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Role of the newer p53 family proteins in malignancy

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The most recently identified members of the p53 family, p63 and p73, share certain structural and functional similarities with p53. Both p63 and p73 can bind to canonical p53-DNA-binding sites, transactivate the promoters of known p53 target genes and induce apoptosis. Despite these similarities there are many important differences. In contrast to *p53*, *p63* and *p73* give rise to multiple distinct protein isoforms that have different functional properties. Upstream signaling pathways involved in the activation of p63 and p73 differ from those involved in p53 activation. Only a subset of the DNA damaging agents that induce p53 can induce p73. Cellular and viral oncoproteins can discriminate between p53 and the newer family members. In addition, the levels of p63 and p73 are affected by certain states of cellular differentiation. Finally, it is becoming clear that the newest members of the p53 family are not classical tumor suppressor genes. In contrast to the high prevalence of *p53* mutations in human cancers, *p63* and *p73* mutations are rare. Indeed, levels of p73 increase during malignant progression. In addition, unlike *p53*-/- mice, mice lacking *p63* and *p73* do not develop tumors, but instead have significant developmental abnormalities. Mutations in *p63* have also been detected in humans with the ectodermal dysplastic syndrome EEC. Further studies are required to determine whether qualitative or quantitative differences in the expression of p63 and p73 isoforms are important in the development of human cancers.

Keywords: p53; p63; p73; apoptosis; tumor suppressor; EEC syndrome

Introduction

p53 one of the most intensively studied genes in human cancer biology. The p53 gene product was first identified twenty years ago as a binding partner for simian virus (SV) 40 T antigen. Only later was it appreciated that p53 is an important tumor suppressor protein that is mutated or inactivated in greater than 50% of human cancers. p53 is a sequence-specific DNA binding transcription factor that responds to certain cytotoxic stresses such as DNA damage by enhancing the transcription of genes that regulate cell-cycle progression as well as programmed cell-death (apoptosis).[1] Most tumor-derived p53 mutations are missense mutations that compromise multiple p53 functions. Cells lacking p53 function are genetically unstable and prone to tumor formation. Furthermore, p53 status may affect response to anti-tumor agents such as chemotherapy and overall prognosis for certain tumors.[2]

Most oncogenes and tumor suppressor genes belong to larger families of related genes. Examples of such gene families include the *Ras* (*K-Ras, N-Ras, H-Ras*), *Myc* (*c-Myc, N-Myc, L-Myc*) and *Rb* (*Rb-1, p107, p130*) families. It had become widely accepted that *p53* provided an exception to this rule because extensive searches in the past failed to reveal any additional *p53* family members. In 1997, however, Daniel Caput and coworkers identified a p53-related gene called *p73*.[3] Shortly thereafter several groups independently isolated the third member of this family, *p63* (also known as *KET, p51, p40* and *p73L*).[4–8] Both p63 and p73 are more closely related to one another than p53. Like p53, p63and p73 can activate transcription from p53-responsive genes and induce apoptosis.[4,6,9] The specific role(s) of p63 and p73 in human cancer are less well understood. Specifically, p63 and p73 are rarely mutated in the tumors examined to date suggesting that they, unlike p53, are not classical tumor suppressor genes. However, the severe defects in mice lacking these genes suggest possible tissue specific functions during development and germ line mutations in p63 have been linked to developmental disorders in humans. This review will outline the similarities and differences between p53, p63, and p73 and will specifically address what is known about the role p63 and p73 in human disease.

Structure

p53 can be thought of as having three major modular domains, namely, an N-terminal transactivation domain (responsible for activating transcription once bound to a specific promoter), a central core DNA-binding domain (responsible for binding to specific DNA sequences) and a

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C-terminal oligomerization domain (which allows 4 p53 molecules to form a homotetramer that is necessary for high-affinity DNA binding). The more recently identified family members, p63 and p73, share significant homology with p53 in these three functional domains.[10] In striking contrast to p53, however, both p63 and p73 give rise to multiple functionally distinct protein isoforms due to alternative promoter utilization and alternative mRNA splicing.

Alternative mRNA isoforms

Different p73 C-termini are produced as a result of alternative splicing of the p73 mRNA (Figure 1). Initially two isoforms of p73, α and β, were identified.[3] In comparison to p73α, p73β is smaller due to removal of exon 13 from the p73 mRNA. This interrupts the p73 open reading frame, yielding a prematurely truncated protein of 499 amino acid residues, including five unique C-terminal residues. Four additional p73 isoforms have now been identified in primary and tumor cells (δ,γ,ε,ζ).[11–15] The structures of these isoforms are shown in Figure 1. In addition, Yang and colleagues recently identified additional p73 isoforms, called ΔN p73α and ΔN p73β, which lack the N-terminal transactivation domain.[16] These isoforms result from the use of an alternative promoter located in intron 3 (Figure 1).

The p63 gene encodes at least six different proteins, which share a common open reading frame.[4] As was true for p73, these multiple isoforms are the result of alternative promoter utilization and alternative splicing (Figure 2). The three TA isoforms, TA-p63 α,β,γ are transcribed from a 5′ promoter and the three ΔN isoforms, ΔN-p63α, ΔN-p63β, and ΔN-p63γ, are transcribed from a 3′ promoter located in intron.[3] The TA symbol refers to the inclusion of the N-terminal transactivation domain, which is lacking in the ΔN isoforms.[4] The different C-termini are the result of alternative mRNA splicing involving 3′ exons. The functional differences among the various p63 and p73 isoforms are discussed below.

Protein subdomains

Transactivation domain. The N-terminal transactivation domain is the least conserved of the three p53 functional domains when comparing the three p53 family members. This domain is 30% identical between p73 and p53, 22% identical between p63 and p53, and 30% identical between p73 and p63.[17] As is true for p53, the transactivation domain of p73 can interact with a co-activator protein called p300 as well as a protein called MDM2 (described in greater detail below). Unlike p53, p73 also contains a second potential transactivation domain within its C-terminus.[18] Most of the splice variants of p73 and p63, with the exception of the N-terminally truncated forms, are able to transcriptionally activate p53 responsive promoters. The potential downstream targets of the newer p53 family members are discussed below.

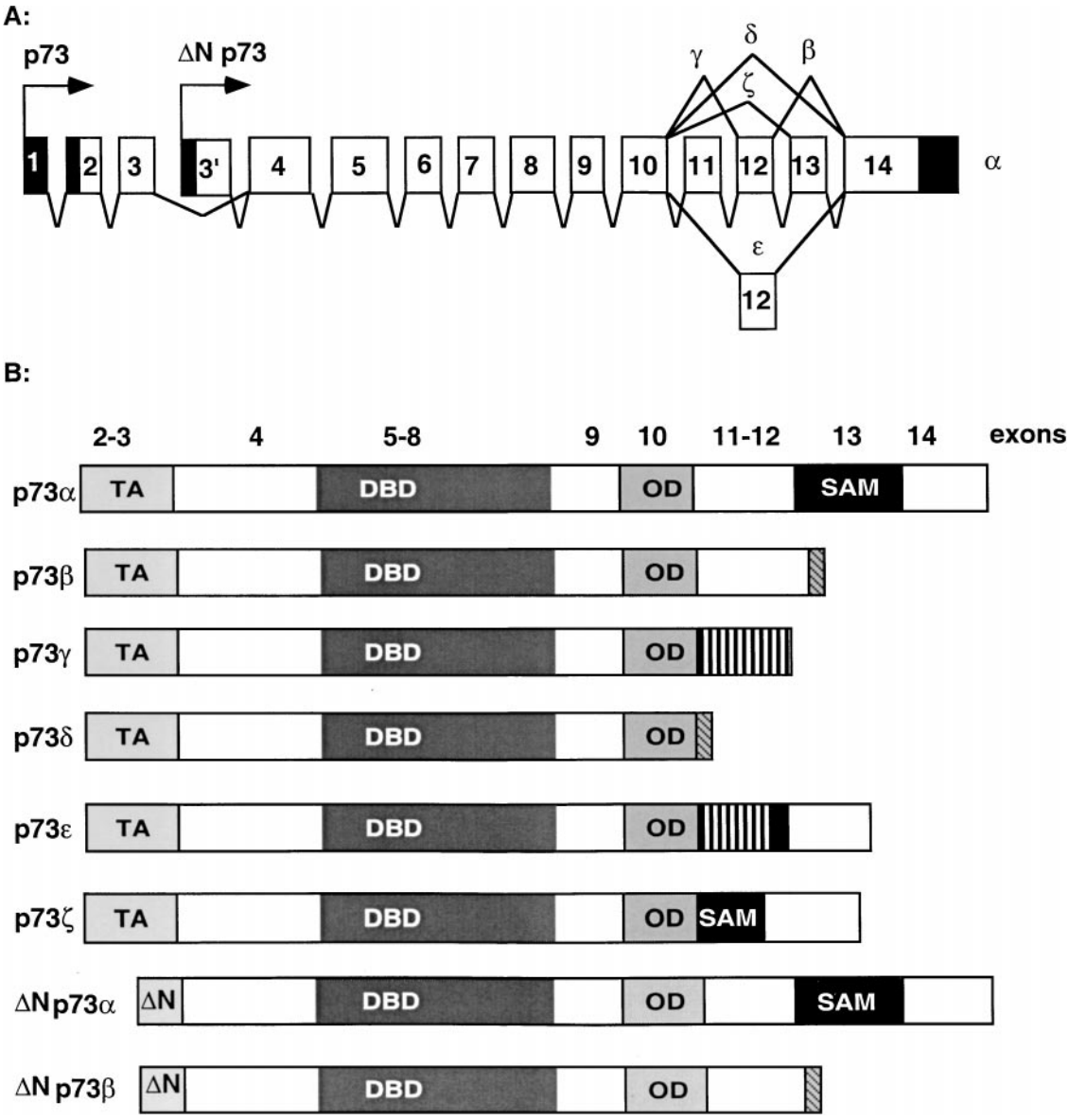
DNA binding domain. The p73 and p63 DNA-binding domains show 63% and 60% identity with p53, respectively. In addition, the residues of p53 that directly contact DNA are conserved in the sequences of p63 and p73.[19] Furthermore, p53 amino acid residues that are commonly altered by tumor-derived missense mutations are likewise conserved. Both p73 and p63 can bind to canonical p53 DNA-binding sites.[20] Whether there are differences among the p53 family members with respect to their optimal DNA-binding sequence(s) *in vivo* is unknown. Indirect evidence for such differences come from studies described below which suggest that the different family members differentially affect 'p53-responsive' promoters in cells.

Oligomerization domain. The C-terminal region of p53 (residues 326–355) is involved in oligomerization and shows 38% homology with p73 residues 351–383 and 38% homology with p63 residues 355–404.[10] p53 binds to DNA as a homotetramer. The p53 family members preferentially form homotetramers rather than heterotetramers with each other.[10] For a given p53 family member the different isoforms described above bind to one another to varying degrees. For example, p73γ binds other p73 isoforms strongly while p73α does not.[12]

SAM Domain. p73 and p63 contain C-terminal extensions that are not similar, or homologous, to p53. The alpha isoforms of p73 and p63 are predicted to encode SAM (sterile alpha motif) domains.[21,22] The SAM domain is a putative protein-protein interaction domain that has been found in multiple signaling proteins, many of which are important in developmental regulation. Although the p73 and p63 SAM domains do not bind to themselves or to one another, these domains may interact with other cellular proteins. Identification of these putative SAM-binding proteins and elucidation of their functions may significantly improve our knowledge of the potential roles of these newer p53 family members in cellular and organismal homeostasis. Interestingly, fusion of SAM domain proteins (TEL) to other regulatory/signaling proteins (*e.g.* PDGFR, AML1) as a result of chromosomal translocations has been described in leukemias and other malignancies.[23,24]

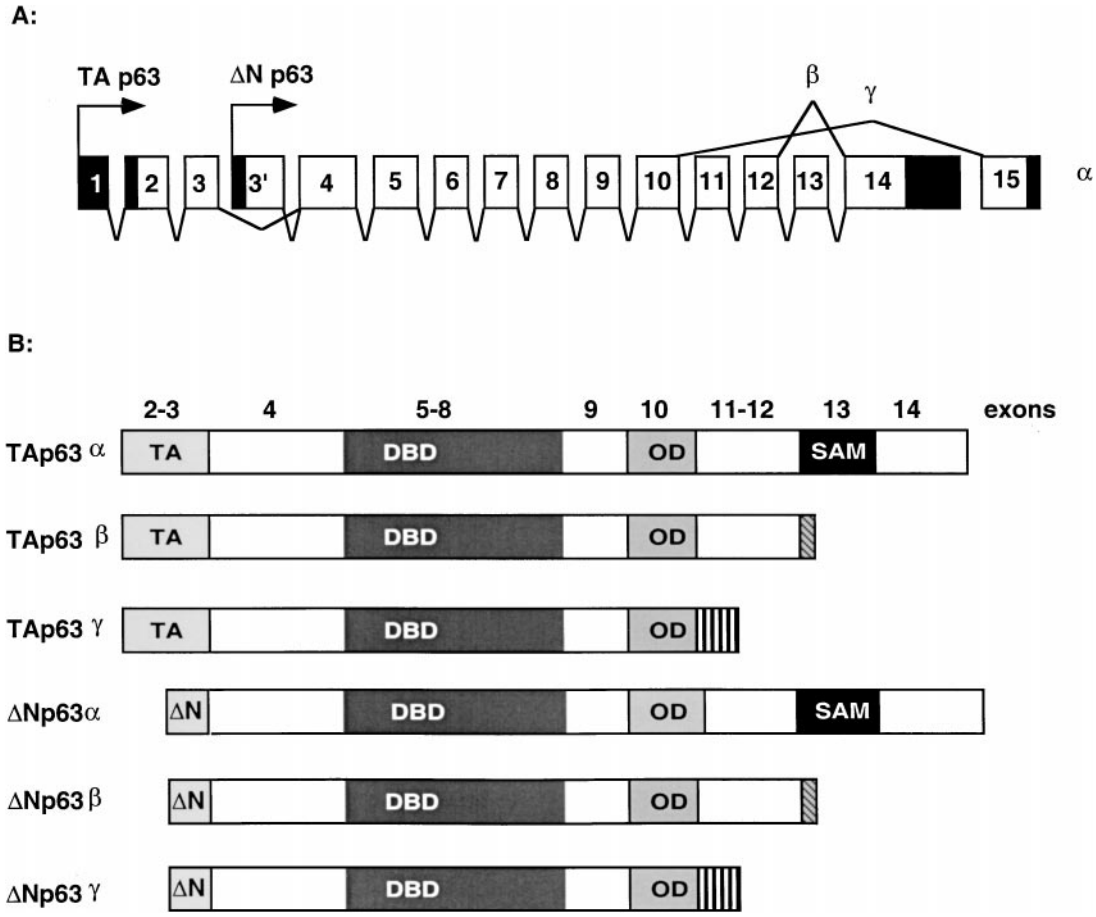
PXXP motifs. p53 contains a proline-rich domain located within residues 60–90. Within this region are five PXXP motifs (P = proline and X = any amino acid).[25] Mutations

Figure 1. (**a**) Structure of *p73* Isoforms: The splicing patterns generating p73 α, β, γ, δ, ζ and ϵ are shown. Transcriptional start sites are indicated by arrows. ΔN p73α and ΔN p73β are transcribed from a cryptic promoter within intron 3 (designated 3′). Untranslated regions are shaded black; (**b**) Functional Domains of p73 Protein: The transactivation (TA), DNA binding (DBD), oligomerization (OD) and sterile alpha motif (SAM) domains are depicted. The p73α exons included in these regions are listed at the top. As a result of alternative splicing of p73 mRNA, the C-terminal protein coding sequences vary. The α, β, δ and ζ forms share the same open reading frame for the majority of the C- terminus, while the γ form has a completely different open reading frame beginning at exon 11 (represented as vertical striped pattern). The C-terminal coding region of ϵ is composed of the γ and then α reading frames. The C-terminus of p73β and δ includes five unique amino acids (represented as diagonal striped pattern). Of note, only p73α, ΔN p73α and p73ζ include the majority of the SAM domain.



and deletions within this domain affect both p53 apoptotic activity and its ability to transcriptionally activate target genes.[25–27] These proline rich regions interact with cellular proteins containing SH3 (src homology 3) domains.[28] These interactions play important roles in cellular signal transduction pathways. Both p63 and p73 contain several PXXP motifs. However, in contrast to the cluster of PXXP motifs in p53, the p63 and p73 PXXP sequences are scattered throughout the coding region. The SH3 domain of the oncoprotein c-abl has been shown to bind p73 via a PXXP sequence located between the p73 DNA binding domain and the predicted oligomerization domain.[29] To date no other studies have examined the role of these PXXP sequences in p73 or p63.

Figure 2. (**a**) Structure of *p63* Isoforms: The splicing patterns generating p63 α, β and γ are shown. ΔN p63α, ΔN p63β and ΔN p63γ are transcribed from a cryptic promoter within intron 3 (designated 3′). Untranslated regions are shaded black. (**b**) Functional Domains of p63 Protein: The transactivation (TA), DNA binding (DBD), oligomerization (OD) and sterile alpha motif (SAM) domains are depicted. The p63α exons included in these regions are listed at the top. As a result of alternative splicing of p63 mRNA the C-terminal protein coding sequences vary. The p63β (ΔN and TA) and p63γ (ΔN and TA) proteins have unique coding sequences represented as diagonal and verical striped patterns, respectively. Only the alpha forms retain the SAM domain coding sequence.



Upstream regulation of p53 family members

The p53 protein is activated in response to a number of cellular stresses including DNA damage, oncogene activation, ribonucleotide depletion and hypoxia.[1] Once activated p53 can induce a growth arrest and/or apoptosis. In this section we will review the similarities and differences in the upstream regulation of p53, p63, and p73.

Growth and differentiation

DeLaurenzi and colleagues identified T-cell stimulation as a mechanism by which p73 is upregulated. They found that stimulation of both cultured and normal peripheral blood lymphocytes with PHA (phytoemagglutinin) causes an increase in p73 mRNA and an associated increase in apoptosis.[13] Another report showed that p73 is upregulated in response to neuronal differentiation agents. Retinoic acid treatment of neuroblastoma cells was associated with increases in p73 protein levels and neuronal differentiation specific markers.[30] Thus, p73 levels may vary during changes in cell growth and differentiation.

DNA damaging agents

Initial studies suggested that p73, in contrast to p53, was not induced by DNA damaging agents such as UV irradiation and actinomycin D.[3] More recently this notion was challenged by several reports which provided evidence that p73 is a target of the non-receptor tyrosine kinase c-abl in response to certain forms of DNA damage. Gong and colleagues showed that p73 is stabilized by cisplatin treatment and by co-expression with c-abl.[31] As described

above, p73 and c-abl form a complex via a p73 PXXP motif and the c-abl SH3 domain.[29]

Furthermore, p73 is phosphorylated by c-abl following gamma-irradiation of cells.[29,32] The pro-apoptotic activity of p73 is potentiated by c-abl and diminished in cells that lack c-abl. Since c-abl is itself phosphorylated and activated by the ataxia-telangiectasia-mutated (ATM) gene product, ATM may also be involved in the pathway leading to c-abl dependent p73 activation.[33] These findings suggest that p73 participates in a mismatch-repair signaling pathway.

In addition to cisplatin, taxol increases p73 accumulation, but ultraviolet light (UV), actinomycin D, or methylmethane sulfonate (MMS) do not in the cell types examined to date.[34] Thus, induction of p73 may be restricted to certain types of DNA damage. Thus far there are no data examining the effect of DNA damage on p63 levels.

Cellular oncoproteins and tumor suppressor proteins

P53 is normally a short-lived protein. The degradation of p53 is now understood in considerable detail. A protein called MDM2 facilitates a process wherein p53 becomes covalently linked to a polyubiquitin tail. This tail serves as a signal that is recognized by a protein degradation complex called the proteasome.[35–37] *MDM2* is itself a p53-target gene. Thus, activation of p53 establishes a negative feedback loop wherein MDM2 limits the accumulation of p53.

Many cellular oncoproteins, including c-myc, E1A, Ras, and E2F1 induce the stabilization, and hence the accumulation, of p53. This is due, at least partly, to the induction of a protein called ARF which, in turn, prevents the MDM2 protein from targeting p53 for degradation.[38,39] Oncogene-induced p53 stabilization would be expected to induce a cell-cycle arrest or apoptosis, either of which would prevent tumorigenesis. Tumors escape this potential failsafe mechanism if, as is commonly the case, they harbor a mutation of p53 or another component of the 'ARF-MDM2-p53 pathway'.[40] For example, some tumors that retain a wild-type p53 allele fail to produce normal levels of p53 due to amplification, and overproduction, of MDM2.

MDM2 binds to a sequence in the p53 N-terminal transactivation domain (residues 17–27) and, in addition to marking p53 for destruction, prevents p53 from serving as a transcriptional activator.[41] The MDM2 binding site in p53 is well conserved in both p63 and p73. Several groups showed that MDM2 binds to p73 and inhibits both p73 dependent transcriptional activation and p73 dependent apoptosis.[42–44] In contrast to p53, however, MDM2 binding does not lead to p73 degradation. Indeed, MDM2 may actually stabilize p73α and β.[42,43] One possible explanation for this resistance to degradation may relate to differences between the p53 and p73 C-termini. Although MDM2 binds to the p53 and p73 N-termini, mutations of the p53 C-terminus block MDM2 mediated degradation of p53.[45] Thus, the C-terminus of p53 plays an as yet poorly understood role with respect to MDM2-dependent proteolysis. It is currently not known whether p63 binds to MDM2 and, if so, whether MDM2 targets p63 for destruction.

Although not specifically mediated by MDM2, p73 degradation is nonetheless likely to involve ubiquitin-dependent-proteolysis since treatment of cells with drugs that inhibit the proteosome results in increased levels of p73.[42,43] Thus, it is possible that p73 stability may be regulated by an as yet unidentified protein such as the MDM2 related protein, MDMX.

Together, these results suggest that oncogenic activation of the ARF/MDM2 pathway results in the accumulation of p53 but not p73. Nonetheless, it is possible that oncogenes utilize other pathways to affect the behavior of p73 (or p63). For example, the p73 promoter contains binding sites for E2F and E2F1 can directly activate p73 transcription.[46,47] Oncogenic activation of p73 may account for the observations of several groups that p73 levels tend to be higher in cancer cells compared with their normal cellular counterparts.[48–54]

The Wilm's Tumor Suppressor gene, *WT-1*, is heterozygously mutated or deleted in a variety of congenital anomaly syndromes and homozygously mutated in up to 15% of Wilm's tumors. WT-1 is a transcription factor and can bind to p53, modulating its ability to activate target genes. Recently Scharnhost and colleagues reported that WT-1, via its so-called zinc-finger motif, binds to p73 and p63.[55] Furthermore, they showed that WT-1 can inhibit p73-induced transcriptional activation of p53 responsive genes. However, in contrast to its stabilizing effect on p53, WT-1 does not stabilize p73α, again suggesting that the mechanisms that regulate the half-lives of p53 and p73 are distinct.

Viral oncoproteins

A number of viral oncoproteins bind to, and inactivate, p53 during the course of viral transformation. Examples include the adenovirus E1B55, human papilloma virus E6 protein, and SV40 T antigen. Notably, these three proteins do not bind to p73.[20,56] Likewise, E6 and SV40 T do not interact with p63 (whether E1B binds to p63 is not known).[57] In fact, p73β can induce growth inhibition and apoptosis in cancer cells that produce E6.[58]

The adenoviral protein E4orf6 also binds to and antagonizes p53, but there have been conflicting reports with respect to the binding of E4orf6 and p73. Dobblestein

and colleagues reported that E4orf6 does not affect p73 stability or inhibit transcriptional activation.[56] Two other groups reported that E4orf6 binds the C-terminus of p73 and blocks transcriptional activation and colony suppression by p73.[59,60]

Finally, Kaida *et al.* recently reported that the human T-cell leukemia virus type 1 (HTLV-1) Tax oncoprotein could inactivate both p73 and p63, as well as p53, through their N-terminal transactivation domains.[61]

Thus certain viral oncoproteins preferentially inactivate p53 while sparing p63 and p73 despite the high degree of similarity between these three proteins. This raises the intriguing possibility that p73 and/or p63 facilitate, rather than inhibit, viral transformation. At a minimum, these findings suggest that the functions of p53, p63, and p73 are not wholly redundant.

Downstream target genes and function

Little is known about which genes are regulated by p63 and p73 under physiological conditions. It is likely that in the next several years advances in genomic technologies will facilitate the identification of unique target genes for each of the p53 family members. For example, DNA microarrays containing thousands of immobilized oligonucleotides or cDNAs can be used to measure changes in the abundance of different mRNAs on a nearly genome-wide scale.[62] Current data suggest p63 and p73 can, at least when overproduced, activate certain 'p53-responsive promoters' leading to p53-like effects (such as apoptosis). For example, p73 and p63 can activate the promoters of several p53-responsive genes implicated in cell-cycle control or apoptosis, including p21, Bax, MDM-2, GADD45, 14-3-3σ cyclin G, and IGFBP3.[3,4,9,12,15,63–65] A caveat with the studies performed to date in this area is that they relied on overproduction of p63 or p73 in cells. In theory, overproduction might mask subtle differences between the various p53 family members. Nonetheless, some differences are emerging. For example, LaThangue and colleagues found that p73 activated GADD45 more efficiently than p53 whereas p53 activated p21 much more strongly than p73.[63] Furthermore, the degree of transactivation for various genes varies between different p73 and p63 C-terminal isoforms suggesting that C-terminal sequences also affect function.[65,66] In many assays, for example, p73β is a more potent transcriptional activator than p73α.[12,63] TA-p63γ, but not TA-p63α, can transactivate the p21 promoter.[4] The ΔN p63 forms lack the N-terminal transactivation domain and are unable to transactivate p53-responsive promoters.[4] This is almost certainly true for ΔN p73 as well. Indeed, the ΔN forms of p63 can block transcriptional activation by the transactivation competent forms of p53, p63, and p73.[4]

Role of p63 and p73 in cancer, disease, and development

Germ line p53 mutations in humans cause the Li-Fraumeni hereditary cancer syndrome. Such patients have a significantly increased risk of developing a spectrum of tumors including breast cancers, sarcomas, brain tumors and leukemias. Similarly, *p53*-/- and *p53*+/- mice develop normally but are prone to multiple tumors including lymphomas and sarcomas.[67] Most sporadic human tumors also harbor mutations, acquired somatically, that directly or indirectly compromise the function of the p53 protein. Thus, the study of mouse and human cancer genetics underscores the importance of p53 inactivation in carcinogenesis.

The similarity of p63 and p73 to p53 fueled interest in whether they too played important roles in cancer. Unlike p53, however, mutations of the newer family members occur rarely in tumors and mice carrying defective p73 and p63 alleles are not tumor prone.[16,68,69] Also in contrast to p53, mice lacking p63 or p73 exhibit multiple developmental abnormalities. The analysis of p73 and p63 in development and cancer are described in greater detail below.

Role of p73 in malignancy

The p73 gene maps to chromosome 1p36, a region of the genome that is frequently deleted in a variety of human tumors including neuroblastoma, melanoma, breast, and colon cancer.[19] In addition, the mouse p73 gene maps to the distal part of chromosome 4 and may be involved in the progression of radiation induced Tcell-lymphomas.[70] The chromosomal location of *p73*, coupled with its structural and functional similarities to *p53*, led to the hypothesis that *p73* was in fact a tumor suppressor gene. In order for p73 to be considered a classical tumor suppressor gene the remaining non-deleted allele would need to be mutated or inactivated in tumors harboring 1p loss. To date, however, extensive studies have revealed only rare p73 mutations in both cell lines and primary tumors, including those with 1p deletions (Table 1). The tumors examined include neuroblastomas, breast, lung, renal prostate, ovarian, bladder, gastric, brain, skin and colorectal cancers.[18,48–54,71–93] While few mutations of p73 have been found, several have had potential functional consequences. Takada and coworkers identified two naturally occurring C-terminal mutations(P405R, P425L) in neuroblastoma which impair p73 transactivational activity.[18] Loss of p73 function may contribute to the pathogenesis of certain lymphomas and leukemias where loss of the p73 mRNA due to promoter hypermethylation has been reported.[93,94]

Some tumor suppressor genes are imprinted, a process leading to the unequal expression of the maternal and

Table 1. Mutational analysis of p73 in primary tumors

Cancer type	Reference	Mutation frequency	Expression level[a]	LOH[b]	Imprinting[c]/methylation
Neuroblastoma	71	0/16	T > N		B 5/6
	18,72	2/140 P405R, P425L			
	73	0/30		10/30	
	74	0/23			
	98				B 4/8
Breast cancer	48	0/8	T > N (29/77)		B 8/14; M 6/14
	75	0/87	T = N	6/46	
	74	1/47 R269Q			
	76	0/77			
Lung cancer	77	0/44		11/26	B 25/26
	49	0/21	T > N (9/10)		M 5/10
	102	1/36 P405R			
	78	0/3	T > N (52/60)		
Renal cancer	79	0/27	T = N		11/12 normal = M; 8/12 tumor = B 2/12 switched allele
Prostate cancer	50	0/106	T > N	2/38	
	82	0/27	T = N		B 27/27
Ovarian cancer	51		T > N	24/56	
	52	0/63	T > N (38/50)	5/10	
Bladder cancer	80		T > N (18/45)		B 12/23
	81	0/23	T > N (22/23)		B 23/23
Colorectal cancer	53	0/82	T > N	8/46	
	74	0/43			
Gastric cancer	83	0/12	Low	12/32	B 32/32
	74	0/31			
Esophageal	84	0/48		2/25	
	85		T > N 9/15	9/14	4/9 LOI, 1/9 switched
Hepatocellular	86	0/48	T = N	5/25	
Cholangiocarcinoma	88		"high" in 17/41		
Brain tumors	54		T > N (ependymomas)		
Oligodendroma	89	0/20			
Melanoma	90	0/17			
	91	0/51		2/10	
Merkel cell ca (skin)	92	1/10 S110L			
ALL	93	0/31			11/35 methylated
AML	111	0/60			M 6/10

[a]Expression level of p73 protein or mRNA (T refers to tumor tissue; N refers to corresponding normal tissue).
[b]LOH = loss of heterozygosity.
[c]Imprinting reported as B for biallelic and M for mono-allelic LOI = loss of imprinting.

paternal alleles of a particular gene in a diploid cell. At the extreme, only the maternal or paternal allele of an imprinted gene is actively transcribed. In such cases, loss of the transcribed allele would deprive a cell of the corresponding protein. Studies of the 1p36 chromosomal region in patients with neuroblastoma suggested that this region of the genome harbored an imprinted tumor suppressor gene.[95] Furthermore, initial studies reported that p73 gene was imprinted, increasing speculation that *p73* was a neuroblastoma gene and offering a potential explanation for the lack of p73 mutations in tumors with 1p36 loss.[3] Subsequent studies, however, have challenged

the notion that p73 is imprinted. A number of studies have documented biallelic expression of p73 in a variety of tissues.[76,79,80,96–98] Furthermore, p73 mRNA levels tend to be higher, and not lower, in tumor tissue compared with surrounding normal tissue. Tumors in which elevated p73mRNA has been described include ependymomas, breast, lung, prostate, ovarian, colorectal, esophageal, and bladder cancer.[48–54] It remains possible that imprinting of *p73* occurs in some tissues and in some individuals. If so, imprinting of *p73* may still contribute to the pathogenesis of some tumors but this remains to be proven.

Although *p73* does not appear to be a classical tumor suppressor gene, for the reasons cited above, it is still possible that modulation of p73 function contributes to cancer initiation or progression in certain settings. Several groups have shown that a subset of p53 mutants can bind to, and inactivate, p73.[99–101] Furthermore the ability of mutant p53 to bind to p73 and block its pro-apoptotic function is enhanced when p53 codon 72 encodes Arg rather than Pro by virtue of a common p53 polymorphism.[100] Of note, the Arg allele is preferentially mutated and retained in squamous cancers arising in p53 Arg/Pro germ line heterozygotes. Additional studies will be required to determine if these last two observations are mechanistically linked.

Finally, by analogy to the ΔN forms of p63 (see also below), ΔN p73 would be expected to block p53 function and thus have anti-apoptotic properties. If so, it will be important to determine whether overproduction of ΔN p73 occurs in cancer.

Role of p63 in malignancy

p63 maps to chromosome 3q27–28 a region that is altered in a variety of cancers including those of the lung, cervix and ovary.[4] As was true for *p73*, *p63* mutations appear to be rare in both primary tumors and cell lines. In the most extensive studies to date, Ikawa and colleagues found only 3 mutations in 101 primary tumors and tumor cell lines, while Sunahara and colleagues found 4 mutations in 80 primary non-small cell lung cancers and no mutations in 85 breast cancers.[53,102] Studies examining p63 mutations in human cancer are summarized in Table 2.[6,103–106] Despite the lack of mutations, alterations in p63 may still play a role in cancer development. In particular, the differential expression of various p63 isoforms may be important as the ΔN forms of p63 can block p53 function and thus might act as oncoproteins. Since p63 is most highly expressed in basal or progenitor layers of many epithelial tissues during development, examination of tumors derived from such tissues (for example, squamous cell carcinomas) may be especially informative. In this regard, Hibi and coworkers have demonstrated high levels of expression of ΔNp63(also called $p40^{AIS}$) in squamous cell carcinomas of the lung and head and neck and have argued that ΔNp63 promotes tumorigenesis in these settings.[105,107] Hall and coworkers noted higher levels of p63 in malignant keratinocytes compared with normal keratinocytes.[108]

Role of family members in development and differentiation

Expression pattern. p53 is expressed in all tissues. In contrast to p53, p73 expression in mice is restricted to the epidermis, sinuses, inner ear and brain.[16] In human cells p73 mRNA has been detected in brain, kidney, placenta, colon, heart, liver, spleen, and skeletal muscle at low levels.[3,12,71] Murine p63 is most highly expressed in proliferating basal cells of the epidermis, cervix, urothelium and prostate.[68] p63 expression in human tissues is highest in skeletal muscle and placenta, but also expressed at lower

Table 2. Mutational analysis of p63 in primary tumors and cell lines

Cancer type	Reference	Mutation frequency	Expression level
Lung cancer (NSCLC)	6,103	4/80 Q31H (2); A148P(1)	
Lung cancer	105	1/14 K298R	T > N 10/14
Cervical cancer	104	0/54	T > N (TAp63γ)
Breast cancer	103	0/85	
Head and neck cancer	105	1/6 E14Q	T > N 6/6
Bladder cancer	112	0/47	T > N 25/47 (TA-p63) T > N 30/47 (ΔNp63)
Primary tumors (neuroblastoma, brain, exophagus, liver, pancreas,colon)	6	1/66 A148P	
Primary cell ines	106	2/35 Q165L	

levels in mammary glands, prostate, trachea, thymus, salivary glands, uterus, heart and lung.[6] For the reasons cited above, it will be important to examine the expression of p63 and p73 with assays that discriminate between the different p63 and p73 isoforms since their biological functions almost certainly differ.

Knockout mouse models

Mice functionally deficient for all p73 isoforms exhibit profound defects including hippocampal dysgenesis, hydrocephalus, chronic infections and inflammation, as well as abnormalities in pheromone sensory pathways.[16] However, these mice do not develop tumors. The increase in infections is not secondary to obvious quantitative or functional deficiencies in granulocytes or lymphocytes, but instead appear related to epithelial barrier dysfunction. The hydrocephalus is caused by an overproduction of cerebrospinal fluid, possibly by epithelial cells of the choroid plexus. The functional loss of p73 leads to disappearance of a specific zone of neurons in the hippocampus. Furthermore, p73 deficient male mice lack interest in females and display attenuated aggressive responses to males; these findings are attributed to defects in the neuroepithelium of both the embryonic and adult vomeronasal organ, an accessory olfactory structure involved in pheromone detection. McKeon and colleagues have suggested that the apparent requirement for p73 in pheromone detection, neurogenesis and cerebrospinal fluid dynamics support a role for p73 in mechanisms of sensing environmental and homeostatic stimuli.[16]

p63 knockout mice exhibit developmental defects that are even more profound than those observed in p73 knockout mice. The findings reported by two independent groups suggest that p63 is essential for several aspects of ectodermal differentiation during embryogenesis.[68,69] The limbs of these mice are absent or truncated as a result of failure of the apical ectodermal ridges to differentiate. Their skin does not progress beyond an early developmental stage, lacks stratification and fails to express differentiation markers such as keratin. There is an absence of all squamous epithelia and their derivatives including hair follicles, teeth, mammary, lacrimal and salivary glands. In addition, there are abnormalities at other sites of squamous epithelia including the tongue, esophagus, proximal stomach, urinary bladder and cervix.[68] These results have led to speculation that p63 directly or indirectly regulates factors involved in ectodermal-mesenchymal communication, which are required for morphogenesis of hair follicles, teeth, mammary bud and the apical ectodermal ridge. In addition, the loss of the regenerative population of epithelial cells in these mice implicate p63 in functions related to the renewal capacity of epithelial stem cells.

In summary, although the newer p53 family members have potential overlapping functions, the striking different phenotypes of p53, p63 and p73 deficient mice emphasize unique roles for each in development and cellular regulation.

Role of p63 in EEC syndrome

Based on the phenotype of *p63* -/- mice, a mutational analysis of *p63* was performed in families with the EEC syndrome (Ectrodactyly, Ectodermal dysplasia, and Cleft lip with or without cleft palate). van Bokhoven and collaborators identified heterozygous p63 mutations in 9 unrelated families with the EEC-like disorder, limb mammary syndrome (LMS).[109] EEC/LMS is a syndrome with autosomal dominant inheritance with highly variable expression and penetrance. Ectodermal dysplasia is manifested by changes in skin, hair, nails, teeth, lacrimal duct, urogenital tract, and can be associated with conductive hearing loss, facial dysmorphism, chronic and recurrent respiratory infections and developmental delay. Most of the mutations (8/9) identified would be predicted to affect p63 DNA-binding capability. Recently two additional p63 mutations have been identified in another limb-malformation syndrome called Split-Hand/Split Foot Malformation (SFHM).[110] These data, together with the striking phenotype of p63 knockout mice, suggest that p63α (and ΔNp63α) play a role in ectodermal development.

Therapeutic implications

p63 and p73, in contrast to p53, are rarely the targets of inactivating mutations in human cancers. Furthermore, overproduction of p73 or p63 can induce apoptosis in p53-defective tumor cells. Thus, one strategy for treating p53-defective tumor cells would be to develop small molecules that lead to the accumulation of transactivation competent forms of p73 or p63. Such molecules might act transcriptionally or posttranscriptionally, With respect to the latter, one could envision several different scenarios. For example, it might be possible to design or discover molecules that stabilize p73 or p63. In addition, it might be possible to develop small molecules that block the interaction of p73 with antagonists such as MDM2 or mutant p53.

It may be proven that the ΔN forms of p63 and p73, by virtue of inhibiting p53-dependent transcription, also play important roles in some types of cancer. If true, it might be possible to develop small molecules that specifically block the functions of these proteins. For example, it might be possible to develop small molecules that specifically prevent these isoforms from binding to DNA.

Conclusions

The recently identified p53 family members p63 and p73 share certain structural and functional similarities with p53. The p53 transactivation, DNA binding, and oligomerization domains are highly conserved among all family members. In addition, like p53, p63 and p73 can form homooligomers, bind canonical p53 DNA-binding sites, transactivate the promoters of known p53 downstream target genes and induce apoptosis. Furthermore, some family members retain the ability to bind to certain cellular proteins involved in transcriptional regulation.

Despite these similarities it is becoming apparent that there are important differences between p53 and the more recently identified cousins. In contrast to *p53*, both *p63* and *p73* give rise to multiple functionally distinct protein isoforms due to alternative promoter utilization and alternative mRNA splicing. Furthermore the ΔN protein forms, which lack the N-terminal transactivation domain, can function as "dominant negative" proteins blocking the functions of the corresponding full length proteins. The upstream signaling pathways involved in activation of the newer family members are also distinct from those of p53. Only a subset of the DNA damaging agents which induce p53 also induce p73. In addition, several unique upstream signals, including T-cell activation and neuronal differentiation, have been identified for p73. Many cellular and viral oncoproteins also discriminate between p53 and the newer family members. Finally, it is becoming apparent that the newer p53 family members are not classical tumor suppressor genes. In particular, these genes are not frequently mutated in the tumors examined to date. To the contrary, p73 levels often increase, rather than decrease, during malignant progression. Mice with deletions in p73 and p63 have significant developmental abnormalities, but unlike mice with p53 deletions, they are not prone to tumor development. Thus, the current state of information suggests that the most recently identified members of the p53 family, p63 and p73, have overlapping as well as distinct biological functions.

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