Subject Review

p63 and p73: Roles in Development and Tumor Formation

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Abstract

The tumor suppressor p53 is critically important in the cellular damage response and is the founding member of a family of proteins. All three genes regulate cell cycle and apoptosis after DNA damage. However, despite a remarkable structural and partly functional similarity among p53, p63, and p73, mouse knockout studies revealed an unexpected functional diversity among them. p63 and p73 knockouts exhibit severe developmental abnormalities but no increased cancer susceptibility, whereas this picture is reversed for p53 knockouts. Neither p63 nor p73 is the target of inactivating mutations in human cancers. Genomic organization is more complex in p63 and p73, largely the result of an alternative internal promoter generating NH_2-terminally deleted dominant-negative proteins that engage in inhibitory circuits within the family. Deregulated dominant-negative p73 isoforms might play an active oncogenic role in some human cancers. Moreover, COOH-terminal extensions specific for p63 and p73 enable further unique protein-protein interactions with regulatory pathways involved in development, differentiation, proliferation, and damage response. Thus, p53 family proteins take on functions within a wide biological spectrum stretching from development (p63 and p73), DNA damage response via apoptosis and cell cycle arrest (p53, TAp63, and TAp73), chemosensitivity of tumors (p53 and TAp73), and immortalization and oncogenesis (ΔNp73).
(Mol Cancer Res 2004;2(7):371–86)

Introduction

p53 controls a powerful stress response by integrating upstream signals from many types of DNA damage and inappropriate oncogenic stimulation, all of which lead to p53 activation. Activated p53 elicits apoptosis, cell cycle arrest, and, in some circumstances, senescence, thereby preventing the formation of tumors (Table 1). Hence, loss of p53 function is a preeminent finding in most human cancers, whether directly through mutation of p53 itself, the most common mechanism (1), impaired nuclear retention of p53 (2, 3), loss of the upstream activator $p14^{ARF}$, or amplification of the p53 antagonist HDM2 (4).

In 1997, two novel family members were identified and termed p73 (5) and p63 (6-10). On the basis of their remarkable structural similarity with p53, p63 and p73 generated instant excitement and quick expectations about their biological functions. Seven years later, we have unearthed striking similarities but also surprising diversities. Both genes give rise to proteins that have (*a*) entirely novel functions and (*b*) p53-related functions. Moreover, the p53-related functions are of either a p53-synergistic or a p53-interfering nature. Both p63 and p73 share >60% amino acid identity with the DNA binding region of p53 (and even higher identity among themselves), including conservation of all DNA contact and structural residues that are hotspots for p53 mutations in human tumors. In addition, p73 shows 38% identity with the p53 tetramerization domain and 29% identity with the p53 transactivation domain (TA). In vertebrates, the p73 and p63 genes are ancestral to p53 and possibly evolved from a common p63/p73 archetype (5, 6).

Gene Architecture of the p53 Family

The gene structure of TP53, TP63, and TP73 is highly conserved from mollusk to human (Fig. 1A and B). The three most conserved domains in all three genes are the NH_2-terminal TA, the central DNA binding domain (DBD), and the COOH-terminal oligomerization domain. TP53 currently has a single promoter but encodes the full-length p53 as well as a long overlooked alternative splice variant of 40 kDa called ΔNp53. ΔNp53 is produced by an alternative splice product that retains intron 2, but because it contains a premature stop codon, internal translation starts at codon 40 (11). ΔNp53 oligomerizes with full-length p53 and interferes with its transcriptional and apoptotic functions. On the other hand, ΔNp53 does not respond to DNA damage but becomes the predominant form during progression into S phase after serum restimulation. Thus, ΔNp53 may play a transient p53 counter-role during normal cell cycle (12). Its potential role in tumors is currently unknown.

TP63 and TP73 have two promoters: P1 in the 5′ untranslated region upstream of the noncoding exon 1 and P2 within the 23 kb spanning intron 3. P1 and P2 promoters produce two diametrically opposing classes of proteins: those containing the TA (TAp63 and TAp73) and those lacking it (ΔNp63 and ΔNp73). ΔNp63 and ΔNp73 occur in human and mouse. In addition, alternative exon splicing of the P1 transcripts of TP63 and TP73 give rise to other isoforms lacking the transactivation (5)

Received 4/21/04; revised 6/8/04; accepted 6/8/04.
Grant support: National Cancer Institute.
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Table 1. p53 Gene Family

	p53	p63	p73
DNA damage response	+++	−/+	++
Apoptosis/cell cycle arrest	+++	+	++
Senescence	+++		+
Developmental function	−	Required for limb and skin formation; essential in stem cell biology of epithelia	Required for central nervous system development of hippocampus, limbic telencephalon, and vomeronasal region; absence of Cajal-Retzius neurons

domain (e.g., ΔN′p73, Ex2Delp73, and Ex2/3Delp73; Fig. 1C; refs. 13-15). Of importance, the ΔNp73 and ΔN′p73 transcripts encode the *same* protein due to the use of a second translational start site because of an upstream premature stop in ΔN′p73 (15). TA proteins mimic p53 function in cell culture including transactivating many p53 target genes and inducing apoptosis, whereas (the collectively called) ΔTA proteins act as dominant-negative inhibitors of themselves and of other family members *in vivo* in the mouse and in transfected human cells (6, 16, 17). Strikingly, the TP63 locus is contained within a frequently amplified region in squamous cell carcinoma (which led to the alternate name of amplified in squamous carcinoma for TP63; ref. 18), and squamous epithelium of the skin and squamous carcinoma produce high levels of ΔNp6α (also called $p68^{AIS}$). Furthermore, ΔNp73 is the predominant TP73 product in the developing mouse nervous system and is required to counteract the proapoptotic action of p53 (see below; refs. 16, 17).

Additional complexity is generated at the COOH terminus: TP73 and TP63 undergo multiple COOH-terminal splicings of exons 10 to 14, skipping one or several exons. Thus far, nine transcripts were found for TP73: α, β, γ, δ, ε, ζ, η, η_1, and ϕ (α being full-length; refs. 15, 19, 20), and three were found for TP63: α, β, and γ (6). The p73 isoforms ϕ, η, and η_1 lack the second COOH-terminal TA and the tetramerization domain encoded by exon 10 (13, 15). In some COOH-terminal isoforms, exon splicing also leads to unique sequences due to frameshifts. For TP63, three isotypes (α, β, and γ) are made. Splicing of different "tails" further modulates the p53-like function of TA proteins, although they do not appear to vary much in their role in tumorigenesis. Structurally, the γ forms of TP73 and TP63 most closely resemble p53 itself, harboring just a small COOH-terminal extension beyond the last 30-amino acid stretch of p53. Surprisingly, whereas TAp63γ (also called p51A) is as powerful as p53 in transactivation and apoptosis assays (6), TAp7γ is rather weak. The α forms of TP73 and TP63 contain an additional highly conserved sterile α motif (SAM). SAMs are protein-protein interaction modules found in a wide variety of proteins implicated in development. In addition, the p73 SAM domain can bind to anionic and zwitterionic lipid membranes (21). The crystal and solution structures of p73 SAM agree with each other and feature a five-helix bundle fold that is characteristic of all SAM domain structures (22, 23). Other SAM-containing proteins are the ETS transcription factor TEL that plays a role in leukemia, the polycomb group of homeotic transcription factors, and the ephrin receptors. Despite predictions of homo- and

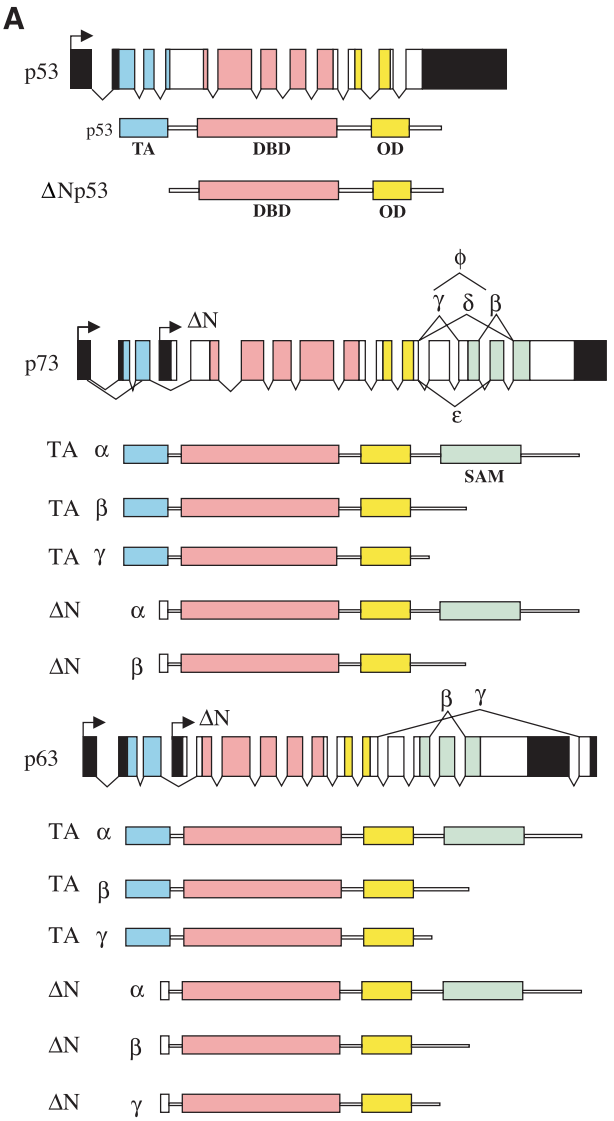

FIGURE 1. A. Gene architecture of the p53 family. The p53 family includes the three genes p53, p63, and p73. They have a modular structure consisting of the TA, the DBD, and the oligomerization domain. All genes are expressed as two major types: full-length proteins containing the TA domain and ΔN proteins missing the TA domain. The products of p73 and p63 are more complex than p53 and contain a COOH-terminal SAM domain and a transactivation inhibitory domain in their α isoforms. p63 and p73 also contain two promoters. The P1 promoter in the 5′ untranslated region produces TA proteins that are transcriptionally active, whereas the P2 promoter produces ΔN proteins with dominant-negative functions toward themselves and toward wild-type p53. In addition, extensive COOH-terminal splicing and, in TP73, additional NH_2-terminal splice variants of the P1 transcript further modulate the p53-like functions of the TA proteins. **B.** Amino acid alignment of human p53, p63, and p73. **C.** Gene architecture of the NH_2 terminus of p73. TAp73 and the NH_2-terminally truncated splice forms Ex2p73, Ex2/3p73, and ΔN′p73 (together with ΔNp73 collectively called ΔTAp73 isoforms) are all generated from the P1 promoter, whereas the P2 promoter in intron 3 produces the dominant-negative ΔNp73, starting with the unique exon 3′. *Arrows,* transcriptional start sites.

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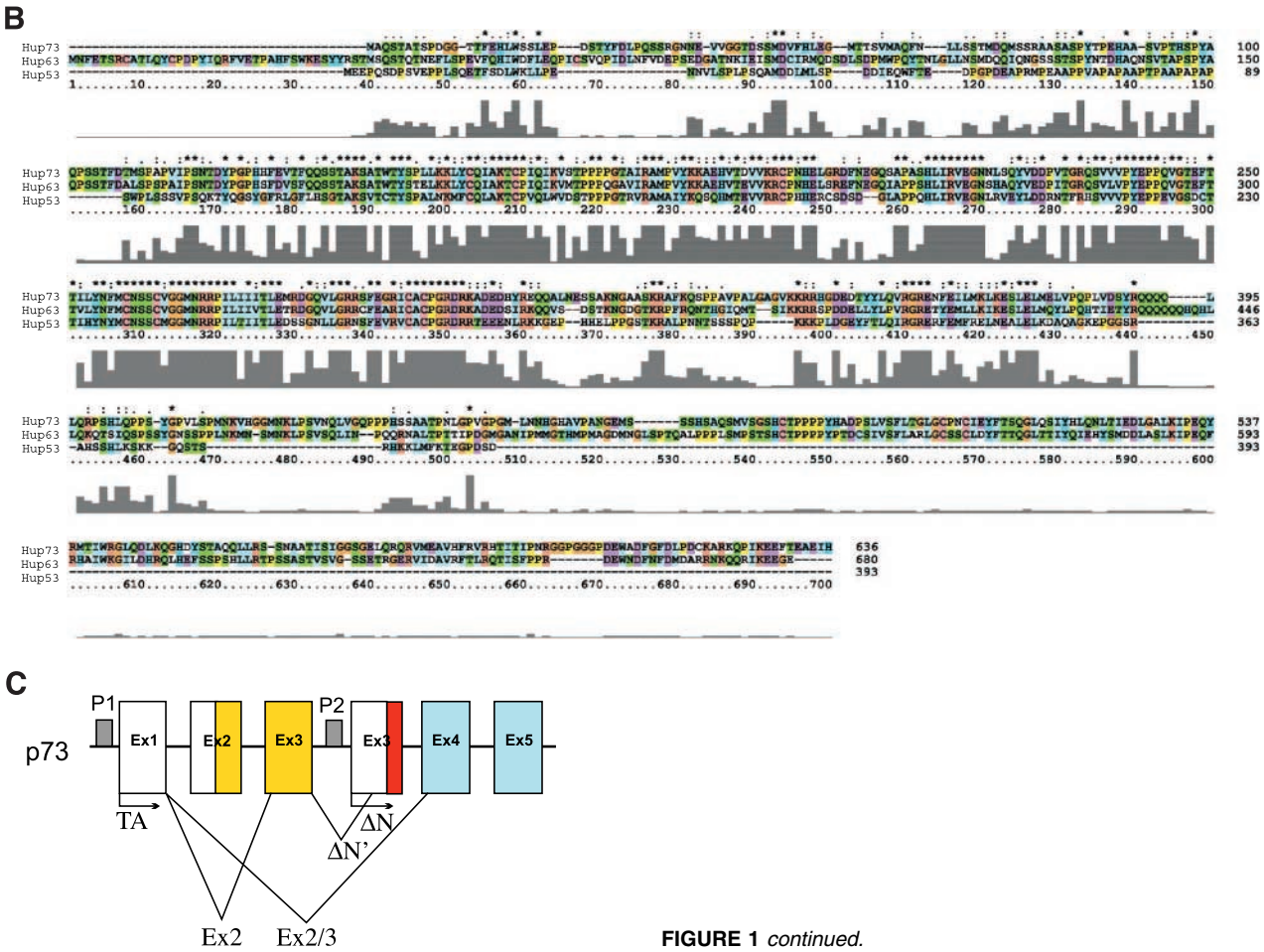

FIGURE 1 *continued.*

hetero-oligomerization of SAM-containing proteins, p73 SAM appears monomeric by experimental analysis, casting doubt whether this domain mediates interaction of p73 with heterologous proteins (23). There are also functional differences between TAp73α and TAp63α. Whereas TAp73α is comparable with p53 in potency in transactivation and apoptosis assays, TAp63α (also called p51B) is very weak (6). One reason for this difference could be that p63α isoforms contain a 27-kDa COOH-terminal region that drastically reduces its transcriptional activity (24). This domain is necessary and sufficient for transcriptional inhibition and acts by binding to a region in the NH_2-terminal TA of p63, which is homologous to the MDM2 binding site in p53. Of note, this transactivation inhibitory domain is biologically important, because patients with deletions in this p63 domain have phenotypes very similar to patients with mutations in the DBD (24).

In summary, by using alternate exon splicing and an alternative promoter, TP73 and TP63 genes can generate an impressive modular complexity by combining a specific "head" with a particular "tail." In practice, this means that our understanding of their biological roles will greatly depend on knowing which forms get expressed under what circumstances.

TP63 and TP73 Play Important Roles in Development and Differentiation

Both genes play important and, despite their structural similarity, surprisingly unique roles in mouse and human development. This is powerfully revealed by the striking developmental phenotypes of p63- and p73-deficient mice (16, 25, 26) and is in contrast to p53-null mice, which are highly tumor prone but lack a developmental phenotype.

TP63

TP63 expression is absolutely essential for limb formation and epidermal morphogenesis (integument and tongue) including the formation of adnexa (teeth, hair, mammary and prostate glands, and sweat and lacrimal glands). p63-null animals show severe limb truncations or absence of limbs and absence of skin and craniofacial malformations. They also fail to develop skin and most epithelial tissues (e.g., prostate and mammary glands). The animals do not survive beyond a few days postnatally. Reminiscent of the knockout phenotype in mice, heterozygous germ line point mutations of p63 in humans cause six rare autosomal dominant developmental disorders with a strong but not absolute genotype-phenotype

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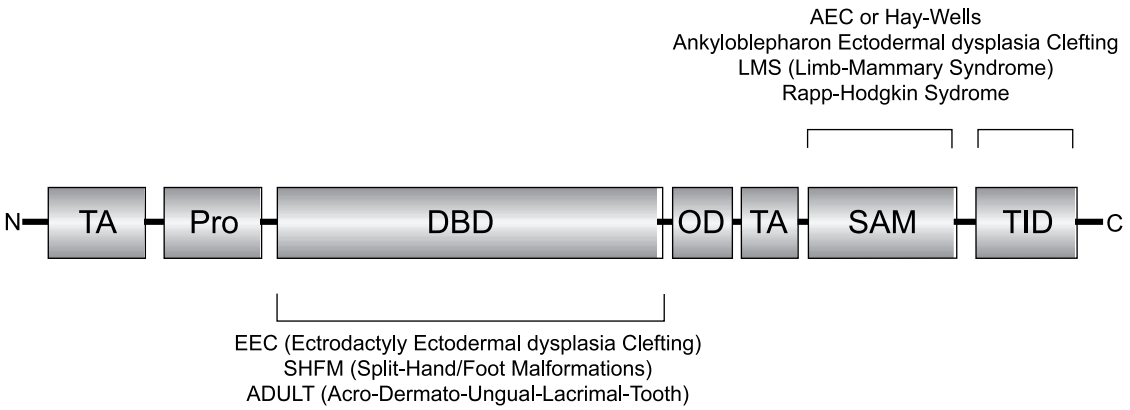

FIGURE 2. Location of p63 point mutations (heterozygous, germ line) in six related human developmental disorders with autosomal dominant transmission and various degrees of limb and facial malformations and ectodermal dysplasia. Mutations are found in the DBD or in the SAM domain/transactivation inhibitory domain. Abbreviations: *Pro*, proline-rich domain; *OD*, oligomerization domain; *TID*, transactivation inhibitory domain.

correlation (with or without ectrodactyly; Fig. 2). Ectrodactyly-ectodermal dysplasia-clefting or the related yet distinct ankyloblepharon-ectodermal dysplasia-clefting or Hay-Wells syndrome was the first discovered. Among 29 p63 mutations found in 90 affected families with ectrodactyly-ectodermal dysplasia-clefting, 28 were missense mutations within the DBD, some of which correspond to the p53 hotspot mutations. These ectrodactyly-ectodermal dysplasia-clefting mutations affect all six major proteins and inhibit DNA binding of the TAp63 forms. Conversely, ectrodactyly-ectodermal dysplasia-clefting mutations in ΔNp63 proteins cause a loss of their dominant-negative properties toward p53 and TAp63γ (27). In contrast, p63 mutations in the ankyloblepharon-ectodermal dysplasia-clefting syndrome are in the SAM domain and affect only the two α isoforms. Ankyloblepharon-ectodermal dysplasia-clefting mutants of p63α have lost interaction with apobec-1 binding protein-1. Without this interaction, the alternatively spliced K-SAM isoform of fibroblast growth factor receptor-2 is not generated (essential for epithelial differentiation), which in turn might lead to inhibition of epithelial differentiation and could perhaps account for the ankyloblepharon-ectodermal dysplasia-clefting phenotype (28). There are four additional related human developmental syndromes with p63 mutations (acro-dermato-ungual-lacrimal-tooth syndrome, limb mammary syndrome, Rapp-Hodgkin syndrome, and split hand-split foot malformation) that extend the genotype-phenotype correlation (29).

Importantly, basal cells of normal human epithelium including the epidermis strongly express p63 proteins, predominantly the ΔNp63 isotype (ratio is ~100:1 of ΔNp63 to TAp63; ref. 6), but lose them as soon as these cells withdraw from the stem cell compartment (30). Consistent with this notion, keratinocyte differentiation is associated with the disappearance of ΔNp63α (31-33), whereas the expression of p53 target genes p21 and 14-3-σ, mediating cell cycle arrest, increase. p63 binds p21 and 14-3-σ promoters and represses them. This repression is reduced in the mutated proteins found in ankyloblepharon-ectodermal dysplasia-clefting syndrome (33). p63 is also indispensable for the differentiation of a transitional urothelium and is expressed in normal bladder urothelium. p63 is lost in most invasive bladder cancers (34).

Together, these data clearly establish a fundamental role of p63 in epithelial stem cell biology and in the apical ectodermal ridge of the limb bud, where p63-expressing cells create a signaling center (30). Whether this role is one in stem cell self-renewal or in stem cell differentiation into stratified epithelium remains a matter of controversy (25, 26). In one model, p63 is required for the ectoderm to commit to epidermal lineages (25, 26), whereas, in the other model, p63 is not required to commit but to maintain the stem cell pool and prevent it from differentiation (29). What appears clearer is that p63 is probably not simply required for the proliferative capacity of stem cells, because their immediate progeny, the TAC cells, are equally proliferative but have already lost p63 expression (30). Zebrafish embryos require ΔNp63 to inhibit p53 and thus allow epidermal proliferation and limb development (35). This study shows an essential and ancient role of ΔNp63 in skin development.

TP73

TP73 also has distinct developmental roles. TP73 expression is required for neurogenesis of specific neural structures, for pheromonal signaling, and for normal fluid dynamics of cerebrospinal fluid (16). The hippocampus is central to learning and memory and continues to develop throughout adulthood. p73-null animals exhibit hippocampal dysgenesis due to the selective loss of large bipolar neurons called Cajal-Retzius in the marginal zone of the cortex and the molecular layers of the hippocampus. These Cajal-Retzius neurons are responsible for cortex organization and coexpress ΔNp73 and the secretory glycoprotein reelin. In addition, p73-null mice have severe malformations of the limbic telencephalon.[3] They also suffer from hydrocephalus (~20%) probably due to hypersecretion of cerebrospinal fluid by the choroid plexus and from a hyperinflammatory response (purulent but sterile excudates) of the respiratory mucosa likely due to mucus hypersecretion. Moreover, the animals are runted and show abnormal reproductive and social behavior due to defects in pheromone detection. The latter abnormality is due to a dysfunction of the vomeronasal organ, which normally expresses high levels of p73.

[3] G. Meyer, personal communication.

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Role of ΔNp73 in Mouse Development

ΔNp73 is the predominant form in the developing mouse brain and might act as a repressor (6, 17). In situ hybridization reveals strong ΔNp73 expression in E12.5 fetal mouse brain in the preplate layer, bed nucleus of stria terminalis, choroid plexus, vomeronasal area, and preoptic area (16). Moreover, ΔNp73 is the only form of p73 found in mouse brain and the sympathetic superior cervical ganglia in P10 neonatal mice (17). Functional studies and knockout mice showed that ΔNp73 plays an essential antiapoptotic role *in vivo*. ΔNp73 is required to counteract p53-mediated neuronal death during the normal "sculpting" of the developing mouse neuronal system (17). Withdrawal of nerve growth factor, an obligate survival factor for mouse sympathetic neurons, leads to p53 induction and p53-dependent cell death. Conversely, nerve growth factor withdrawal leads to a decrease of ΔNp73. Importantly, sympathetic neurons are rescued from cell death after nerve growth factor withdrawal when ΔNp73 levels are maintained by viral delivery. Likewise, sympathetic neurons are rescued from Adp53-mediated neuronal death by coinfected AdΔNp73. In pull-down assays, mixed protein complexes of p53/ΔNp73 were demonstrated, suggesting one biochemical basis for transdominance in addition to possible promoter competition. Together, these data firmly put ΔNp73 downstream of nerve growth factor in the nerve growth factor survival pathway. It also explains why $p73^{-/-}$ mice, missing all forms of p73 including protective ΔNp73, undergo accelerated neuronal death in postnatal superior cervical ganglia (17).

In tissue culture models, p73 also plays a role in differentiation of several cell lineages. TP73 expression increases during retinoic acid–induced and spontaneous differentiation of neuroblastoma cells (36, 37). In addition, ectopic TAp73β but not p53 induce morphologic and biochemical markers of neuroblastoma differentiation (36). Moreover, expression of specific COOH-terminal isoforms correlates with normal myeloid differentiation. p73α and p73β are associated with normal myeloid differentiation, whereas p73γ, p73δ, p73ε, and p73θ are associated with leukemic blasts. In fact, p73ε is specific for leukemic blast cells (38). Similarly, TAp73γ and TAp73δ may play a role in the terminal differentiation of human skin keratinocytes (39). This suggests a p73-specific differentiation role that is not shared by p53 and, for the most part, not shared by p63 either. p53 has an important developmental role in early mouse embryogenesis (E7-8d) as revealed when the autoregulatory feedback loop with MDM2 is removed and p53 levels remain uncontrolled (40, 41). Nevertheless, in stark contrast to TP63- and TP73-null mice, TP53-null mice make it through development with essentially no problems (with the exception of rare exencephaly in females; refs. 42, 43). A commonly offered explanation is that p53 functions are covered by redundant p63 and p73 functions. At least in theory, this idea could now be tested, although generating double or even triple knockouts might be a daunting task. The concept of substitution, however, is inconsistent with the finding that ΔN isoforms rather than TA isoforms are the predominant proteins of TP63 and TP73 during development. Indeed, the very fact that TP63- and TP73-deficient mice have a phenotype could be viewed as evidence for the important *in vivo* role of ΔN isoforms during development because, conversely, p53 cannot substitute for those forms.

Of note, p73-deficient mice lack spontaneous tumor formation even after a 2-year observation period (16). Although the tumor rate after mutagenic challenge or the tumor rate of double p53/p73-null mice is currently unknown, this result is another clear difference between p53 and the other family members. It indicates that if TP73 and TP63 do have a role in tumor formation, it might be a complex one, which is probably not revealed by simply eliminating the entire gene.

p63 and p73 Expression in Normal Human Tissues

p73 gene expression occurs at very low levels in all normal human tissues studied (37, 44), making detection difficult. p63, mainly its ΔN form, occurs at higher levels and is readily detectable at the protein level. In embryonic epidermis, p63 is the molecular switch for initiation of an epithelial stratification program (45). In postnatal epidermis, p63 expression is restricted to the nuclei of basal cells of normal epithelia (skin, esophagus, tonsil, prostate, urothelium, ectocervix, and vagina) and to certain populations of basal cells in glandular structures of prostate, breast, and bronchi (6, 46). Specifically, p63 is expressed in myoepithelial cells of the breast and considered to be the specific marker for those cells in normal breast tissue (47, 48). p63 expression in prostate is restricted to basal cells, making it an excellent diagnostic marker in prostate cancer. The vast majority of prostate cancers and preinvasive prostate intraepithelial neoplasia lesions have lost p63 expression. Basal cells play important roles in differentiation and carcinogenesis of the prostate (49, 50).

Transcriptional and Apoptotic Activity of p63 and p73

In general, many functional parallels are found among p53, TAp73, and TAp63 on the one hand and among ΔNp73 and ΔNp63 on the other hand. When ectopically overexpressed in cell culture, p73α and p73β closely mimic the transcriptional activity and biological function of p53. p73β and, to a lesser extent, p73α bind to canonical p53 DNA binding sites and transactivate many p53-responsive promoters (51-54), although relative efficiencies on a given p53 target promoter may differ from p53 and also differ among various COOH-terminal isoforms of TAp73 and TAp63 (53, 54). In reporter assays, p73-responsive promoters include well-known p53 target genes involved in antiproliferative and proapoptotic cellular stress responses such as $p21^{WAF1}$, 14-3-3σ, GADD45, BTG2, PIG3 (53), ribonucleotide reductase p53R2 (55), and IGFBP3 (56). Bax transactivation is controversial (53, 56). TAp73α and TAp73β also induce MDM2. Conversely, ectopic p73 overexpression leads to transcriptional repression of vascular endothelial growth factor, analogous to the ability of p53 to transcriptionally suppress vascular endothelial growth factor (57). Although there are probably still dozens of common targets that have not yet been described or discovered, it will be important to identify p63/p73-preferred or p63/p73-specific targets. For example, 14 novel target genes that are differentially regulated by various p53 family members were recently identified (58). It is worth noting, however, that binding of endogenous p63/p73 to their putative target gene promoters

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has not been demonstrated yet *in vivo* by chromatin immunoprecipitation (59). In summary, there are common and perhaps preferred target genes for each p53 family member and their isoforms. Small interfering RNA strategies specifically designed against these isoforms will clarify the biological functions of each p53 family member.

p73 has its own unique determinants for transactivation and growth suppression. The domains of p73β (the most potent form in transactivation and growth arrest) necessary for inducing cell cycle arrest are the TA domain, the DBD, and the tetramerization domain. However, unlike p53, p73-mediated apoptosis does not require PXXP region adjacent to the TA domain or the entire COOH-terminal region. Interestingly, PXXP motifs, although dispensable for p73 function, render p73 inactive in transactivation when deleted (60). ΔTAp73 could mediate hyperphosphorylation of retinoblastoma, resulting in enhanced E2F activity and thus possibly also interfering with the retinoblastoma tumor suppressor pathway (61).

A surprising "essential cooperativity" among family members for transcriptional function was recently found. In response to DNA damage, induction of $p21^{WAF1}$ (mediating cell cycle arrest) occurred normally in $p63^{-/-}$ and $p73^{-/-}$ single null mouse embryo fibroblasts (MEFs) and $p63/p73^{-/-}$ double null MEFs. However, in double null MEFs, the induction of Bax, Noxa, and PERP genes (thought to mediate apoptosis) was suppressed. Chromatin immunoprecipitation assays confirmed that there is no binding of p53 to the Bax, PERP, and NOXA promoters in the absence of p63 or p73, whereas, conversely, p63 still binds to them in $p53^{-/-}$ single null MEFs. These data demonstrate that either p63 or p73 are essential for p53-induced apoptosis (62). Ectopic p73 promotes apoptosis in human tumor cell lines independent of their p53 status (5, 51). In fact, in a subset of cancer cell lines, p73β is more efficient in inducing apoptosis than p53 itself (63). Potency differences exist among the COOH-terminal isoforms. Overexpression of p73α, p73β, and p73δ suppresses focus formation of p53-deficient Saos-2 cells, whereas p73γ fails or suppresses only very poorly (51, 63, 64). Similarly, TAp63α lacks significant transcriptional and apoptotic ability, whereas TAp63γ is very potent in both (6). However, TAp63γ activity is controversial. Cells overexpressing TAp63γ, TAp63α, ΔNp63α, and ΔNp63γ showed poor or no detectable apoptosis compared with overexpressed p53 or p73α. Although TAp63γ yielded clear p21 induction, it showed only moderate activity for apoptosis (65). Using gene profiling via microarrays, ΔNp63α and TAp63α regulate a broad spectrum of genes with diverse roles in cellular function but possess opposing regulatory effects toward each other (66).

Regulation of p73 and p63 Protein Stability and Transcriptional Activity

Proteasomes are mediating the turnover of p73 proteins because proteasome inhibitors stabilize p73 isoforms (67). We determined the individual half-lives of all NH_2- and COOH-terminal isoforms and found that they differ only moderately. However, when coexpressed in various cell types, TAp73α/β proteins become markedly stabilized by ΔNp73α/β but, in doing so, render them functionally inactive. This is mediated via hetero-oligomerization by the transdominant ΔNp73. In contrast, p53 protein fails to accumulate via ΔNp73 coexpression. In the ongoing debate whether TAp73 is a relevant tumor suppressor, we suggest that increased TAp73 protein levels should be interpreted with caution when levels are the only criteria that can be used to deduce TAp73 activity. This is particularly the case in primary tumors in which functional studies are not possible (68).

In sharp contrast to p53, however, p73 degradation is not mediated by MDM2. The molecular basis for the MDM2 resistance of p73 was found by systematic motif swapping. Region 92-112 of p53, which is absent in p73, was identified to confer MDM2 degradability to p53 (69). p73 protein is also resistant to human papillomavirus (HPV) E6, which together with E6-AP mediates hyperactive degradation of p53 in HPV-infected cells (67, 70). This relationship might have some bearing in tumors with increased p73 expression (see below). Just as MDM2 does not mediate p73 degradation, $p19^{ARF}$, which stabilizes p53 levels by antagonizing the degrading action of MDM2, has not been shown to stabilize p73 protein. One potential consequence of the differential MDM2 sensitivity between p53 and p73 was seen in tissue culture: ectopic coexpression of p73 leads to a selective decrease of ectopic p53 and endogenous induced p53 because p53 is susceptible to MDM2, whereas p73 is not (71). This suggests a potential down-modulation of p53 by high levels of TAp73 (because MDM2 is also a p73 target), an interesting family twist to keep in mind with respect to tumor formation. On a transcriptional level, however, the negative feedback regulation between the two genes is preserved. MDM2 is transcriptionally activated by p73 and in turn negatively regulates the transcriptional ability of p73, just as it functions toward p53 (67, 72, 73). However, the mechanism is again distinct from p53. The binding to MDM2 causes the disruption of physical and functional interaction with p300/cAMP-responsive element binding protein by competing with p73 for binding to the NH_2 terminus of p300/cAMP-responsive element binding protein (73). Degradation of p73 might be linked to small ubiquitin-related modifier-1 (74). On the other hand, the novel Hect domain-containing NEDD4-like E3 ubiquitin ligase NEDL2 binds to p73 via its PY motif in the COOH-terminal region (75). p53, which lacks the PY motif, does not bind to NEDL2. Overexpression of NEDL2 results in the ubiquitination of p73; however, rather than mediating degradation, it enhances the steady-state level of p73 and its ability to transactivate p53/p73-responsive promoters. The differential binding of NEDL2 to p53 family members is thus another factor that might contribute to their functional divergence. Likewise, the NAD(P)H:quinone oxidoreductase-1 stabilizes p73α (as well as p53) but not p73β by binding of its SAM domain to NQO1, which protects p73α from 20S proteasomal degradation that is independent of MDM2. This NQO1-mediated stabilization of p73α and p53 provides one explanation why NQO1 knockout mice have a cancer phenotype and humans with inactive NQO1 polymorphisms are susceptible to cancer (76).

The ankyrin-rich, Src holomogy 3 domain, proline-rich proteins ASPP1 and ASPP2 stimulate the apoptotic function of p53, p63, and p73 (77, 78). By binding to the DBD of p53, p63, and p73, ASPP1 and ASPP2 stimulate the transactivation

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function of all three proteins on the promoters of Bax, PIG3, and PUMA but not MDM2 or $p21^{WAF-1/CIP1}$. Hence, ASPP1 and ASPP2 are the first two identified common activators of all p53 family members. The transcriptional coactivator Yes-associated protein potentiates TAp73-mediated transactivation of Bax after DNA damage. Conversely, Akt phosphorylates Yes-associated protein, which induces interaction with 14-3-3, relocation of Yes-associated protein to the cytoplasm, and attenuation of p73-mediated apoptosis (Fig. 3; ref. 79).

SAM-containing p63α forms are more stable because the transactivation inhibitory domain, intramolecularly bound to the TA domain, interferes with p63α degradation. This pool of protein is ready to be used in case of a stimulus such as DNA damage or a developmental signal (24). In general, TAp63 isoforms (half-life ~6 minutes *in vitro*) are much less stable than ΔNp63 isoforms (half-lifes >5 hours). The specific DNA binding activity of TAp63 must be essential for its protein stability because the disease-related DNA binding mutants of TAp63 are very stable. Whereas MDM2 binds to TAp63, MDM2 is unlikely to be the E3 ubiquitin ligase for p63 because ectopic delivery of MDM2 has no significant effect on TAp63 levels (80). However, p63α and p63γ are exported from the nucleus to the cytoplasm when coexpressed with MDM2, suggesting that MDM2 is capable of inhibiting the apoptotic function of p63 by removing it from the nucleus but not by directing its degradation (80). Rather, as seen in p73, p63 degradation might also be linked to a COOH-terminal small ubiquitin-related modifier-1 site. In an additional family twist, however, ΔNp63 isoform stability may also be regulated independently of proteasomes. A protein complex between ΔNp63α and p53, mediated by both DBDs, promotes p63 degradation via a caspase-1-specific pathway (81). This result may explain the observation that UV irradiation of cultured keratinocytes suppresses ΔNp63 levels (82). Of note, a check-and-balance system may exist: whereas ΔNp63 is a transcriptional inhibitor of p53, p53 is a stability inhibitor of ΔNp63. This relationship also points toward another level of intimate functional cross-talk among p53 family members, a theme that will surface again and again. In summary, the respective E3 ubiquitin ligases for p63 and p73 remain to be identified.

Post-Translational Modifications During Activation

p53 stabilization and activation by genotoxic stress is associated with multiple post-translational modifications at the NH_2 and COOH termini of p53 *in vivo*. In close temporal relationship to stress, the NH_2 terminus undergoes heavy phosphorylation (Ser^{15}, Ser^{20}, Ser^{33}, Ser^{37}, Ser^{46}, Thr^{18}, and Thr^{81}), which is thought to stabilize the protein by interfering with MDM2 binding, thereby disrupting the constitutively targeted degradation. The COOH terminus also undergoes site-specific phosphorylation (Ser^{315} and Ser^{392}), acetylation (Lys^{320}, Lys^{373}, and Lys^{382}), and sumoylation (Lys^{386}). The COOH-terminal modifications are thought to activate the transcriptional activity of p53 (83). So-called stress kinases (e.g., ATM, ATR, and Chk2), which detect genotoxic stress and initiate signal transduction, are *in vivo* kinases for specific p53 serine residues, whereas the histone acetyltransferases p300/cAMP-responsive element binding protein and PCAF (which at the same time are transcriptional coactivators) acetylate p53.

Modification differences for p73 are starting to be worked out (Fig. 3). Serine phosphorylation has been reported for p73 (84). In addition, p73α undergoes phosphorylation at Tyr^{99} by c-*abl* in response to γ-IR that in turn activates p73 for apoptosis (85, 86). This is due to a direct interaction between the PXXP motifs of p73 and the Src holomogy 3 domain of c-*abl*. Interestingly, Tyr^{99} phosphorylation activates p73 but does not stabilize the protein. On the other hand, cisplatin also activates p73 function and stabilizes the protein but does not tyrosine phosphorylate it. Sumoylation of COOH-terminal

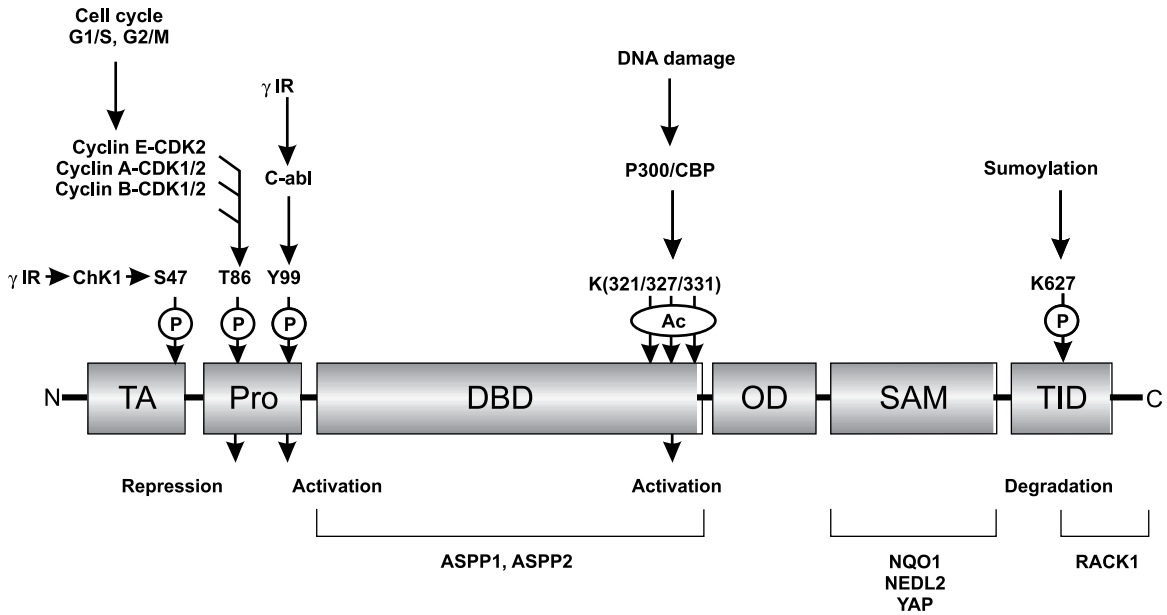

FIGURE 3. Post-translational modifications of p73 and proteins interacting with p73.

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Lys^{627} occurs specifically in p73α but not in p73β *in vitro*. However, in contrast to sumoylation of p53, which activates its transcriptional activity, sumoylation of p73 promotes its degradation (74). p63 does not have PXXP motifs, and modification studies for p63 have not been reported.

p73 proteins may also play a role in the regulation of cell growth. p73 physically interacts with various cyclins and certain cyclin-CDK complexes (cyclin A-CDK1/2, cyclin B-CDK1/2, and cyclin E-CDK2), which can phosphorylate various p73 isoforms *in vitro* at Thr^{86}. This cell cycle–dependent phosphorylation inhibits p73 to induce endogenous p21 expression (87). p73 is a physiologic target of the cyclin B-CDK1 mitotic kinase complex *in vivo*, which results in a decreased ability of p73 to bind DNA and activate transcription in mitotic cells. Both p73α and p73β isoforms are hyperphosphorylated in normal mitotic cells (88). Recently, the receptor for activated C kinase-1 was found to interact with the extreme COOH terminus of p73α, although receptor for activated C kinase is notorious for being "fished" out in yeast two hybrids by a wide variety of baits. Nevertheless, when overexpressed, receptor for activated C kinase-1 inhibits p73α-mediated transcription of p73 target genes and p73α-dependent apoptosis (89). DNA damage induces acetylation of p73 at Lys^{321}, Lys^{327}, and Lys^{331} by the acetyltransferase p300/cAMP-responsive element binding protein. Nonacetylated p73 is defective in inducing proapoptotic genes such as $p53^{AIP1}$ but retains the ability to activate other target genes such as p21. This indicates that DNA damage-dependent p73 acetylation, like in p53, potentiates the apoptotic function of p73 by selectively increasing its ability to induce the transcription of proapoptotic target genes (90).

Role of p73 and p63 in Tumors: p73 Is Not a Classic Knudson-Type Tumor Suppressor

p73 maps to chromosome 1p36.33, which frequently undergoes loss of heterozygosity in breast and colon cancer, neuroblastoma, oligodendroglioma, and melanoma. This fact, in conjunction with the functional similarity to p53, originally led to the proposal that p73 is a tumor suppressor gene (5). Genetic data on most cancer types (with the notable exception of leukemias and lymphomas), however, exclude p73 as a classic Knudson-type tumor suppressor, which by definition is targeted to undergo loss of expression or function during tumorigenesis. To date, in a total of >1,100 primary tumors, loss of function mutations in p73 are vanishingly rare (0.6%). Moreover, imprinting of the p73 locus, initially thought to be an epigenetic explanation to satisfy the two-hit hypothesis (because it would only require one hit of loss of heterozygosity against the transcribed allele), is rather uncommon and, if present, varies from tissue to tissue and person to person and does not correlate with p73 expression levels (37, 91-93). In fact, in lung, esophageal, and renal carcinoma, the second p73 allele is specifically activated in tumors (loss of imprinting; refs. 94-96). As an additional difference from p53, p73 protein fails to be inactivated by most of the major viral oncoproteins that inactivate p53 [i.e., SV40 T-antigen (97) and Ad E1B 55 kDa (98)]. For HPV E6, although clearly not inducing p73 degradation (67, 70, 99), controversy exists whether E6 of low- and high-risk strains inactivates the transcription function of p73 (99, 100). However, some viral protein products do target p73. p73 transcriptional activity is inhibited by Ad E4orf6 (101) and human T-cell lymphotrophic virus 1 Tax (102). p63 also fails to interact with SV40 T-antigen and the HPV E6 protein (103).

Alteration of p73 Expression in Human Cancer

Surprisingly, work on multiple primary tumor types and cell lines from our laboratory and confirmed by others showed that the most common identifiable cancer-specific alteration is overexpression of various isoforms of the wild-type TP73 rather than a loss of expression (5). This suggests that TP73 plays an oncogenic role in tumorigenesis. The single exceptions to this picture might be lymphoid malignancies and, possibly, bladder cancer. Although overexpression of p73 gene was found in B-cell chronic lymphocytic leukemia (104) and during differentiation of myeloid leukemic cells (38), TP73 has been found to be transcriptionally silenced in some lymphoblastic leukemias and lymphomas due to hypermethylation (105, 106). Likewise, based on one immunocytochemical study with prognostic analysis, invasive high-grade bladder cancers, which had lost p73 (and p63) staining, had a poorer clinical outcome (107).

To date, significant prevalence of p73 overexpression has been found in 12 different tumor types including tumors of breast (91), neuroblastoma (37), lung (95, 108), esophagus (96), stomach (109), colon (110), bladder (111, 112), ovarian cancer (70% of cases in one cohort; refs. 113-115), ependymoma (115), liver cancer (116), cholangiocellular carcinoma (117), chronic myelogenous leukemia blast crisis and acute myelogenous leukemia (118, 119), colon carcinoma (120, 121), and head and neck squamous carcinoma (associated with distant metastasis; refs. 122-124). Most studies measure overexpression of full-length p73 mRNA (TAp73) by reverse transcription-PCR, but a few studies also measure overexpression of TAp73 protein(s) by either immunoblot or immunocytochemistry. For example, we found overexpression of TAp73 transcripts (5- to 25-fold) in 38% of 77 invasive breast cancers relative to normal breast tissue and in five of seven breast cancer cell lines (13- to 73-fold; ref. 91). Likewise, we found overexpression of TAp73 transcripts in a subset of neuroblastoma (8- to 80-fold) and in 12 of 14 neuroblastoma cell lines (8- to 90-fold; ref. 37). A close correlation between p73 mRNA levels and protein levels was shown in ovarian carcinoma cell lines (113). In a series of 193 patients with hepatocellular carcinoma, 32% of tumors showed detectable (high) p73 by immunocytochemistry and in situ hybridization, whereas all normal tissue had undetectable levels (low; ref. 116). Of note, primary tumors and tumor cell lines with p73 overexpression tend to simultaneously overexpress a complex profile of shorter COOH-terminal splice variants (p73γ, p73δ, p73ε, and p73ϕ), whereas the normal tissue of origin is limited to the expression of p73α and p73β (91). Importantly, patients with high global p73 protein expression had a worse survival than patients with undetectable levels (116, 121).

There is an emerging sense that the dominant-negative ΔTAp73 isoforms rather than TAp73 might be the physiologically relevant components of tumor-associated p73

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overexpression, functionally overriding an often concomitant increase in TAp73 expression. This might have escaped notice because many of the early p73 overexpression studies in human cancers determined total p73 levels (all isoforms). Therefore, up-regulation of ΔTAp73 forms likely contributed to the elevated total p73 levels found previously in human cancers. Although, to date, only a few limited studies of tumors (breast cancer, gynecologic cancers, hepatocellular carcinoma, and neuroblastoma) focused on ΔTAp73, highly prevalent, tumor-specific up-regulation of ΔNp73 or ΔN′p73 (producing the same protein) has already been found in all of them (125-131). For example, in a study of 100 ovarian carcinomas, we found that ΔN′p73 transcripts are overexpressed in 91% (126). In hepatocellular carcinoma, ΔTAp73 is up-regulated compared with normal liver (127), and in various gynecologic cancers, we found that ΔNp73 is overexpressed in 73% of cases compared with the patients' matched normal tissues of origin (125). Moreover, 31% of 52 breast cancers overexpressed ΔNp73 compared with normal breast tissue (125). Of note, ΔNp73 overexpression appears to have a clinical impact at least in some cancer types. ΔNp73 was found to be an independent prognostic marker for reduced progression-free and overall survival in neuroblastoma patients (130).

Alteration of p63 Expression in Human Cancer

TP63 is not a tumor suppressor. The analysis of p63 in cancers of patients with germ line mutations or somatic mutations indicates similar lack of mutations but up-regulation of dominant-negative forms. For example, no p63 mutations were found in 47 bladder cancers (132) or 68 squamous cell carcinoma of the head and neck (124). Only 1 missense mutation ($Ala^{148}Pro$) of 66 various human tumors and 2 missense mutations in 35 tumor cell lines were found (8).

The human TP63 gene is on chromosome 3q27-28 within a region that is frequently amplified in squamous cell, cervical, and prostate carcinomas. Some lung cancers and squamous cell carcinomas of the head and neck show p63 overexpression associated with a modest increase in TP63 copy numbers (18).[4] Importantly, although many amplified in squamous carcinoma isoforms are produced in those tumors, the majority are dominant-negative ΔNp63 forms (mainly $p40^{AIS}$). $p40^{AIS}$ acts like an oncogene in nude mice and in Rat1a focus formation assays (18). Similar findings exist in nasopharyngeal carcinoma, which almost always has functional wild-type p53. In 25 primary nasopharyngeal carcinomas, all tumor cells overexpressed predominantly ΔNp63, which in normal nasopharyngeal epithelium is limited to proliferating basal and suprabasal cells (133). In esophageal squamous cell carcinoma, ΔNp63 is the major isotype expressed throughout. In contrast, in normal esophagus, p63 staining is restricted to the basal and suprabasal cell layers (122, 134). Thus, the maintenance of the ΔNp63 isoforms in squamous cancers may contribute to keeping the cells in a stem cell–like phenotype, thereby promoting tumor growth. On the other hand, ΔNp63α (also known as chronic ulcerative stomatitis protein) is frequently undetectable in cutaneous lesions like basal cell carcinoma, basal cell nevus syndrome, and nevus sebaceous in contrast to normal skin where it is strongly expressed in basal cells (135).

In prostate, p63 staining is a reliable marker of basal cells (49, 136) and stains positively in basal cell hyperplasia; however, prostatic adenocarcinoma, devoid of basal cells, is negative. This can be used clinically for differential diagnosis (136).

Up-regulation of ΔNp63 was also found in 30 of 47 bladder cancers (132). Interestingly, TAp63 was concomitantly down-regulated in 25 of those 47 tumors. However, another study on 160 bladder transitional cell carcinomas examined this relationship more closely. Whereas 93% of low-grade papillary superficial tumors expressed p63, only 68% of intermediate- and high-grade superficial tumors were positive. Moreover, there was a striking drop to 16% when tumors progressed in stage from superficial to invasive. Thus, loss of p63 in transitional cell carcinoma occurs with a progressive loss of urothelial differentiation associated with stage and grade (34, 137). p63 is expressed in myoeptithelial cells of ducts in the breast (48). p63 is expressed in a minority of breast carcinoma (mainly ductal carcinomas, rarely in carcinoma in situ) and is not expressed in invasive carcinoma (34, 47, 48, 138, 139). However, lack of p63 expression cannot be used as a reliable marker of invasiveness in breast ductal carcinoma in situ (140). In less differentiated gastric carcinoma, p63 is highly expressed in both TA and ΔN forms, suggesting that it can promote neoplastic growth in gastric epithelium (141).

Upstream Components That Signal to p73 and p63

p73 is able to mediate death stimuli from three different tumor surveillance pathways *in vivo*: oncogenes, DNA damage, and T-cell receptor hyperactivation.

p73 Is Activated to Mediate Apoptosis by Cellular and Viral Oncogenes

We and others recently established that the cellular and viral oncogenes E2F1, c-Myc, and E1A can induce and activate the endogenous TAp73α and TAp73β proteins for target gene transactivation, apoptosis, and growth suppression in p53-deficient human tumor cells (142-145). E2F1 is a direct transcriptional activator by binding to several E2F1-responsive elements within the P1 promoter of TP73 (143, 145). This is specific for TP73 because E2F1 does not activate the TP63 promoter, suggesting that this promoter is devoid of an E2F1 response element (145). Because oncogene deregulation of E2F1 and c-Myc are one of the most common genetic alterations in human tumors, these findings might provide a physiologic mechanism for TAp73 overexpression in tumors. Taken together, these data establish another important link between p73 and human cancer.

Moreover, during E2F1-mediated apoptosis in primary MEFs, a striking nonadditive cooperation between wild-type p53 and p73 exists (145). Whereas wild-type MEFs show 77% apoptosis after forced E2F1 expression, $p53^{-/-}$ MEFs

[4] The authors therefore named the amplified locus amplified in squamous carcinoma.

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(containing p73) and $p73^{-/-}$ MEFs (containing p53) both show reduced apoptosis after forced E2F1 expression with 12% and 15%, respectively. This reduced apoptosis of $p73^{-/-}$ MEFs, despite the presence of wild-type p53, is consistent with an important synergistic but independent signal emanating from TAp73 that cooperates with p53 to induce oncogene-triggered death in a tumor surveillance pathway.

p73 is required for antigen-induced death of circulating peripheral T cells after T-cell receptor activation and for tumor necrosis factor-α-induced death of thymocytes (immature T cells). This death pathway is mediated via the E2F1-p73 (144, 146). Conversely, the survival of antigen-stimulated T cells requires nuclear factor κB–mediated inhibition of p73 expression (146). Consistent with this notion, E2F1-null mice exhibit a marked disruption of lymphatic homeostasis with increased numbers of T cells and splenomegaly, suggesting that p73 plays a role in tumor surveillance pathways of lymphoid cells (147, 148). Moreover, the p73 gene is transcriptionally silenced in acute lymphoblastic leukemia and Burkitt's lymphoma due to hypermethylation (105, 106, 149, 150). This appears to be restricted to lymphoid tumors because neither other hematopoietic malignancies nor solid tumors show p73 hypermethylation (105, 149). Interestingly, in radiation-induced T-cell lymphomas of the mouse, the p73 locus undergoes loss of heterozygosity in 33% of the cases (151). Thus, in lymphoid tumors, p73 shows some genetic features of a classic tumor suppressor gene. Early growth response factor-1, an immediate early gene that is activated by mitogens in quiescent post-mitotic neurons, induces apoptosis in neuroblastoma cells. This apoptosis seems to be mediated by p73, which is elevated in cells overexpressing early growth response factor-1 (152).

Very scant data are available on the activation of p63 by oncogenes. Both epidermal growth factor receptor and p63 are overexpressed in a significant number of head and neck squamous cell carcinoma. A pharmacologic inhibitor of epidermal growth factor receptor in a head and neck squamous cell carcinoma line led to a decrease on p63 levels, suggesting that p63 is a downstream target of epidermal growth factor receptor signaling (153).

p73 and p63 Are Activated to Mediate Apoptosis by a Spectrum of DNA Damage

Endogenous p73 is activated for apoptosis in response to cisplatin, taxol, and γ-IR in a pathway that depends on the nonreceptor tyrosine kinase c-*abl* (85, 86, 154), supporting a model in which some DNA damage signals are channeled through c-*abl* to p73. Hence, one would predict that p73-deficient cells should have defective DNA damage checkpoint controls. This seems to be borne out by the observation that p53/p73 double null MEFs are more resistant to killing by cisplatin and taxol than p53 single null MEFs (62). Endogenous p73 protein is also rapidly induced by camptothecin treatment,[5] etoposide, cisplatin, and doxorubicin (155-157). Thus, DNA damage-dependent activation of p73 might be partly responsible for p53-independent apoptosis.

[5] U.M. Moll, unpublished observation.

Ectopic TAp63γ in a mouse erythroleukemia line is rapidly stabilized and induces WAF1 after treatment with UV, γ-IR, or actinomycin D (158). Surprisingly, stabilized TAp63γ was associated with erythroid differentiation rather than apoptosis, as was seen with ectopic p53. Because ectopic TAp63γ without additional DNA damage caused apoptosis in baby hamster kidney cells (6), it hints at a functional versatility of TAp63γ to induce differentiation under genotoxic circumstances.

Mechanisms of Transdominance: Heterocomplex Formation and Promoter Competition

Previously, we and others demonstrated physical interaction between oncogenic and antioncogenic family members as one of two mechanisms for their transdominant interference with the suppressor functions of wild-type p53 and TAp73 (20, 61, 125, 159). Mixed protein complexes were found between endogenous ΔNp73α or ΔNp73αβ on the one hand and either wild-type p53, TAp73α, orTAp73β on the other hand in primary human tumors, cultured human tumor cells, and mouse neurons. The stoichiometric ratio of TA/ΔNp73 could be a determinant in tumor formation. The slightest decrease in this ratio might be sufficient to convert TAp73 from a tumor suppressor to an oncogene.

Concerning p53 mutants, physical interactions between certain but not all human p53 mutants and TAp73 or TAp63 proteins were found in coimmunoprecipitation assays, and these interactions correlate with functional transdominance. In contrast, complexes between wild-type p53 and p73 are not observed in mammalian cells (17, 52, 160, 161). Unexpectedly, protein contact occurs between the DBD of mutant p53 and the DBD plus oligomerization domain of p73 (162-164) rather than between the respective oligomerization domains. In cotransfections, mixed heterocomplexes were shown between p53 mutants $p53Ala^{143}$, $p53Leu^{173}$, $p53His^{175}$, $p53Cys^{220}$, $p53Trp^{248}$, or $p53Gly^{281}$ and TAp73α, TAp73γ, and TAp73δ (52, 160, 162, 164) or TAp63 (164). Physiologic complexes were found in five tumor cell lines between endogenous mutant p53 and p73 (160, 162). Functionally, formation of such stable complexes leads to a loss of p73- and p63-mediated transactivation and proapoptotic abilities. Moreover, E2F1-induced p73 transactivation, apoptosis, and colony suppression was inhibited by coexpressed $p53His^{175}$ (143). Interestingly, the Arg/Pro polymorphism at codon 72 of mutant p53 is a biological determinant for binding and inactivation of p73, with 72R mutants of p53 being inhibitory, whereas 72P mutants are not (160, 165).

This functional inhibition of TAp73/p73 by some p53 mutants mirrors the ability of many transdominant missense p53 mutants to abrogate wild-type p53 function (166, 167). It suggests that in tumors that express both TAp73 and mutant p53 (typically at very high levels due to deficient MDM2-mediated degradation), the function of TAp73 and TAp63 might be inactivated. Moreover, these functional interactions define a network that could result in a "two birds with one stone" effect for at least some inactivating p53 mutations. If this occurs in primary human tumors, it might have far-reaching consequences because (*a*) it argues for a transdominant

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inhibition of the tumor suppressor function of TAp73 isoforms during tumor development, (*b*) it could be the underlying mechanism for the gain-of-function activity of certain p53 mutants, and (*c*) it might further increase chemoresistance in cancer therapy of established tumors. p53 is exceptional among tumor suppressors in that it selects for the overexpression of missense mutants rather than for loss of expression as most other suppressor genes do. This gain-of-function results in increased tumorigenicity compared with p53-null parental cells, increased resistance to cancer agents, and increased genomic instability due to abrogation of the mitotic spindle checkpoint (168-170). Conceivably, p63 also participates in this network. On the other hand, it should be noted that some p53 mutants clearly are recessive toward TAp73 (e.g., p53His^{283} and p53Tyr^{277}; ref. 164) and do not interfere with its action.

With respect to p63, tumor-derived p53 mutants can associate with p63 through their core domains. This interaction impairs transcriptional activity of p63 and could contribute in promoting tumorigenesis and conferring selective survival advantage to cancer cells (162).

Promoter competition by ΔNp73 at TAp73/p53 response elements is another transdominant mechanism (20, 171). It is conceivable that ΔNp73 or ΔNp63 homo-oligomers might have a stronger affinity to certain target gene promoters than wild-type p53. In those cases, p53 inhibition could occur due to competition at the level of target gene access. In the wild-type p53-containing ovarian carcinoma cell line A2780, coexpression of increasing amounts of either TAp73α, TAp73β, TAp73γ, or TAp73ε inhibits specific DNA binding and transcriptional activity of p53 in the absence of hetero-oligomer formation (161, 172).

In short, the biological consequences of deregulated TP73 and TP63 expression might be diametrically different depending on the isoform stoichometry (ΔNp73/p63 versus TAp73/p63) and presence or absence of mutant p53.

An Autoregulatory Feedback Loop Exists Among p53, TAp73, and ΔNp73

p53 and TAp73 regulate ΔNp73 but not ΔNp63 levels by binding to the p73 P2 promoter and inducing its transcription. A p73-specific responsive element was mapped within the P2 region (159). This generates a negative feedback loop analogous to the p53-MDM2 loop that in turn negatively regulates the activity of p53 and p73 (159, 171, 173, 174). ΔNp73 blocks p53 and TAp73 activity through heterocomplex formation (20, 125, 159) or through promoter competition (20, 171) and thus contributes to the termination of the p53/p73 response in cells that do not undergo apoptosis. In contrast to ΔNp73, ΔNp63 expression is transcriptionally repressed by p53 (175).

p73 and Chemosensitivity

Endogenous p73 protein levels increase in response to cisplatin and Adriamycin (86, 90, 154). Although originally thought to respond only to a limited spectrum, it is now clear that TAp73 (α more than β) is induced by a wider variety of chemotherapeutic agents (Adriamycin, cisplatin, taxol, and etoposide) in different tumor cell lines (157, 165). p73 accumulation is due to increased transcription and increased protein stabilization and leads to induction of apoptotic target genes such as apoptosis-induced protein-1. Conversely, blocking

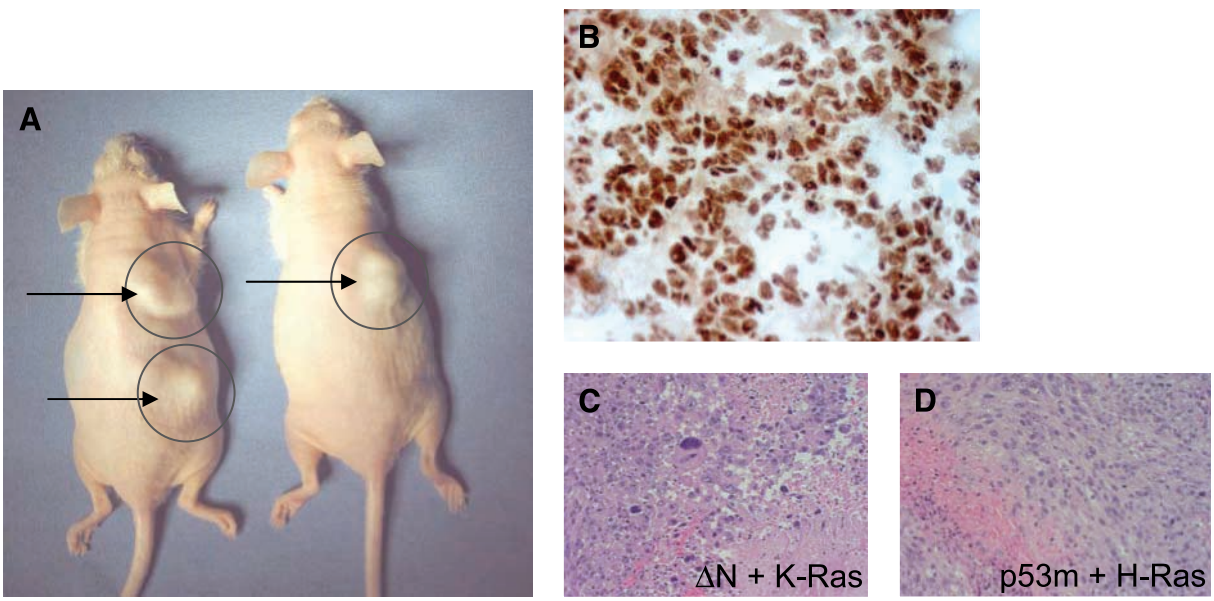

FIGURE 4. ΔNp73-expressing primary cells are tumorigenic in nude mice. **A.** Nude mice injected with ΔNp73 and oncogenic Ras-expressing MEFs develop tumors. **B.** Immunohistochemical examination shows nuclear ΔNp73 expression in tumor cells from **A**. **C** and **D.** Histologically, ΔNp73 and Ras-coexpressing tumors from **A** are anaplastic fibrosarcomas and resemble fibrosarcomas produced in MEF control cells injected with mutant p53 R175H and oncogenic Ras.

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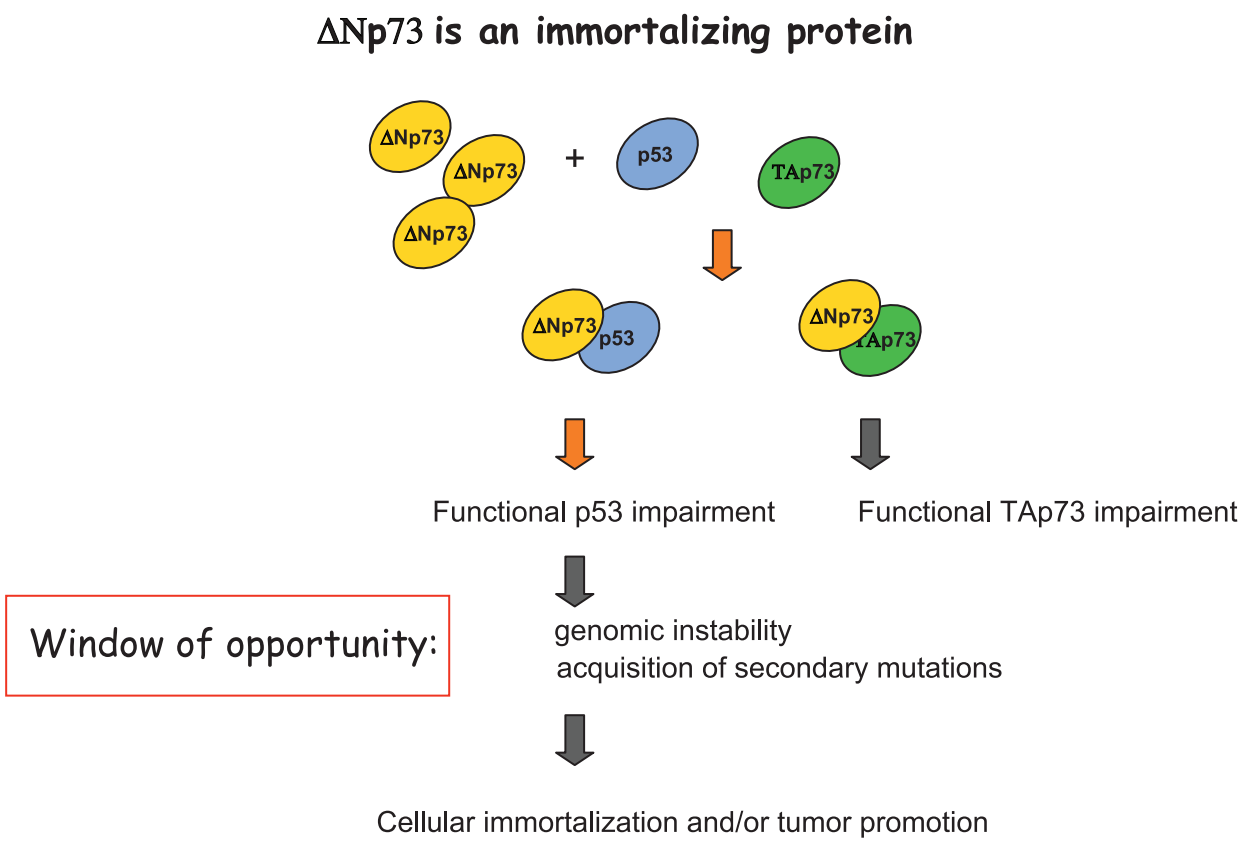

FIGURE 5. Proposed mechanism of the action of ΔNp73 in tumor promotion. ΔNp73 promotes immortalization of primary MEF cells by a factor of 10^3 and cooperates with oncogenic Ras in their transformation. Mechanistically, ΔNp73 counteracts the growth-restraining actions of p53 and TAp73 either temporarily or permanently, thus creating a window of opportunity for the acquisition of secondary mutations and/or genomic instability.

TAp73 function (either by the inhibitory p73DD fragment or by p73 small interfering RNA) leads to enhanced chemoresistance, which is independent of the p53 gene status. Of note, whereas the presence of p73 is essential for p53 to induce apoptosis in fibroblasts (62), p73 on the other hand can induce apoptosis in cells that lack functional p53 (157). This confirms the importance of p73 in the response to chemotherapeutic agents (165).

In cell culture, overexpression of antiapoptotic p73 isoforms can also block chemotherapy-induced apoptosis in wild-type p53 tumor cells (125, 173). Moreover, overproduction of certain p53 mutants can block p73 function and chemotherapy-induced apoptosis (52, 164, 176). This effect is most strongly linked to the Arg^{72} polymorphism of the p53 gene (157, 160, 165) and is mediated by stable hetero-oligomers involving the DBDs. Bergamaschi et al. have used different cell lines forced to express a series of p53 mutants as either Arg (72R) or Pro (72P) versions at codon 72. Only Arg mutants correlated with chemoresistance. These data were mirrored in a series of polymorphic head and neck cancer patients with the same p53 mutants: 72R patients showed poor response to chemotherapy and shorter survival (165). Conversely, down-modulation of endogenous p53 mutants enhances chemosensitivity in p53-defective mutant cells (157). Consequently, a promising therapeutic approach includes the use of small interfering RNA specifically directed against particular p53 mutants, which might restore chemosensitivity of tumor.

Potential Application of p63/p73 in Gene Therapy of p53-Inactivated Tumors

Some authors suggest the use of p73β in gene therapy as a substitute for p53. For example, cervical cancers caused by HPV are resistant to p53 gene therapy possibly because HPV E6 protein degrades p53 by ubiquitin-mediated proteolysis. However, p73β is resistant to HPV E6-mediated proteolysis, induces apoptosis, and is a potent inhibitor of cancer colony growth *in vitro* (p73α was a less effective suppressor of the cell growth; ref. 100). Furthermore, colorectal cancer cells that are resistant to p53-mediated cell death undergo apoptosis after adenovirus-mediated p73β and p63γ gene transfer (177). In addition, some pancreatic adenocarcinoma lines lacking functional wild-type p53 are completely resistant to p53-mediated apoptosis. However, p73β is capable of efficiently kill these cells (178). This p73-mediated cell death is probably mediated by $p53^{AIP1}$, an important mediator of p53/p73-dependent apoptosis. $p53^{AIP1}$ is not activated by p53 because, in these particular cells, p53 is not phosphorylated at Ser^{46}, which is essential for transcriptional activation of $p53^{AIP1}$ by p53.

p73 and p63 Appear to Play a Role in Cancer—but as an Oncogene or as a Suppressor Gene?

Clearly, p73 plays an important role in human tumors *in vivo*. However, the current picture of the role of p73 in

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human cancer is a puzzling ying-yang, given the diametrically opposing functions of the two types of concomitantly expressed gene products and inhibitory family network of interactions. However, some observations seem to fall into place now: the p53-synergistic action of TAp73 after DNA damage or oncogene deregulation in primary cells might be an additional fail-safe mechanism against neoplastic transformation. This, however, makes the frequent overexpression of TAp73 in many human tumors all the more puzzling. On the other hand, there is striking evidence that ΔTAp73/p63 forms are overexpressed in human tumors (91, 125) and perhaps preferably in wild-type p53 tumors (126) and could act as oncogenes *in vivo*. ΔTAp73/p63 inactivates p53, TAp73, and TAp63 in their role to induce apoptosis and cell cycle arrest and inhibits their suppressive activity in colony formation (125). In addition, TAp73 is inactivated by dominant-negative interference from mutant p53. Moreover, ΔNp73 functions as an immortalizing oncogene. We recently showed that ΔNp73 promotes immortalization of primary MEFs and cooperates with Ras in driving their transformation *in vivo* (Figs. 4 and 5; ref. 179). Stiewe et al. have found that ΔTAp73 overexpression results in malignant transformation of NIH3T3 fibroblasts and tumor growth in nude mice (127). How can we decide on the true role? We feel that, ultimately, the fact that TP73 is virtually never targeted by inactivating mutations *in vivo* strongly suggests that it is indeed the oncogenic ΔTAp73 forms that are the truly critical ones during tumor formation and progression. However, a large body of primary tumor analysis will be required to test if overexpression of ΔTAp73 isoforms can be linked to p53 status and clinical outcome.

Conclusions

Inactivation of the p53 tumor suppressor is the single most common genetic defect in human cancer. The discovery of two close structural homologues, p63 and p73, generated instant excitement and quick expectations about their biological functions. We now know that, in development, both genes clearly have novel p53-independent functions. p63 is involved in epithelial stem cell regeneration, and p73 is involved in hippocampal neurogenesis, pheromonal pathways, and ependymal cell function. To determine the role of these p53 homologues in tumor biology is still a challenge, but we have made progress. It is already clear that they are not classic Knudson-type tumor suppressors. However, the existence of p53-like and p53-inhibitory versions of TP73 and TP63, plus intimate functional cross-talk among all family members, endows these genes with both tumor suppressor and oncogenic roles. To determine which of these ying-yang roles are important in cancer, more future clinicopathologic studies correlating relative overexpression ratios of these opposing subgroups, p53 mutation status, and clinical outcome might be one of the best available tools.

Acknowledgments

We thank P. Pancoska for technical assistance. We apologize to our colleagues in the field whose contributions were not cited due to space limitations.

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