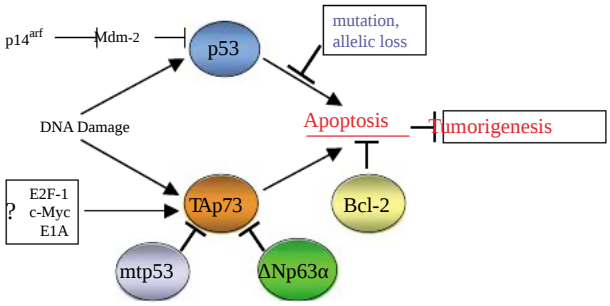


**Figure 2** Pathways involving the p53 family functional network in squamous cell carcinoma. DNA damage and oncogenic stress (through $p14^{arf}$) trigger p53 activation, leading to selective pressure for its mutation or loss to avoid apoptosis. Similarly, DNA damage and potentially oncogenic stress induce TAp73 expression and activation. TAp73 activity can be suppressed through overexpression of DNp63a or certain missense p53 mutants (mtp53). Alternatively, apoptosis can be averted in DNp63a-negative tumors through overexpression of bcl-2, which inhibits the proapoptotic function of TAp73 target genes.

## Upstream regulation of p63 and p73 transcription

### *Oncogenic stress induces TAp73 expression*

As illustrated by the above examples, regulation of *p63* and *p73* expression in different tumor and tissue types dictates to a large degree how these genes contribute to human cancer. Both *p63* and *p73* exhibit transcriptional regulation through distinct, largely non-overlapping upstream pathways. Although most studies have focused their attention on the P1 promoters of *p63* and *p73* that drive expression of the respective TA isoforms, recent work has begun to explore the internal promoters found within intron 3 (P2) that regulate transcription of DNp63 and DNp73. Thus far, it appears that the P1 and P2 promoters of both genes possess distinct regulatory elements. In the case of p73, endogenous TAp73a and TAp73b are known to be induced by oncogenic stress through activation of E2F-1 and potentially c-Myc, as well as expression of the viral oncoprotein E1A (Melino

*et al*., 2002). E2F-1 regulates TAp73 transcription directly by binding to E2F sites within the P1 promoter (Irwin *et al*., 2000; Seelan *et al*., 2002). The view that TAp73 is an important endogenous E2F-1 target gene is supported by recent work on the mechanism by which E1A increases TAp73 levels. E1A expression in tumor cells induces both TAp73 mRNA and protein, and this induction is dependent on both the Rb-binding domain within E1A and the E2F-1-binding site within the p73 P1 promoter (Flinterman *et al*., 2005). Furthermore, E1A-induced activation of p73 is sufficient to cause apoptosis in p53-mutant tumor cells, in keeping with the notion that activation of p73 in this setting is functionally relevant (Flinterman *et al*., 2005). Additional evidence suggests that E2F-1-dependent transcription of TAp73 may be important to the DNA damage response in p53-mutant cells. The checkpoint kinases Chk1 and Chk2 can regulate TAp73 transcription following genotoxic insults via stabilization and consequent activation of E2F-1 (Urist *et al*., 2004). The role of the E2F-1-TAp73 pathway in normal cells is more controversial, as different experimental approaches have led to different conclusions as to whether induction of p73 is required *in vivo* for E2F-1-dependent apoptosis in lymphocytes (Wan and DeGregori, 2003; Senoo *et al*., 2004). Another area of controversy is the role of c-myc in the regulation of TAp73 expression and activity. Studies have demonstrated the ability of c-myc to increase TAp73 protein levels, as well as the possibility of both negative and positive effects on p73-dependent transcription through physical interaction between c-myc and TAp73 (Zaika *et al*., 2001; Uramoto *et al*., 2002; Watanabe *et al*., 2002). Finally, several studies have pointed to an important role for DNA methylation in the regulation of TAp73 expression. One intriguing example is methylation within the TAp73 first intron that affects sites for the ZEB transcriptional repressor (Fontemaggi *et al*., 2001). Lack of ZEB binding as a result of methylation of its binding site appears to be a mechanism for tissue-specific regulation of TAp73 during cellular differentiation. In contrast to TAp73, few direct mechanisms that regulate transcription of TAp63 have been defined. It does seem clear, however, that TAp63 is not induced by prominent regulators of TAp73 such as E2F-1 (Waltermann *et al*., 2003).

*Expression of p63 and p73 is regulated through novel feedback loops*

Several potential autoregulatory feedback loops have been proposed for the regulation of *p53* family members. The P2 promoter of *p73* has been shown to be positively regulated by p53 and TAp73, leading to the induction of DNp73 (Grob *et al*., 2001; Nakagawa *et al*., 2002). These findings imply a potential negative feedback loop that may restrict p53 and TAp73-dependent transcription. Both positive and negative regulation of DNp63 mRNA and protein have been shown by different groups to be mediated by both p53 and DNp63 itself (Harmes *et al*., 2003; Waltermann *et al*., 2003; Antonini *et al*., 2006; Romano *et al*., 2006). DNp63 may also be positively regulated by TAp63g in a manner that is independent of p53, suggesting yet another possible feedback mechanism (Li *et al*., 2006). Additional feedback mechanisms with potential relevance to human cancer have been proposed and involve distinct p63 target genes. For example, DNp63a expression is promoted by epidermal growth factor receptor (EGFR) signaling through activation of the phosphatidylinositol 3-kinase (PI3K) pathway in both normal keratinocytes and SCC cells (Barbieri *et al*., 2003; Matheny *et al*., 2003). In turn, EGFR itself is a transcriptional target of p63 (Nishi *et al*., 2001; Carroll *et al*., 2006), implying positive feedback regulation through p63. Similarly, Notch signaling, which is important in many human tumors and which limits proliferation and promotes differentiation in keratinocytes, has been shown to inhibit DNp63a transcription. DNp63a, however, appears to function as a direct repressor of some Notch-induced genes, suggesting equilibrium between Notch and p63 activity in keratinocytes and potentially some cancers (Nguyen *et al*., 2006). Detailed functional interrogation of these pathways in tumor cells will be important to define which of these interactions may be relevant in a cancer-specific context.

## Regulation of p63 and p73 activity

*Ubiquitin-like modifications regulate both p63 and p73*

In addition to regulation through altered transcription, multiple post-translational mechanisms are being uncovered that regulate the expression and function of particular p63 and p73 isoforms. These mechanisms are likely to have important implications for future therapeutic targeting of p63 and p73 in human cancer. One emerging theme in p63/p73 regulation involves ubiquitin and ubiquitin-like modifications (Watson and Irwin, 2006). MDM2 is an E3 ubiquitin ligase that functions as an essential negative regulator of p53 through ubiquitination and subsequent proteasome degradation (Momand *et al*., 2000). Notably, all three essential residues of p53 that contact MDM2 (F19, W23 and L26) are conserved in both TAp63 and TAp73 (Kussie *et al*., 1996; Kaghad *et al*., 1997; Yang *et al*., 1998). Although it has been shown that MDM2 can associate with TAp73, MDM2 apparently does not induce ubiquitination or degradation even though it does inhibit p73 transcriptional activity (Balint *et al*., 1999; Zeng *et al*., 1999). A recent study suggests that MDM2-dependent inhibition of TAp73 may be required for cellular transformation. A short isoform of p21 ras, p19 ras, was found to suppress ras-induced transformation by abrogating the p73-MDM2 interaction, leading to p73 activation (Jeong *et al*., 2006). Whether MDM2 associates significantly with p63 or plays a role in regulating p63 stability and activity remains inconclusive (Little and Jochemsen, 2001; Calabro *et al*., 2002).

Additional E3 ubiquitin ligases have been found to interact with p73 and p63. NEDL2, a NEDD4-related E3 ubiquitin ligase has been shown to bind to a C-terminal proline-rich (PY) motif found in p73a and b isoforms (Miyazaki *et al*., 2003). NEDL2-dependent ubiquitination of p73 promotes its stabilization and increased TAp73 transcriptional activity by an unknown mechanism. In contrast, another NEDD4-related E3 ligase named Itch has been shown to interact with the PY motif of endogenous p63a and p73a (both TA and DN isoforms), thereby promoting their proteasome-dependent degradation (Rossi *et al*., 2005, 2006a, b). Itch does not interact with p53, which lacks a PY motif. Consistent with these findings, primary keratinocytes derived from Itch knockout mice have increased levels of DNp63a (Rossi *et al*., 2006a). The p73a and p63a isoforms can also be regulated by conjugation with the small ubiquitin-like modifier SUMO-1. Sumoylation was shown to modestly inhibit the transcriptional activity of p73 and p63 (Minty *et al*., 2000; Ghioni *et al*., 2005). However, the relatively small fraction of total cellular p63 and p73 found to be sumoylated may not be sufficient to significantly inhibit their overall transactivational functions (Blandino and Dobbelstein, 2004). Another unresolved issue regarding these findings is that neither the E3 ubiquitin nor SUMO ligases have been shown to clearly distinguish between TA and DN isoforms of p63 and p73.

*Kinase-dependent pathways contribute to both positive and negative regulation of p63 and p73*

Multiple phosphorylation events are likely to be important for regulation of p63 and p73 in both normal and tumor cells. TAp73, an important mediator of the DNA damage response in tumor cells, is phosphorylated at Tyr-99 by the c-Abl kinase in response to a subset of DNA damaging agents, potentiating p73-dependent apoptosis (Agami *et al*., 1999; Gong *et al*., 1999; Yuan *et al*., 1999). Phosphorylation of p73 promotes its association with the prolyl isomerase Pin1, which in turn enhances acetylation of p73 by p300 (Mantovani *et al*., 2004). Both p73 acetylation and its association with Pin1 have been shown to stabilize p73 following chemotherapeutic exposure. Another DNA damage-induced pathway for p73 regulation involves association of TAp73a with the checkpoint kinase Chk1 (Gonzalez *et al*., 2003). This association results in Ser-47 phosphorylation of TAp73a and increased proapoptotic activity. Kinase-dependent signaling also contributes to negative regulation of TAp73 activity. Kinase pathways that appear to be involved in restraining p73 proapoptotic activity include the PI3K/AKT pathway, the PKA pathway and the CDK pathway (Basu *et al*., 2003; Gaiddon *et al*., 2003; Hanamoto *et al*., 2005). One such example is phosphorylation-dependent regulation of the Yes-associated protein, YAP, a potent co-activator of p73-dependent transcription (Strano *et al*., 2001). AKT-dependent inhibition of TAp73a involves phosphorylation of YAP, which promotes association of YAP with 14-3-3 and subsequent nuclear export (Basu *et al*., 2003). Data suggest that YAP might be particularly important in promoting the proapoptotic function of TAp73 following DNA damage (Strano *et al*., 2005).

The precise contribution of phosphorylation to the regulation of DNp63 and DNp73 isoforms remains largely unknown. Increased phosphorylation of DNp63a at Ser-66/68 and Ser-361 was observed in response to ultraviolet (UV) radiation and chemotherapeutic exposure (Westfall *et al*., 2005). This phosphorylation correlated with downregulation of DNp63a in epithelial cells following these stimuli. A similar effect was demonstrated following treatment with the chemotherapy agent cisplatin. DNp63a was phosphorylated in response to cisplatin treatment, which promoted its association with stratifin (also known as 14-3-3s) (Fomenkov *et al*., 2004). This interaction was shown to mediate nuclear export of DNp63a into the cytoplasm where RACK1 (receptor for activated protein C kinase 1) targets DNp63a for proteasomal degradation. RACK1 itself does not contain any motifs characteristic of E3 ubiquitin ligases, but it remains conceivable that RACK1 facilitates the degradation of DNp63a by recruiting an unidentified E3 ubiquitin ligase. Of note, DNp73 is also known to undergo rapid downregulation following treatment with DNA damaging agents (Maisse *et al*., 2004). Although it is unknown whether this degradation is phosphorylation-dependent, it was shown to be independent of the Itch ubiquitin ligase, which is itself downregulated following DNA damage (Rossi *et al*., 2005). Despite many unanswered questions, a consistent picture emerges whereby certain forms of DNA damage induce an apoptotic response mediated at least in part through degradation of anti-apoptotic DN isoforms and stabilization of proapoptotic TA isoforms.

## Oncogenesis versus tumor suppression mediated by p63 and p73

Collectively, the data summarized above point to important contributions of both p63 and p73 to human tumorigenesis. The complexity in defining their specific contribution results to a large degree from tissue- and tumor-specific differences in isoform expression. As noted above, defective apoptosis observed in *p63*- and *p73*-null mouse fibroblasts is likely to reflect loss of the respective proapoptotic TA isoforms (Flores *et al*., 2002). Similarly, the tumor-prone phenotype of the respective heterozygous mice may be linked mechanistically to loss of these particular isoforms. Tumors arising in *p63*- and *p73*-heterozygous mice are known to exhibit loss of the respective wild-type alleles, presumably reflecting a selective pressure to inactivate the TA isoforms (Flores *et al*., 2005). Nevertheless, human cancers rarely utilize mutation or deletion of *p63* and *p73* as mechanisms for inactivation of these genes. In a subset of tumors, inhibition is achieved by methylation-induced promoter silencing. In others, overexpression of

either DNp63 or DNp73 isoforms likely contributes to functional suppression of TAp63, TAp73, and probably p53, thereby promoting tumorigenesis.

Inhibition of apoptosis mediated by TAp63/TAp73 isoforms is likely to explain one contribution of DNp63 and DNp73 to human cancer. However, it still remains to be determined whether apoptosis is the sole function of TAp63 and TAp73 that is essential for their proposed tumor-suppressive property. In addition, it remains to be established to what degree DNp63 and DNp73 isoforms function exclusively as trans-suppressors of TA isoforms, or, perhaps more likely, whether they regulate additional TA-independent pathways. As noted, TAp73 isoforms are well-established mediators of apoptosis in both normal and tumor cells. TAp63 isoforms are not as prominent in this regard, although recent data support their role as context-specific proapoptotic factors (Jacobs *et al*., 2005; Suh *et al*., 2006). Nevertheless, several additional p63- and p73-dependent pathways relevant to human cancer have been defined. Evidence exists for contributions of p63 and p73 target genes to proliferation, differentiation, cellular adhesion, growth factor signaling, angiogenesis and other pathways (Senoo *et al*., 2002; Barbieri *et al*., 2005; Cam *et al*., 2006; Carroll *et al*., 2006; Nguyen *et al*., 2006; Troung *et al*., 2006). These functions may be particularly relevant for p63, which seems to exhibit a multifaceted developmental role that is less tightly linked to suppression of apoptosis than that of p73. More sophisticated *in vivo* models, coupled with isoform-specific knockout and RNAi knockdown approaches will be required to clarify which among these pathways are most relevant for human tumorigenesis.

## Future therapeutic targeting of p63 and p73 in human cancer

Ultimately, it is hoped that understanding how p63 and p73 contribute to tumorigenesis may lead to new therapeutic approaches for the relevant cancers. In the near term, expression of particular p63 and/or p73 isoforms may prove to be useful clinical markers of sensitivity to specific chemotherapeutic agents. For example, recent data have suggested that expression of p63 in SCCs is correlated with the therapeutic response to cisplatin (Zangen *et al*., 2005). This observation may be linked to the ability of cisplatin to specifically activate TAp73, which is co-overexpressed with DNp63a in SCC tumors but not normal epithelial cells (Deyoung *et al*., 2006). DNp63a normally suppresses the potent pro-apoptotic activity of TAp73 in tumor cells, but cisplatin treatment induces profound downregulation of DNp63a (Figure 3) (Fomenkov *et al*., 2004). At the same time, TAp73 undergoes a variety of post-translational modifications that are required for its activation following cisplatin treatment (Oberst *et al*., 2005). Thus, down-regulation of DNp63a and activation of TAp73 mediate cisplatin sensitivity in these tumors. We recently showed that ablation of TAp73 isoforms in SCC cells induced

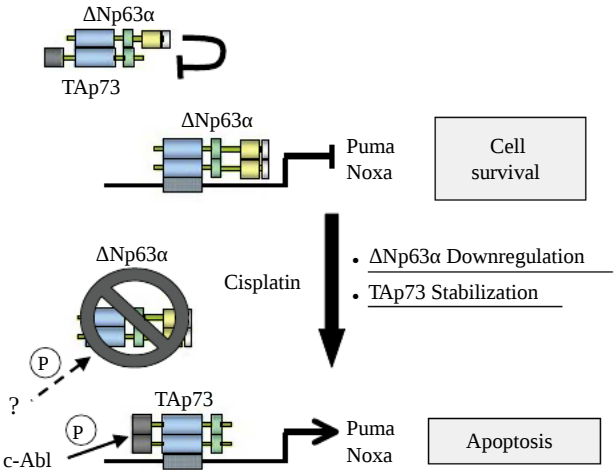


**Figure 3** p63/p73 pathway mediates cisplatin sensitivity in squamous carcinoma cells. In proliferating tumor cells, DNp63a inhibits the proapoptotic transcriptional activity of TAp73 through both direct physical interaction and through direct binding to the promoters of TAp73 target genes. Cisplatin treatment induces DNp63a downregulation and TAp73 stabilization, thereby activating the TAp73-dependent apoptotic program. Phosphorylation of TAp73 by c-Abl and potentially other kinases is important for its activation following DNA damage. Phosphorylation may also contribute to downregulation of DNp63a in this context. Note that proteins are shown schematically as dimers but in fact are thought to bind DNA as tetramers.

marked cisplatin resistance, supporting a TAp73-dependent pathway for sensitivity in these cells (Rocco *et al*., 2006). Given that they are direct mediators of chemotherapy-induced apoptosis, expression of these particular p63 and p73 isoforms could predict a favorable therapeutic response in a variety of tumors.

Much remains to be learned about the mechanisms that mediate the complex interplay between different p63 and p73 isoforms under conditions such as genotoxic stress. Beyond providing a potential means to predict chemosensitivity, understanding these mechanisms may provide new and more specific ways to target these proteins themselves for cancer therapy. Although promising p53-directed therapies have been slow to emerge, it is conceivable that p63 and p73 might be more amenable to therapeutic targeting. In support of this concept, recent data from several groups have demonstrated that established tumor cells remain dependent in some fashion on p63/p73 expression (Barbieri *et al*., 2006; Cam *et al*., 2006; Rocco *et al*., 2006). Thus, ablation of DNp73, which is overexpressed in rhabdomyosarcoma cells, can lead to growth arrest and differentiation (Cam *et al*., 2006). Similarly knockdown of DNp63, overexpressed in SCCs, induces apoptosis (Rocco *et al*., 2006).

Of particular interest for future targeting of p63 and p73 are the variety of post-translational modifications that regulate the stability and activity of particular isoforms. Thus, inhibiting ubiquitin or SUMO ligases that target TAp63/TAp73 isoforms could stabilize these proteins and thereby promote a selective therapeutic response in tumors that overexpress these isoforms.

Unfortunately, the apparent lack of TA/DN isoform specificity in the modifications identified to date may limit the usefulness of this approach. Alternatively, targeting specific kinases may be a more attractive possibility, given the ability of several kinase pathways to inhibit the function of p73. Theoretically, targeting the appropriate kinase in the correct tumor-specific context could have profound effects through p63- or p73-dependent pathways. Such therapeutic effects hold some promise of sparing normal tissues, given the large number of examples of tumor-specific expression of particular p63 and p73 isoforms. Undoubtedly, a more detailed understanding of the diverse contributions of p63 and p73 to human cancer could ultimately yield a variety of more effective therapeutic strategies for refractory malignancies.

**Acknowledgements**

The authors thank James Rocco, Nabeel Bardeesy and Chee-Onn Leong for helpful discussions and for critical review of the paper. This work was supported by NIH/NICDR DE-15945, by the Mary Kay Ash Charitable Foundation, and by the Tracey Davis Memorial Fund.

**References**

Agami R, Blandino G, Oren M, Shaul Y. (1999). Interaction of c-abl and p73a and their collaboration to induce apoptosis. *Nature* **399**: 809–813.

Antonini D, Rossi B, Han R, Minichiello A, Di Palma T, Corrado M *et al*. (2006). An autoregulatory loop directs the tissue-specific expression of p63 through a long-range evolutionarily conserved enhancer. *Mol Cell Biol* **26**: 3308–3318.

Balint E, Bates S, Vousden KH. (1999). Mdm2 binds p73 alpha without targeting degradation. *Oncogene* **18**: 3923–3929.

Barbieri CE, Barton CE, Pietenpol JA. (2003). Delta Np63 alpha expression is regulated by the phosphoinositide 3-kinase pathway. *J Biol Chem* **278**: 51408–51414.

Barbieri CE, Perez CA, Johnson KN, Ely KA, Billheimer D, Pietenpol JA. (2005). IGFBP-3 is a direct target of transcriptional regulation by DeltaNp63alpha in squamous epithelium. *Cancer Res* **65**: 2314–2320.

Barbieri CE, Pietenpol JA. (2006). p63 and epithelial biology. *Exp Cell Res* **312**: 695–706.

Barbieri CE, Tang LJ, Brown KA, Pietenpol JA. (2006). Loss of p63 leads to increased cell migration and up-regulation of genes involved in invasion and metastasis. *Cancer Res* **66**: 7589–7597.

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

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

Bjorkqvist AM, Husgafvel-Pursiainen K, Anttila S, Karjalainen A, Tammilehto L, Mattson K *et al*. (1998). DNA gains in 3q occur frequently in squamous cell carcinoma of the lung, but not in adenocarcinoma. *Genes Chromosomes Cancer* **22**: 79–82.

Blandino G, Dobbelstein M. (2004). p73 and p63: why do we still need them? *Cell Cycle* **3**: 886–894.

Blint E, Phillips AC, Kozlov S, Stewart CL, Vousden KH. (2002). Induction of p57(KIP2) expression by p73beta. *Proc Natl Acad Sci USA* **99**: 3529–3534.

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

Calabro V, Mansueto G, Parisi T, Vivo M, Calogero RA, La Mantia G. (2002). The human MDM2 oncoprotein increases the transcriptional activity and the protein level of the p53 homolog p63. *J Biol Chem* **277**: 2674–2681.

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

Carroll DK, Carroll JS, Leong CO, Cheng F, Brown M, Mills AA *et al*. (2006). p63 regulates an adhesion programme and cell survival in epithelial cells. *Nat Cell Biol* **8**: 551–561.

Chan WM, Siu WY, Lau A, Poon RY. (2004). How many mutant p53 molecules are needed to inactivate a tetramer? *Mol Cell Biol* **24**: 3536–3551.

Chi SG, Chang SG, Lee SJ, Lee CH, Kim JI, Park JH. (1999a). Elevated and biallelic expression of p73 is associated with progression of human bladder cancer. *Cancer Res* **59**: 2791–2793.

Chi SW, Ayed A, Arrowsmith CH. (1999b). Solution structure of a conserved C-terminal domain of p73 with structural homology to the SAM domain. *EMBO J* **18**: 4438–4445.

Choi HR, Batsakis JG, Zhan F, Sturgis E, Luna MA, El-Naggar AK. (2002). Differential expression of p53 gene family members p63 and p73 in head and neck squamous tumorigenesis. *Hum Pathol* **33**: 158–164.

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

Corn PG, Kuerbitz SJ, van Noesel MM, Esteller M, Compitello N, Baylin SB *et al*. (1999). Transcriptional silencing of the p73 gene in acute lymphoblastic leukemia and Burkitt's lymphoma is associated with $5^0$ CpG island methylation. *Cancer Res* **59**: 3352–3356.

Courtois S, de Fromentel CC, Hainaut P. (2004). p53 protein variants: structural and functional similarities with p63 and p73 isoforms. *Oncogene* **23**: 631–638.

Cuadros M, Ribas G, Fernandez V, Rivas C, Benitez J, Martinez-Delgado B. (2006). Allelic expression and quantitative RT-PCR study of TAp73 and DeltaNp73 in non-Hodgkin's lymphomas. *Leuk Res* **30**: 170–177.

Cui R, He J, Mei R, de Fromentel CC, Martel-Planche G, Taniere P *et al*. (2005). Expression of p53, p63, and p73 isoforms in squamous cell carcinoma and adenocarcinoma of esophagus. *Biochem Biophys Res Commun* **336**: 339–345.

Davison TS, Vagner C, Kaghad M, Ayed A, Caput D, Arrowsmith CH. (1999). p73 and p63 are homotetramers capable of weak heterotypic interactions with each other but not with p53. *J Biol Chem* **274**: 18709–18714.

De Laurenzi V, Costanzo A, Barcaroli D, Terrinoni A, Falco M, Annicchiarico-Petruzzelli M *et al*. (1998). Two new p73

splice variants, gamma and delta, with different transcriptional activity. *J Exp Med* **188**: 1763–1768.

De Laurenzi V, Melino G. (2000). Evolution of functions within the p53/p63/p73 family. *Ann NY Acad Sci* **926**: 90–100.

De Laurenzi V, Rossi A, Terrinoni A, Barcaroli D, Levrero M, Costanzo A *et al*. (2000). p63 and p73 transactivate differentiation gene promoters in human keratinocytes. *Biochem Biophys Res Commun* **273**: 342–346.

Deyoung MP, Johannessen CM, Leong CO, Faquin W, Rocco JW, Ellisen LW. (2006). Tumor-Specific p73 Up-regulation Mediates p63 Dependence in Squamous Cell Carcinoma. *Cancer Res* **66**: 9362–9368.

Di Como CJ, Gaiddon C, Prives C. (1999). p73 function is inhibited by tumor-derived p53 mutants in mammalian cells. *Mol Cell Biol* **19**: 1438–1449.

Dohn M, Zhang S, Chen X. (2001). p63a and DNp63a can induce cell arrest and apoptosis and differentially regulate p53 target genes. *Oncogene* **20**: 3193–3205.

Dominguez G, Silva JM, Silva J, Garcia JM, Sanchez A, Navarro A *et al*. (2001). Wild type p73 overexpression and high-grade malignancy in breast cancer. *Breast Cancer Res Treat* **66**: 183–190.

Dominguez G, Garcia JM, Pena C, Silva J, Garcia V, Martinez L *et al*. (2006). DeltaTAp73 upregulation correlates with poor prognosis in human tumors: putative *in vivo* network involving p73 isoforms, p53, and E2F-1. *J Clin Oncol* **24**: 805–815.

Douc-Rasy S, Barrois M, Echeynne M, Kaghad M, Blanc E, Raguenez G *et al*. (2002). DeltaN-p73alpha accumulates in human neuroblastic tumors. *Am J Pathol* **160**: 631–639.

Fillippovich I, Sorokina N, Gatei M, Haupt Y, Hobson K, Moallem E *et al*. (2001). Transactivation-deficient p73alpha (p73Deltaexon2) inhibits apoptosis and competes with p53. *Oncogene* **20**: 514–522.

Flinterman M, Guelen L, Ezzati-Nik S, Killick R, Melino G, Tominaga K *et al*. (2005). E1A activates transcription of p73 and Noxa to induce apoptosis. *J Biol Chem* **280**: 5945–5959.

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

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

Fomenkov A, Zangen R, Huang YP, Osada M, Guo Z, Fomenkov T *et al*. (2004). RACK1 and stratifin target DeltaNp63alpha for a proteasome degradation in head and neck squamous cell carcinoma cells upon DNA damage. *Cell Cycle* **3**: 1285–1295.

Fontemaggi G, Gurtner A, Strano S, Higashi Y, Sacchi A, Piaggio G *et al*. (2001). The transcriptional repressor ZEB regulates p73 expression at the crossroad between proliferation and differentiation. *Mol Cell Biol* **21**: 8461–8470.

Gaiddon C, Lokshin M, Ahn J, Zhang T, Prives C. (2001). 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* **21**: 1874–1887.

Gaiddon C, Lokshin M, Gross I, Levasseur D, Taya Y, Loeffler JP *et al*. (2003). Cyclin-dependent kinases phosphorylate p73 at threonine 86 in a cell cycle-dependent manner and negatively regulate p73. *J Biol Chem* **278**: 27421–27431.

Ghioni P, Bolognese F, Duijf PH, Van Bokhoven H, Mantovani R, Guerrini L. (2002). Complex transcriptional effects of p63 isoforms: identification of novel activation and repression domains. *Mol Cell Biol* **22**: 8659–8668.

Ghioni P, D'Alessandra Y, Mansueto G, Jaffray E, Hay RT, La Mantia G *et al*. (2005). The protein stability and transcriptional activity of p63alpha are regulated by SUMO-1 conjugation. *Cell Cycle* **4**: 183–190.

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

Gonzalez S, Prives C, Cordon-Cardo C. (2003). p73alpha regulation by Chk1 in response to DNA damage. *Mol Cell Biol* **23**: 8161–8171.

Green H, Easley K, Iuchi S. (2003). Marker succession during the development of keratinocytes from cultured human embryonic stem cells. *Proc Natl Acad Sci USA* **100**: 15625–15630.

Grob TJ, Novak U, Maisse C, Barcaroli D, Luthi AU, Pirnia F *et al*. (2001). Human delta Np73 regulates a dominant negative feedback loop for TAp73 and p53. *Cell Death Differ* **8**: 1213–1223.

Hagiwara K, McMenamin MG, Miura K, Harris CC. (1999). Mutational analysis of the p63/p73L/p51/p40/CUSP/KET gene in human cancer cell lines using intronic primers. *Cancer Res* **59**: 4165–4169.

Hanamoto T, Ozaki T, Furuya K, Hosoda M, Hayashi S, Nakanishi M *et al*. (2005). Identification of protein kinase A catalytic subunit beta as a novel binding partner of p73 and regulation of p73 function. *J Biol Chem* **280**: 16665–16675.

Harmes DC, Bresnick E, Lubin EA, Watson JK, Heim KE, Curtin JC *et al*. (2003). Positive and negative regulation of deltaN-p63 promoter activity by p53 and deltaN-p63-alpha contributes to differential regulation of p53 target genes. *Oncogene* **22**: 7607–7616.

Harms K, Nozell S, Chen X. (2004). The common and distinct target genes of the p53 family transcription factors. *Cell Mol Life Sci* **61**: 822–842.

Helton ES, Zhu J, Chen X. (2006). The unique NH2-terminally deleted (DeltaN) residues, the PXXP motif, and the PPXY motif are required for the transcriptional activity of the DeltaN variant of p63. *J Biol Chem* **281**: 2533–2542.

Hibi K, Trink B, Patturajan M, Westra WH, Caballero OL, Hill DE *et al*. (2000). AIS is an oncogene amplified in squamous cell carcinoma. *Proc Natl Acad Sci USA* **97**: 5462–5467.

Hu H, Xia SH, Li AD, Xu X, Cai Y, Han YL *et al*. (2002). Elevated expression of p63 protein in human esophageal squamous cell carcinomas. *Int J Cancer* **102**: 580–583.

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

Irwin MS, Kondo K, Marin MC, Cheng LS, Hahn WC, Kaelin Jr WG. (2003). Chemosensitivity linked to p73 function. *Cancer Cell* **3**: 403–410.

Ishimoto O, Kawahara C, Enjo K, Obinata M, Nukiwa T, Ikawa S. (2002). Possible oncogenic potential of DeltaNp73: a newly identified isoform of human p73. *Cancer Res* **62**: 636–641.

Jacobs WB, Govoni G, Ho D, Atwal JK, Barnabe-Heider F, Keyes WM *et al*. (2005). p63 is an essential proapoptotic protein during neural development. *Neuron* **48**: 743–756.

Jeong MH, Bae J, Kim WH, Yoo SM, Kim JW, Song PI *et al*. (2006). p19ras interacts with and activates p73 by involving the MDM2 protein. *J Biol Chem* **281**: 8707–8715.

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

Kamiya M, Nakazato Y. (2002). The expression of p73, p21 and MDM2 proteins in gliomas. *J Neurooncol* **59**: 143–149.
Kawano S, Miller CW, Gombart AF, Bartram CR, Matsuo Y, Asou H *et al*. (1999). Loss of p73 gene expression in leukemias/lymphomas due to hypermethylation. *Blood* **94**: 1113–1120.
Keyes WM, Wu Y, Vogel H, Guo X, Lowe SW, Mills AA. (2005). p63 deficiency activates a program of cellular senescence and leads to accelerated aging. *Genes Dev* **19**: 1986–1999.
Keyes WM, Vogel H, Koster MI, Guo X, Qi Y, Petherbridge KM *et al*. (2006). p63 heterozygous mutant mice are not prone to spontaneous or chemically induced tumors. *Proc Natl Acad Sci USA* **103**: 8435–8440.
Koga F, Kawakami S, Fujii Y, Saito K, Ohtsuka Y, Iwai A *et al*. (2003). Impaired p63 expression associates with poor prognosis and uroplakin III expression in invasive urothelial carcinoma of the bladder. *Clin Cancer Res* **9**: 5501–5507.
Koker MM, Kleer CG. (2004). p63 expression in breast cancer: a highly sensitive and specific marker of metaplastic carcinoma. *Am J Surg Pathol* **28**: 1506–1512.
Kovalev S, Marchenko N, Swendeman S, LaQuaglia M, Moll UM. (1998). Expression level, allelic origin, and mutation analysis of the p73 gene in neuroblastoma tumors and cell lines. *Cell Growth Differ* **9**: 897–903.
Kussie PH, Gorina S, Marechal V, Elenbaas B, Moreau J, Levine AJ *et al*. (1996). Structure of the MDM2 oncoprotein bound to the p53 tumor suppressor transactivation domain. *Science* **274**: 948–953.
Lang GA, Iwakuma T, Suh YA, Liu G, Rao VA, Parant JM *et al*. (2004). Gain of function of a p53 hot spot mutation in a mouse model of Li–Fraumeni syndrome. *Cell* **119**: 861–872.
Leupin N, Luthi A, Novak U, Grob TJ, Hugli B, Graber H *et al*. (2004). P73 status in B-cell chronic lymphocytic leukaemia. *Leuk Lymphoma* **45**: 1205–1207.
Li N, Li H, Cherukuri P, Farzan S, Harmes DC, DiRenzo J. (2006). TA-p63-gamma regulates expression of DeltaN-p63 in a manner that is sensitive to p53. *Oncogene* **25**: 2349–2359.
Little NA, Jochemsen AG. (2001). Hdmx and Mdm2 can repress transcription activation by p53 but not by p63. *Oncogene* **20**: 4576–4580.
Liu G, Nozell S, Xiao H, Chen X. (2004). DeltaNp73beta is active in transactivation and growth suppression. *Mol Cell Biol* **24**: 487–501.
Liu SS, Chan KY, Cheung AN, Liao XY, Leung TW, Ngan HY. (2006). Expression of deltaNp73 and TAp73alpha independently associated with radiosensitivities and prognoses in cervical squamous cell carcinoma. *Clin Cancer Res* **12**: 3922–3927.
Maisse C, Munarriz E, Barcaroli D, Melino G, De Laurenzi V. (2004). DNA damage induces the rapid and selective degradation of the DeltaNp73 isoform, allowing apoptosis to occur. *Cell Death Differ* **11**: 685–687.
Mantovani F, Piazza S, Gostissa M, Strano S, Zacchi P, Mantovani R *et al*. (2004). Pin1 links the activities of c-Abl and p300 in regulating p73 function. *Mol Cell* **14**: 625–636.
Marin MC, Jost CA, Brooks LA, Irwin MS, O'Nions J, Tidy JA *et al*. (2000). A common polymorphism acts as an intragenic modifier of mutant p53 behaviour. *Nat Genet* **25**: 47–54.
Massion PP, Taflan PM, Jamshedur Rahman SM, Yildiz P, Shyr Y, Edgerton ME *et al*. (2003). Significance of p63 amplification and overexpression in lung cancer development and prognosis. *Cancer Res* **63**: 7113–7121.
Matheny KE, Barbieri CE, Sniezek JC, Arteaga CL, Pietenpol JA. (2003). Inhibition of epidermal growth factor receptor signaling decreases p63 expression in head and neck squamous carcinoma cells. *Laryngoscope* **113**: 936–939.
Melino G, De Laurenzi V, Vousden KH. (2002). p73: Friend or foe in tumorigenesis. *Nat Rev Cancer* **2**: 605–615.
Melino G, Lu X, Gasco M, Crook T, Knight RA. (2003). Functional regulation of p73 and p63: development and cancer. *Trends Biochem Sci* **28**: 663–670.
Mills AA. (2006). p63: oncogene or tumor suppressor? *Curr Opin Genet Dev* **16**: 38–44.
Mills AA, Zheng B, Wang XJ, Vogel H, Roop DR, Bradley A. (1999). p63 is a p53 homologue required for limb and epidermal morphogenesis. *Nature* **398**: 708–713.
Minty A, Dumont X, Kaghad M, Caput D. (2000). Covalent modification of p73alpha by SUMO-1. Two-hybrid screening with p73 identifies novel SUMO-1-interacting proteins and a SUMO-1 interaction motif. *J Biol Chem* **275**: 36316–36323.
Miyazaki K, Ozaki T, Kato C, Hanamoto T, Fujita T, Irino S *et al*. (2003). A novel HECT-type E3 ubiquitin ligase, NEDL2, stabilizes p73 and enhances its transcriptional activity. *Biochem Biophys Res Commun* **308**: 106–113.
Moll UM, Slade N. (2004). p63 and p73: roles in development and tumor formation. *Mol Cancer Res* **2**: 371–386.
Momand J, Wu HH, Dasgupta G. (2000). MDM2 – master regulator of the p53 tumor suppressor protein. *Gene* **242**: 15–29.
Muller M, Schilling T, Sayan AE, Kairat A, Lorenz K, Schulze-Bergkamen H *et al*. (2005). TAp73/DeltaNp73 influences apoptotic response, chemosensitivity and prognosis in hepatocellular carcinoma. *Cell Death Differ* **12**: 1564–1577.
Nakagawa T, Takahashi M, Ozaki T, Watanabe Ki K, Todo S, Mizuguchi H *et al*. (2002). Autoinhibitory regulation of p73 by Delta Np73 to modulate cell survival and death through a p73-specific target element within the Delta Np73 promoter. *Mol Cell Biol* **22**: 2575–2585.
Nguyen BC, Lefort K, Mandinova A, Antonini D, Devgan V, Della Gatta G *et al*. (2006). Cross-regulation between Notch and p63 in keratinocyte commitment to differentiation. *Genes Dev* **20**: 1028–1042.
Nishi H, Senoo M, Nishi KH, Murphy BA, Rikiyama T, Matsumura Y *et al*. (2001). p53 homologue p63 represses epidermal growth factor receptor gene expression. *J Biol Chem* **276**: 41717–41724.
Nylander K, Vojtesek B, Nenutil R, Lindgren B, Roos G, Zhanxiang W *et al*. (2002). Differential expression of p63 isoforms in normal tissues and neoplastic cells. *J Pathol* **198**: 417–427.
Oberst A, Rossi M, Salomoni P, Pandolfi PP, Oren M, Melino G *et al*. (2005). Regulation of the p73 protein stability and degradation. *Biochem Biophys Res Commun* **331**: 707–712.
Park BJ, Lee SJ, Kim JI, Lee SJ, Lee CH, Chang SG *et al*. (2000). Frequent alteration of p63 expression in human primary bladder carcinomas. *Cancer Res* **60**: 3370–3374.
Parsa R, Yang A, McKeon F, Green H. (1999). Association of p63 with proliferative potential in normal and neoplastic keratinocytes. *J Invest Dermatol* **113**: 1099–1104.
Patturajan M, Nomoto S, Sommer M, Fomenkov A, Hibi K, Zangen R *et al*. (2002). DeltaNp63 induces beta-catenin nuclear accumulation and signaling. *Cancer Cell* **1**: 369–379.
Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA *et al*. (2000). Molecular portraits of human breast tumours. *Nature* **406**: 747–752.

Peters UR, Tschan MP, Kreuzer KA, Baskaynak G, Lass U, Tobler A *et al*. (1999). Distinct expression patterns of the p53-homologue p73 in malignant and normal hematopoiesis assessed by a novel real-time reverse transcription-polymerase chain reaction assay and protein analysis. *Cancer Res* **59**: 4233–4236.

Petrenko O, Zaika A, Moll UM. (2003). deltaNp73 facilitates cell immortalization and cooperates with oncogenic Ras in cellular transformation *in vivo*. *Mol Cell Biol* **23**: 5540–5555.

Pozniak CD, Radinovic S, Yang A, McKeon F, Kaplan DR, Miller FD. (2000). An anti-apoptotic role for the p53 family member, p73, during developmental neuron death. *Science* **289**: 257–258.

Ramalho FS, Ramalho LN, Della Porta L, Zucoloto S. (2006). Comparative immunohistochemical expression of p63 in human cholangiocarcinoma and hepatocellular carcinoma. *J Gastroenterol Hepatol* **21**: 1276–1280.

Redon R, Muller D, Caulee K, Wanherdrick K, Abecassis J, du Manoir S. (2001). A simple specific pattern of chromosomal aberrations at early stages of head and neck squamous cell carcinomas: PIK3CA but not p63 gene as a likely target of 3q26-qter gains. *Cancer Res* **61**: 4122–4129.

Reis-Filho JS, Milanezi F, Amendoeira I, Albergaria A, Schmitt FC. (2003). Distribution of p63, a novel myoepithelial marker, in fine-needle aspiration biopsies of the breast: an analysis of 82 samples. *Cancer* **99**: 172–179.

Ribeiro-Silva A, Ramalho LN, Garcia SB, Brandao DF, Chahud F, Zucoloto S. (2005). p63 correlates with both BRCA1 and cytokeratin 5 in invasive breast carcinomas: further evidence for the pathogenesis of the basal phenotype of breast cancer. *Histopathology* **47**: 458–466.

Rizzo MG, Giombini E, Diverio D, Vignetti M, Sacchi A, Testa U *et al*. (2004). Analysis of p73 expression pattern in acute myeloid leukemias: lack of DeltaN-p73 expression is a frequent feature of acute promyelocytic leukemia. *Leukemia* **18**: 1804–1809.

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

Romano RA, Birkaya B, Sinha S. (2006). Defining the regulatory elements in the proximal promoter of DeltaNp63 in keratinocytes: Potential roles for Sp1/Sp3, NF-Y, and p63. *J Invest Dermatol* **126**: 1469–1479.

Rossi M, Aqeilan RI, Neale M, Candi E, Salomoni P, Knight RA *et al*. (2006a). The E3 ubiquitin ligase Itch controls the protein stability of p63. *Proc Natl Acad Sci USA* **103**: 12753–12758.

Rossi M, De Laurenzi V, Munarriz E, Green DR, Liu YC, Vousden KH *et al*. (2005). The ubiquitin-protein ligase Itch regulates p73 stability. *EMBO J* **24**: 836–848.

Rossi M, De Simone M, Pollice A, Santoro R, La Mantia G, Guerrini L *et al*. (2006b). Itch/AIP4 associates with and promotes p63 protein degradation. *Cell Cycle* **5**: 1816–1822.

Seelan RS, Irwin M, van der Stoop P, Qian C, Kaelin Jr WG, Liu W. (2002). The human p73 promoter: characterization and identification of functional E2F binding sites. *Neoplasia* **4**: 195–203.

Senoo M, Matsumura Y, Habu S. (2002). TAp63g (p51A) and dNp63a (p73L), two major isoforms of the *p63* gene, exert opposite effects on the vascular endothelial growth factor (VEGF) gene expression. *Oncogene* **21**: 2455–2465.

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

Serber Z, Lai HC, Yang A, Ou HD, Sigal MS, Kelly AE *et al*. (2002). A C-terminal inhibitory domain controls the activity of p63 by an intramolecular mechanism. *Mol Cell Biol* **22**: 8601–8611.

Sniezek JC, Matheny KE, Westfall MD, Pietenpol JA. (2004). Dominant negative p63 isoform expression in head and neck squamous cell carcinoma. *Laryngoscope* **114**: 2063–2072.

Stiewe T, Putzer BM. (2002). Role of p73 in malignancy: tumor suppressor or oncogene? *Cell Death Differ* **9**: 237–245.

Stiewe T, Theseling CC, Putzer BM. (2002). Transactivation-deficient Delta TA-p73 inhibits p53 by direct competition for DNA binding: implications for tumorigenesis. *J Biol Chem* **277**: 14177–14185.

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

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

Suh EK, Yang A, Kettenbach A, Bamberger C, Michaelis AH, Zhu Z *et al*. (2006). p63 protects the female germ line during meiotic arrest. *Nature* **444**: 624–628.

Sunahara M, Shishikura T, Takahashi M, Todo S, Yamamoto N, Kimura H *et al*. (1999). Mutational analysis of p51A/TAp63gamma, a p53 homolog, in non-small cell lung cancer and breast cancer. *Oncogene* **18**: 3761–3765.

Tannapfel A, Schmelzer S, Benicke M, Klimpfinger M, Kohlhaw K, Mossner J *et al*. (2001). Expression of the p53 homologues p63 and p73 in multiple simultaneous gastric cancer. *J Pathol* **195**: 163–170.

Thanos CD, Bowie JU. (1999). p53 Family members p63 and p73 are SAM domain-containing proteins. *Protein Sci* **8**: 1708–1710.

Tokuchi Y, Hashimoto T, Kobayashi Y, Hayashi M, Nishida K, Hayashi S *et al*. (1999). The expression of p73 is increased in lung cancer, independent of p53 gene alteration. *Br J Cancer* **80**: 1623–1629.

Tonon G, Wong KK, Maulik G, Brennan C, Feng B, Zhang Y *et al*. (2005). High-resolution genomic profiles of human lung cancer. *Proc Natl Acad Sci USA* **102**: 9625–9630.

Troung AB, Kretz M, Ridky TW, Kimmel R, Khavari PA. (2006). p63 regulates proliferation and differentiation of developmentally mature keratinocytes. *Genes Dev* **20**: 3185–3197.

Tyner SD, Venkatachalam S, Choi J, Jones S, Ghebranious N, Igelmann H *et al*. (2002). p53 mutant mice that display early ageing-associated phenotypes. *Nature* **415**: 45–53.

Uramoto H, Izumi H, Ise T, Tada M, Uchiumi T, Kuwano M *et al*. (2002). p73 Interacts with c-Myc to regulate Y-box-binding protein-1 expression. *J Biol Chem* **277**: 31694–31702.

Urist M, Tanaka T, Poyurovsky MV, Prives C. (2004). p73 induction after DNA damage is regulated by checkpoint kinases Chk1 and Chk2. *Genes Dev* **18**: 3041–3054.

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

van Bokhoven H, Brunner HG. (2002). Splitting p63. *Am J Hum Genet* **71**: 1–13.

Vogelstein B, Lane D, Levine AJ. (2000). Surfing the p53 network. *Nature* **408**: 307–310.

Vousden KH. (2000). P53: Death Star. *Cell* **103**: 691–694.

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

Waltermann A, Kartasheva NN, Dobbelstein M. (2003). Differential regulation of p63 and p73 expression. *Oncogene* **22**: 5686–5693.

Wan YY, DeGregori J. (2003). The survival of antigen-stimulated T cells requires NFkappaB-mediated inhibition of p73 expression. *Immunity* **18**: 331–342.

Wang X, Mori I, Tang W, Nakamura M, Nakamura Y, Sato M *et al*. (2002). p63 expression in normal, hyperplastic and malignant breast tissues. *Breast Cancer* **9**: 216–219.

Wang TY, Chen BF, Yang YC, Chen H, Wang Y, Cviko A *et al*. (2001). Histologic and immunophenotypic classification of cervical carcinomas by expression of the p53 homologue p63: a study of 250 cases. *Hum Pathol* **32**: 479–486.

Watanabe K, Ozaki T, Nakagawa T, Miyazaki K, Takahashi M, Hosoda M *et al*. (2002). Physical interaction of p73 with c-Myc and MM1, a c-Myc-binding protein, and modulation of the p73 function. *J Biol Chem* **277**: 15113–15123.

Watson IR, Irwin MS. (2006). Ubiquitin and ubiquitin-like modifications of the p53 family. *Neoplasia* **8**: 655–666.

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

Westfall MD, Mays DJ, Sniezek JC, Pietenpol JA. (2003). The DNp63alpha phosphoprotein binds the p21 and 14-3-3sigma promoters *in vivo* and has transcriptional repressor activity that is reduced by Hay-Wells syndrome-derived mutants. *Mol Cell Biol* **23**: 2264–2276.

Westfall MD, Joyner AS, Barbieri CE, Livingstone M, Pietenpol JA. (2005). Ultraviolet radiation induces phosphorylation and ubiquitin-mediated degradation of DeltaNp63alpha. *Cell Cycle* **4**: 710–716.

Yamaguchi H, Inokuchi K, Sakuma Y, Dan K. (2001). Mutation of the p51/p63 gene is associated with blastic crisis in chronic myelogenous leukemia. *Leukemia* **15**: 1729–1734.

Yang A, Kaghad M, Wang Y, Gillett E, Fleming MD, Dotsch V *et al*. (1998). p63, a p53 homolog at 3q2–29, encodes multiple products with transactivating, death inducing, and dominant negative activities. *Mol Cell* **2**: 305–316.

Yang A, McKeon F. (2000). p63 and p73: p53 mimics, menaces and more. *Nat Rev* **1**: 199–207.

Yang A, Schweitzer R, Sun D, Kaghad M, Walker N, Bronson RT *et al*. (1999). p63 is essential for regenerative proliferation in limb, craniofacial and epithelial development. *Nature* **398**: 714–718.

Yang A, Walker N, Bronson RT, Kaghad M, Oosterwegel M, Bonnin J *et al*. (2000). p73-deficient mice have neurological, pheromonal and inflammatory defects but lack spontaneous tumors. *Nature* **404**: 99–103.

Yang A, Kaghad M, Caput D, McKeon F. (2002). On the shoulders of giants: p63, p73 and the rise of p53. *Trends Genet* **18**: 90–95.

Yang A, Zhu Z, Kapranov P, McKeon F, Church GM, Gingeras TR *et al*. (2006). Relationship between p63 binding, DNA sequence, transcription activity and biological function in human cells. *Mol Cell* **24**: 593–602.

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

Zaika A, Irwin M, Sansome C, Moll UM. (2001). Oncogenes induce and activate endogenous p73 protein. *J Biol Chem* **276**: 11310–11316.

Zaika AI, Slade N, Erster SH, Sansome C, Joseph TW, Pearl M *et al*. (2002). DeltaNp73, a dominant-negative inhibitor of wild-type p53 and TAp73, is up-regulated in human tumors. *J Exp Med* **196**: 765–780.

Zamo A, Malpeli G, Scarpa A, Doglioni C, Chilosi M, Menestrina F. (2005). Expression of TP73L is a helpful diagnostic marker of primary mediastinal large B-cell lymphomas. *Mod Pathol* **18**: 1448–1453.

Zangen R, Ratovitski E, Sidransky D. (2005). DeltaNp63alpha Levels Correlate with Clinical Tumor Response to Cisplatin. *Cell Cycle* **4**: 1313–1315.

Zeng X, Chen L, Jost CA, Maya R, Keller D, Wang X *et al*. (1999). MDM2 suppresses p73 function without promoting p73 degradation. *Mol Cell Biol* **19**: 3257–3266.

Zhu J, Jiang J, Zhou W, Chen X. (1998). The potential tumor suppressor p73 differentially regulates cellular p53 target genes. *Cancer Res* **58**: 5061–5065.