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Review

Role of p73 in malignancy: tumor suppressor or oncogene?

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Abstract

The recently identified p53 family member, p73, shows substantial structural and functional homology with p53. However, despite the established role of p53 as a proto-type tumor suppressor, a similar function of p73 in malignancy is questionable. Overexpression of p73 can activate typical p53-responsive genes, and activation of p73 has been implicated in apoptotic cell death induced by aberrant cell proliferation and some forms of DNA-damage. These data together with the localization of *TP73* on chromosome 1p36, a region frequently deleted in a variety of human tumors, led to the hypothesis that p73 has tumor suppressor activity just like p53. However, unlike p53−/− mice, p73 knockout mice do not develop tumors. Extensive studies on primary tumor tissues have revealed overexpression of wild-type p73 in the absence of p73 mutations instead, suggesting that p73 may augment, rather than inhibit tumor development. In contrast to p53, differential splicing of the *TP73* gene locus gives rise to a complex pattern of interacting p73 isoforms with antagonistic functions. In fact, induction of apoptosis by increased levels of p73 can be blocked by both p53 mutants and the N-terminally truncated p73 isoforms, which were recently shown to possess oncogenic potential. In the light of these new findings the contradictory role of p73 in malignancy will be discussed.
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Abbreviations: LOH, loss of heterozygosity; LOI, loss of imprinting; TA, transactivation domain; DBD, DNA binding domain; OD, oligomerization domain; PR, proline-rich domain; SAM, steric alpha motif-like domain; ΔTA, lacking the aminoterminal transactivation domain; RT–PCR, reverse transcriptase-polymerase chain reaction; TCR-AICD, T-cell receptor activation-induced cell death; RB, retinoblastoma tumor suppressor

Introduction

In our search for the underlying causes of cancer, *TP53* is the most intensively studied gene. Integrating numerous signals that control cell life and death, p53 has evolved from a potential oncogene to the prototype tumor suppressor gene. As p53 regulates the expression of genes involved in cell cycle arrest and apoptosis in response to genotoxic damage or cellular stress, failure of p53 function consequently leads to uncontrolled cell growth – a defining feature of cancer cells.[1] It is therefore no wonder that p53 is the most frequent site of genetic alterations found in human cancers.[2] For almost two decades, p53 made scientists believe that it is unique in form and function. Therefore, the recent discovery of two *TP53*-related genes, *TP73* and *TP63* (*KET, TP51, p40, p73L*, and *AIS*), raised the possibility that other tumor suppressors exist which share the power of p53 in preventing cancer formation.[3–5] This review gives a summary of our current knowledge about the curious role of p73 in malignancies and discusses possible diagnostic and therapeutic implications.

TP73 gene locus: complexity of isoforms

p63 and p73 contain the characteristic features of the p53 protein – an acidic, aminoterminal transactivation (TA) domain, a proline-rich domain (PR), a central core DNA-binding (DBD) and a carboxy-terminal oligomerization domain (OD).[3] Consistently, p73 showed many p53-like properties: it could bind to p53 DNA target sites, transactivate p53-responsive genes and induce cell cycle arrest or apoptosis.[3,6–8]

C-terminal diversity

Unlike the *TP53* gene, which shows only little alternative splicing, both *TP63* and *TP73* give rise to a complexity of multiple protein isoforms due to alternative promoter usage[8,9] and differential mRNA splicing (Figure 1A).[3,10–14] Most of the alternative splicing occurs at the 3′ end, involves exons 10 to 13, and generates transcripts encoding protein isoforms with different C-terminal structures (Figure 1B). At present, at least six alternatively spliced p73 mRNAs named α, β, γ, δ, ε, and ζ have been identified in normal cells that share the N-terminal transactivation domain, the DNA-binding domain and the oligomerization domain. In addition, the recently discovered short isoforms θ, η, and $\eta 1$ lack the p73 C-terminal transactivation domain and the oligomerization domain encoded by exon 10.[15] In contrast to all other C-terminal isoforms, the η isoform has so far only been detected in neoplastic but not in normal cells.[15] The existence of several C-terminal splice variants with completely different C-terminal sequence motifs is an intriguing feature of p73, yet the specific function of these different p73 isoforms, if any, is still unknown. What makes the complexity of C-terminal isoforms even more difficult to assess is, that none of the smaller

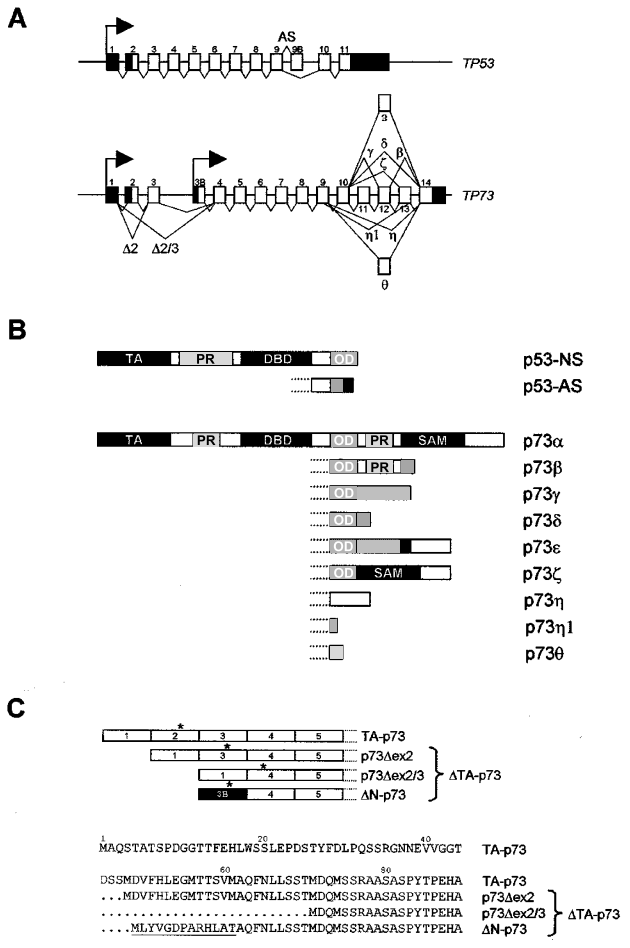

Figure 1 Gene structure of *TP53* and *TP73*. (**A**) *TP53* generates two splice variants, p53-NS (normal splice) and p53-AS (alternative splice). *TP73* C-terminal isoforms α, β, γ, δ, ε, ζ, η, η1, and θ are generated by alternative splicing of exons 10 to 14. *TP73* N-terminal isoforms comprise full-length (transactivating) TA-p73 and N-terminally truncated p73 isoforms ΔTA-p73 (p73Δex2, p73Δex2/3 and ΔN-p73). (**B**) Functional domains of p53 and the various C-terminal p73 protein isoforms. The transactivation (TA), DNA binding (DBD), oligomerization (OD), steric alpha motif-like (SAM), and proline-rich (PR) domains are shown. Alternative splice p53 undergoes splicing, which results in a replacement of p53 amino acids 364–390 with 17 unique amino acids. Identical amino acid sequence motifs in the different p73 C-terminal regions are indicated by equal shading. (**C**) N-terminal *TP73* splicing pattern (upper half) and amino acid alignment of ΔTA-p73 proteins (lower half). The underlined amino acids are unique to the ΔTA-p73 isoforms ΔN-p73 and are derived from the alternative exon 3B located in intron 3. The translations start is indicated (*)

isoforms has been detected on the protein level – so far all experimental evidence of these C-terminal isoforms is based on detection by RT–PCR. Current commercially available antibodies only react with p73α and p73β and these two isoforms are therefore the only ones that have been detected unambiguously on the protein level.

N-terminal diversity

In addition to the carboxy-terminal diversity reported for human p73, the analysis of murine p73 by Yang *et al*.[16] revealed a transcript (ΔN-p73) that lacks the N-terminal transactivation domain and is derived from an alternative promoter located in intron 3. Recently, the human homologue (termed ΔN-p73) transcribed from an alternative promoter was identified.[17] Apart from this physiological transcript, N-terminally truncated p73 proteins in cancer cells are also encoded by N-terminal splice variants that lack either exon 2 or both exon 2 and 3 (Figure 1C) (Stiewe and Pützer, unpublished data).[3,12,14,18] Together, in human cancer cells three different p73 messages (p73Δex2, p73Δex2/3 and ΔN-p73) encode N-terminally truncated p73 proteins (designated ΔTA-p73). However, the relative contribution of these transcripts to the expression of the N-terminally truncated p73 proteins that could be identified in human tumor cell extracts is not clear at present (Stiewe and Pützer, unpublished data). Since the N-terminal domain harbors the major transactivation domain of p73, the ΔTA-p73 isoforms are all transactivation-defective and act as dominant-negative inhibitors of full-length p73 and p53. By antagonizing the pro-apoptotic p53 family members the ΔTA-p73 isoforms serve as anti-apoptotic proteins.[9] Therefore, the use of two different promoters combined with alternative splicing yields essentially two classes of proteins: those containing an acidic amino terminus (TA-p73) analogous to the transactivation domain of p53; and those with a truncated amino terminus (ΔTA-p73) that lack this region. Inherent to this unusual gene structure is the idea of 'two genes in one', whereby TA-p73 products embody the necessary pieces for p53-like function, whereas ΔTA-p73 products act entirely opposite.[8] With regard to human cancer it is therefore an eminent question, whether p73 acts p53-like as a tumor suppressor or rather opposite as an oncogene.

Signaling by p73

The ability of p53 to inhibit cell growth is due, at least in part, to its ability to bind to specific DNA sequences and activate the transcription of target genes which encode proteins involved in cell cycle control (e.g. *CDKN1A/p21, GADD45, HDM2, PCNA, 14-3-3σ*, *BTG2*) and apoptosis (e.g. *BAX, KILLER/DR5*, redox-related p53-induced genes like *PIG3, NOXA, PIDD, PUMA, p53AIP1*).[1] When overexpressed p73 also binds to p53 DNA target sites, transactivates p53-responsive genes and is capable of inducing cell cycle arrest and apoptosis in mammalian cells in a p53-like manner.[3,6,7] The effects of p73, however, appear to be cell-context dependent, as Fang *et al*.[19] showed that induced expression of both p73α and p73β can also lead to irreversible growth arrest with markers of replicative senescence in EJ bladder carcinoma cells. However, most of these studies relied on overexpression of p73 in cells, therefore possibly masking subtle differences between the various p53 family members. In addition, there are no reports confirming binding of endogenous p73 to potential target gene promoters by chromatin immunoprecipitation *in vivo*.

Nonetheless, there is evidence that p73 can activate many but not all of the previously identified p53 cellular target genes.[20] For example *14-3-3σ* is strongly induced by p73, whereas *p21* and *HDM2* are strongly induced by p53.[20] This raises the possibility that target genes exist, that are specifically regulated by p73 but not by p53, which would provide new insight into nonredundant functions of

the p53 family members. A first step in this direction was the identification of aquaporin 3 (*AQP3*), a glycerol and water transporter, as a p73-responsive gene using a cDNA subtraction method. It is speculated, that in p73-deficient mice lack of *AQP3* induction accounts for the defects in production or reabsorption of cerebrospinal fluid, resulting in hydrocephalus.[21] Common p53/p73-target genes on the one hand and experimental evidence for exclusive p73- (or p53-) targets on the other hand indicate, that p73 in general appears both similar to and different from p53 in its signaling pathways.

Furthermore, there is evidence from several reports indicating a quantitative difference in the transcriptional activity of the various p73 splice variants. In many assays, for example, the β-isoform is a more potent transcriptional activator than p73α.[10,20,22,23] Likewise p73β is more potent than p73α as an inducer of apoptosis, suggesting that p73α contains an 'inhibitory' region not included in the β-isoform. Consistently, a C-terminal deletion mutant of p73α lacking the putative inhibitory region showed a significantly higher level of transcriptional activity than wild-type p73α.[24,25] Moreover, the transcriptional activity of p73β was reduced *in trans* by co-expression with either p73α or p73ε, which bears an identical C-terminal structure as p73α.[25] This suppression effect is most likely mediated by inter-variant associations as it depends on the presence of the oligomerization domain. These observations indicate that p73-mediated gene expression is regulated by the interaction of all p73 isoforms present in the cell. The current data therefore indicate the existence of transciptional specificity among the p53-family members with pronounced differences between p53 and p73 on the one hand and between the various p73 isoforms on the other hand.

p73 in cancer

The data presented above all demonstrate that signaling by p73 is different yet similar to what is known for p53, suggesting a comparable function as a tumor suppressor gene in human cancers. However, these data cannot easily be reconciled with data obtained from knock-out mice.

p73 knockout mice

p73-deficiency causes profound developmental defects, including hippocampal dysgenesis, hydrocephalus, chronic infections and inflammation, as well as abnormalities in pheromone sensory pathways revealing a unique role for p73 in neurogenesis, sensory pathways and homeostatic control.[16] However, in contrast to p53-deficient mice, which develop spontaneous tumors at high frequencies in various organs,[26] those lacking p73 show no increased susceptibility to spontaneous tumorigenesis.[16] At first sight, these data argue against a tumor suppressor activity of p73, but it has to be kept in mind, that p73 encodes two antagonistic genes in one – TA-p73 and ΔN-p73. The knockout mice lack both p73 isoforms so that the relative balance of pro- and anti-apoptotic isoforms remains unchanged. Studies of knockout mice in which specific isoforms, such as full-length or ΔN-p73, are deleted, should provide further insight into the unique functions of each isoform and might disclose a specific function of one or the other isoform in tumorigenesis.

Loss of heterozygosity and imprinting

The mouse p73 gene maps to the distal part of chromosome 4, which undergoes frequent loss of heterozygosity in γ-radiation-induced T-cell lymphomas.[27] The human *TP73* gene maps to chromosome 1p36.3, a region commonly deleted in various tumor entities including neuroblastoma, colorectal cancer, melanoma and breast cancer.[3] Fine mapping studies on the *TP73* locus confirmed its localization within the smallest region of overlap (SRO),[28] prompted the assumption that p73 was indeed the 1p36 tumor suppressor gene long sought after and initiated an extensive analysis of the p73 status of more than 1000 primary tumors (Table 1). Unfortunately, the majority of studies failed to detect mutations in both cell lines and tumors, including tumors with 1p36 deletions, emphasizing that p73 is no classical tumor suppressor according to Knudson's two-hit hypothesis. Initially, the failure to detect mutations in the remaining allele was interpreted with the localization of the *TP73* gene in an imprinting region explaining the monoallelic expression of p73 observed in many normal tissues.[3] If true, loss of the transcribed allele, such as through 1p36 deletion, would be sufficient to make a cell functionally null for p73. However, although some studies demonstrated monoallelic expression in certain tissues and tumors, a number of different studies have demonstrated loss of imprinting (LOI), biallelic expression of p73 or allele switching (Table 1). Thus, information about the imprinting of p73 in cancers is still limited and confusing. If LOI is a common event, p73 might even function to enhance tumor cell growth like an oncogene.

Promoter silencing

Loss of p73 expression appears to be an unfrequent finding in general. Transcriptional silencing due to promoter hypermethylation has only been reported in certain hematological malignancies such as primary acute lymphoblastic leukemias (ALLs) and Burkitt lymphomas.[29,30] In contrast, increased expression of p73 was reported in chronic myelogenous leukemia blast crisis, acute myelogenous leukemia, and B-cell chronic lymphocytic leukemias (B-CLL) with a positive correlation of p73 overexpression and a high-risk B-CLL phenotype.[31,32]

Mutations of p73 in primary cancers

Despite extensive studies of p73 in a variety of primary cancers, only few tumors with missense mutation of p73 have been found (Table 1). For example, two mutations (P405R and P425L), one somatic and one germline, were found in primary neuroblastoma. The P425L substitution reduced the ability of p73α to transactivate various p53-responsive promoters and to suppress cell growth in p53-deficient Saos-2 cells. In contrast, p73β(P425L) was as effective as wild-type p73β in transactivation and growth inhibition. On the other hand, the P405R substitution had no significant effect on both the transcriptional activity and the growth-suppressive ability of p73α or p73β.[33] The lack of correlation between the

Table 1 Loss of heterozygosity, mutation and expression level in p73 in primary cancers

Cancer type	Mutations	Expression level[c]	LOH[a]	Imprinting[b]/ methylation
Neuroblastoma				
Kovalev 1998, Cell Growth Differ. 9: 897	0/16	T>N		B 5/6
Ichimiya 1999, Oncogene 18: 1061	2/140 (P405R, P425L)		28/151	
Ejeskar 1999, Int. J. Mol. Med. 3: 585	0/30		10/30	
Han 1999, Eur. J. Surg. Oncol. 25: 194	0/23			
Liu 2000, Int. J. Oncol. 16:181	0/31			B 4/8
Yang 2000, Int. J. Mol. Med. 5:379	0/30		5/33	
Kong 2000, Neoplasia 1:80	0/32		3/32	
Matos 2001, Pediatr. Hematol. Oncol. 18: 37		T>N		B 7/11
Lung cancer				
Nomoto 1998, Cancer Res. 58: 1380	0/44		11/26	B 25/26
Mai 1998, Cancer Res. 58: 2347	0/21	T>N (9/10)		M 5/10
Ikawa 1999, Cell Death Differ. 6: 1154	1/36 (P405R)		11/33	
Tokuchi 1999, Br. J. Cancer 80: 1623	0/3	T>N (52/60)		
Nicholson 2001, Cancer Res. 61: 5636	0/10		10/48	
Colorectal cancer				
Sunahara 1998, Int. J. Oncol. 13: 319	0/82	T>N	8/46	
Han 1999, Eur. J. Surg. Oncol. 25: 194	0/43			
Gastric cancer				
Han 1999, Eur. J. Surg. Oncol. 25: 194	0/31			
Yokozaki 1999, Int. J. Cancer 83: 192	0/12	low p73 (6/8)	12/32	B 32/32
Kang 2000, Clin. Cancer Res. 6: 1767	0/39	T>N (14/16)		B 5/21
Esophageal cancer				
Nimura 1998, Int. J. Cancer 78: 437	0/48		2/25	
Cai 2000, Carcinogenesis 21: 683		T>N (9/15)	9/14	4/9 LOI, S1/9
Hepatocellular carcinoma				
Mihara 1999, Br. J. Cancer 79: 164	0/48	T=N	5/25	
Tannapfel 1000, J. Natl. Cancer Inst. 91: 1154		T>N (61/193)		
Herath 2000, Hepatology 31: 601		T>N	14/35	
Peng 2000, Anticancer Res. 20: 1487	1/22		2/11	7/8
Cholangiocellular carcinoma				
Tannapfel 1999, Br. J. Cancer 80: 1069		high p73 (17/41)		
Renal cancer				
Mai 1998, Oncogene 17: 1739	0/27	T=N		M 11/12N, B 8/12T, S 2/12T
Breast cancer				
Zaika 1999, Cancer Res. 59: 3257	0/8	T>N (29/77)		B 4/8)
Shishikura 1999, Int. J. Cancer 84: 321	0/87	T=N	6/46	
Han 1999, Eur. J. Surg. Oncol. 25: 194	1/47 (R269Q)			
Scwartz 1999, Breast Cancer Res. Treat. 58: 25	0/3	T>N (52/60)		
Dominguez 2000, Breast Cancer Res. Treat. 63: 17			6/67	
Ahomadegbe 2000, Oncogene 19: 5413		T<N	7/22	M 12/16
Dominguez 2001, Breast Cancer Res. Treat. 66: 183		T>N (19/70)	10/59	
Prostate cancer				
Takahashi 1998, Cancer Res. 58: 2076	0/106	T>N	2/38	
Yokomizo 1999, Prostate 39: 94	0/27	T=N		B 27/27
Melanoma				
Kroiss 1998, Melanoma res. 8: 504	0/17			
Schittek 1999, Int. J. Cancer 82: 583	0/51		2/10	
Herbst 1999, Arch. Dermatol. Res. 291: 362			1/17	
Ovarian cancer				
Imyanitov 1999, Oncogene 18: 4640		T>N	24/56	
Codegoni 1999, Ann. Oncol. 10:949			2/51	
Ng 2000, Oncogene 19: 1885	0/63	T>N (38/50)	5/10	
Zwahlen 2000, Int. J. Cancer 88: 66		T>N		
Chen 2000, Clin. Cancer Res. 6: 3910		T>N	2/24	B 22/24
Bladder cancer				
Yokomizo 1999, Oncogene 18: 1629	0/23	T>N (22/23)		B23/23
Chi 1999, Cancer Res. 59: 2791		T>N (18/45)		B12/23
CNS-Tumors				
Loiseau 1999, Neurosci. Lett. 263: 173		T>N		
Mai 1998, Cancer Res, 59: 2791	0/20			
Lomas 2001, Cancer Genet. Cytogenet. 129: 88	1/30 (N204S)			
Nozaki 2001, Brain Pathol. 11. 296	0/27	T>N		
Hematological malignancies				
Corn 1999, Cancer Res. 59: 3352	0/31			11/35 methylated
Stirewalt 1999, Leukemia 13: 985	0/60			M 6/10
Novak 2001, Ann. Oncol. 12: 981		T>N		

[a]Loss of heterozygosity. [b]Imprinting reported as B for biallelic, M for mono-allelic and S for switched. [c]T: tumor tissue, N: corresponding normal tissue

infrequent mutations and their growth-suppressive function underline that human cancers typically maintain expression of functionally active p73.

Overexpression of wild-type p73

As already mentioned above, the majority of studies that have not only analyzed the p73 status on the genomic level, but also measured p73 expression, have identified higher p73 mRNA and protein levels in tumor tissues compared with surrounding normal tissue (Table 1). Whereas levels of p73 expression are typically so low that it is often difficult to detect the transcripts by Northern blot or the protein by Western blot analysis, p73 expression among cancers is variable and some tumor cell lines and primary tumor samples express readily detectable levels (Stiewe and Pützer, unpublished data).[34] In particular, neuroblastomas, ependymomas, hepatocellular and cholangiocellular carcinomas, lung, prostate, colorectal, gastric, breast, bladder, ovarian, and esophageal cancers have all been reported to have elevated p73 levels compared with their normal tissue counterparts (Table 1).

Importantly, p73 overexpression appears to be positively correlated with prognostically relevant parameters. Tannapfel *et al*.[35] identified p73-overexpression as an independent prognostic factor in a large study of 193 patients with hepatocellular carcinomas. The mean survival time for p73-positive tumors was 127 days *versus* 462 days for those without expression.[35] Similarly, p73-overexpression correlated with advanced tumor grade in meningiomas, which are typically p53 wild-type. In low grade meningiomas p73 was undetectable in 10 of 25 cases and expressed at low levels in the remainder. In contrast, p73 expression was detectable in all malignant meningiomas.[36] In breast cancer, a significant association of p73 overexpression and tumors with lymph node metastases, vascular invasion and higher pathologic stage was demonstrated.[37] Likewise, p73α protein expression positively correlated with higher risk B-CLL stages.[32] All these correlations between high level expression of p73 and various prognostic parameters make it unlikely that p73 overexpression is only secondary to other transforming events and suggest that p73 is implicated in tumorigenesis and possibly functions as a dominant oncogene to enhance tumor progression and therapeutic resistance. The first experimental evidence in this direction stems from the observation that p73α overexpression is associated with resistance to treatment with DNA-damaging agents in a human ovarian cancer cell line.[38] So far, however, there are no definite answers as to (i) which p73 isoforms are actually overexpressed and ultimately responsible for the possible oncogenic activity and (ii) which factors are the underlying causes for p73 overexpression in tumor tissues.

Upregulation of p73 by oncogenes

Most human cancers harbor aberrations of cell-cycle control, which result in deregulated and elevated activity of the cell-cycle promoting transcription factor E2F1.[39] Deregulation of E2F1 by mitogenic oncogenes including Ras, c-*myc*, v-abl and E1A is believed to trigger an apoptotic checkpoint, leading to accumulation of p53 and p53-dependent apoptosis.[39] This is due, at least partly, to the induction of the tumor suppressor ARF, which in turn prevents MDM2 from targeting p53 for degradation. MDM2 binds to a sequence in the p53 N-terminal transactivation domain and, in addition to marking p53 for destruction, prevents p53 from serving as a transcriptional activator. MDM2 also inhibits both p73 dependent transactivation and p73 dependent apoptosis,[40,41] however, in contrast to p53, MDM2 binding does not lead to p73 degradation, so that oncogenic activation of the ARF/MDM2 pathway results in the accumulation of p53 but not p73. Whereas p53 is indirectly activated in response to oncogenes such as E2F1, we and others have recently shown, that E2F1 regulates p73 levels directly, through recognition and transactivation of the *TP73* promoter which contains several E2F-binding sites.[42,43] Further evidence that p73 is regulated by E2F1 includes the observation that p73 levels fall as cells are induced to exit the cell cycle after serum withdrawal and reaccumulates upon S-phase entry, a period in the cell cycle in which E2F-responsive genes are actively transcribed.[42,43] E2F1-mediated p73 upregulation leads to activation of the p73 transcription function, as shown by p73-responsive reporter activity and by induction of known endogenous p73 target genes.[42] Importantly, E2F1-mediated activation of endogenous p73 induces apoptosis in human tumor cells and conversely, inactivation of p73 by a dominant negative p73 inhibitor, a p73 anti-sense construct or expression of dominant-negative mutant p53 inhibits E2F1-induced apoptosis.[42] p73$-/-$ MEFs were equally protected from E2F1-induced apoptosis as MEFs lacking p53[43] and, unlike p53, activation of p73 by E2F1 is also required for TCR-AICD (T-cell receptor activation-induced cell death) in lymphocytes.[44] In addition, other oncogenes including E1A and c-*myc* were also shown to signal to p73 *in vivo* and to enlist p73 to induce apoptosis in tumor cells.[45] Analyses of E1A mutants indicated that the apoptotic activity of E1A in the absence of functional p53 correlates closely with the ability to bind the key regulators of E2F1-induced apoptosis, p300/CBP and RB.[46] It is therefore conceivable, that at least the effect of E1A on p73 can be attributed to activation of E2F1. However, it still remains possible that E1A and c-*myc* exert additional effects on p73 unrelated to E2F1. Increased levels of p73 in cancer tissues might therefore be attibutable to increased transactivation of the TP73 gene by tumor-associated deregulation of E2F1 by various oncogenes.

Inhibition of p73 by mutant p53

However, since E2F1-induced upregulation of p73-expression has been shown to cause apoptosis,[42,43] sustained overexpression of p73 would therefore require inhibition of its inherent pro-apoptotic activity. This could be achieved by p53 mutants which inhibit the putative tumor suppressor action of p73 in a dominant negative fashion by generating defective heterooligomers with wild-type p73. In cotransfection assays, the R175H and R248W mutants of p53 coprecipitate with p73α.[47] Furthermore, in a p53 reporter assay, transactivation activity of p73α is partially inhibited by the p53 mutants, which correlates with a reduction in p73α-mediated apoptosis.[47] The inhibition of p73-dependent transactivation and apoptosis

appears to be mediated by direct protein–protein interaction.[47,48] Initially, the high degree of homology between the oligomerization domains of p53 and p73 suggested the possiblity that these proteins may form heterotetramers, and Kaghad *et al*.[3] reported that p73β but not p73α can interact modestly with p53 in a yeast two-hybrid assay. However, Davison *et al*.[49] using purified oligomerization domains of p53 and p73, failed to find any interaction. Further studies revealed, that the core domain of mutant p53 is sufficient for the association with wild-type p73, whereas both the specific DNA binding and the oligomerization domains of p73 are required.[50,51] One characteristic of p53 required for its binding to p73 is recognition by the monoclonal antibody PAb240, which recognizes the p53 core domain in a mutant conformation.[51] Thus, the core domain of mutant p53, which has always been regarded as a 'dead' domain since it cannot bind and activate p53 target genes, now acquires a protein–protein interaction capacity that might contribute to the gain of function activities of mutant p53 by sequestering and inactivating proteins like p73.[50] In addition, the association between p53 mutants and p73 is governed by a common polymorphism at codon 72 of p53 that encodes Arg or Pro, with Arg leading to a stronger interaction with p73.[48] This might explain why Arg-p53 mutants cooperated with Ras to transform cells and inhibit p73-dependent apoptosis more effectively than their Pro-counterparts. Importantly, the Arg allele is preferentially mutated and retained in squamous cell tumors of the skin and vulva arising in Arg/Pro germline heterozygotes.[48] Together, inactivation of p73 by certain p53 mutants appears to provide a selective advantage in promoting tumorigenesis.

However, reduction of p73's apoptotic potential by mutant p53 cannot account for all cases of p73 overexpression. Although e.g. in esophageal cancer samples p53 defects were significantly correlated with elevated expression of p73,[52] several studies on other primary tumors have failed to find a significant correlation between p53 mutational status and p73 overexpression. In a study of Zaika *et al*.[34] only three of eight breast cancers with p73 overexpression harbored p53 mutations, whereas five expressed wild-type p53. This mutation frequency of 38% is identical to the one determined in a group of eight randomly chosen breast cancers with normal levels of p73 expression. Similarly, no correlation between p53 mutations and p73 overexpression could be detected in lung cancers and ovarian cancer cell lines and tissues.

Inhibition of p73 by specific p73-isoforms

As described above, the *TP73* gene encodes a large variety of different isoforms with in part antagonistic functions. Higher levels of total p73 mRNA and protein appear to be associated with a more complex p73 isoform pattern in ovarian cancer,[18] breast cancer,[34] meningiomas, B-CLL, and myeloid leukemias. Whereas in normal tissues the larger transcripts p73α and p73β are the predominant isoforms, samples with p73 overexpression tend to express higher levels of the smaller transcripts γ, δ, ε, and ζ. p73γ is a very weak transcriptional activator and repressor of colony formation.[10] However, although p73γ activates transcription poorly by itself, it has no inhibitory effect on coexpressed p73β.[10] In contrast, p73ε has been shown to inhibit p73β, which consistently is the strongest transactivator of p53 targets in ectopic expression.[25] Interestingly, the epsilon isoform is expressed in leukemic cells but completely absent in mature myeloid cells.[53] Since the various p73 isoforms form homotypic interactions the relative abundance of p73 isoforms in the cell certainly influences the composition of the resulting p73 tetramers and their transcriptional activity and pro-apoptotic potential, so that overexpression of the smaller variants might lead to inhibition of tumor suppressor activities of p73. However, further work on the complex regulatory network generated by the multiple C-terminal isoforms is needed in order to assess their role in tumorigenesis.

Apart from the C-terminal variants, especially the N-terminal isoforms have to be considered as p73 inhibitors. As shown by Pozniak *et al*.[9] murine ΔN-p73 is a potent anti-apoptotic protein, which rescues sympathetic neurons from apoptosis induced by nerve growth factor (NGF) withdrawal or p53 overexpression. Murine ΔN-p73β interacts with p53 and inhibits p53-dependent neuronal apoptosis and because sympathetic neuron death following NGF withdrawal is p53-dependent, ΔN-p73 possibly inhibits neuronal apoptosis by acting as a direct antagonist to p53.[9] Consistently, p73 deficient mice, in which all p73 isoforms are deleted, display increased levels of apoptosis in developing sympathetic neurons. Apart from its function as a p53-antagonist, ΔN-p73 also inhibits p73-dependent transcription.[16] Just recently, a human homologue of murine ΔN-p73, which is also transcribed from an alternative promoter in intron 3, was identified.[17]

In addition, in human tumor cells N-terminally truncated p73 proteins (ΔTA-p73) are also encoded by N-terminal splice variants that lack either exon 2 or both exon 2 and 3 (Figure 1C) (Stiewe and Pützer, unpublished data).[3,12,14,18] In an analysis of ovarian cancers expression of the p73 transcript lacking exon 2 was exclusively detected in cancer cell lines and invasive tumor tissues, but not in semi-malignant borderline tumors.[18] In a multifocal neuroblastoma, the expression pattern of the p73Δex transcript in different tumor areas correlated with distinct clinico-biological characteristics.[12] All ΔTA-p73 isoforms were shown to have a dominant negative effect towards transactivation and apoptosis-induction by both p53 and full-length p73 (Stiewe and Pützer, unpublished data).[14] Since inhibition of the tumor suppressor p53 is a common theme in human tumorigenesis and many inhibitors of p53 act as transforming oncogenes, it is intriguing to speculate that tumor progression actually selects for overexpression of the anti-apoptotic p73 isoforms. In fact, we recently demonstrated increased expression of ΔTA-p73 on the transcript and protein level in cancer cell lines and tumor tissues, which correlated with the expression level of total p73 (Stiewe and Pützer, unpublished data). Therefore, it is a very interesting observation, that the N-terminally truncated p73 isoforms promote anchorage-independent growth of NIH3T3 cells, a characteristic feature of transformed cells. In addition, nude mice injected with ΔTA-p73 expressing NIH3T3 cells rapidly developed tumors (Stiewe and Pützer, unpublished data). On the

one hand, it is conceivable that ΔTA-p73 antagonizes the tumor suppressor action of p53 and its own p53-like isoform (TA-p73) either by direct competitive binding to the same cognate p53 DNA binding sites or by engaging in heteromeric complexes that are defective for specific DNA binding. On the other hand, it is also possible, that ΔTA-p73 possesses growth-promoting, potentially oncogenic activities on its own.

However, the overall effect of p73 activity in the tumor cell appears to promote transformation or tumor growth so that deregulated expression might be actively selected for during tumor development. The inherent, growth-inhibiting functions of full-length TA-p73 might be neutralized by homo- or heterotypic interactions with mutant p53, C-terminal p73 variants and/or ΔTA-p73, all of which are commonly found in human cancers. The resulting tumor-associated change in p73 subunit composition could account for a shift in the net function of p73 from pro- to anti-apoptotic and allow the growth-promoting, potentially oncogenic functions of p73 to outweigh the growth-inhibiting, tumor suppressive activities. The current data therefore support the hypothesis that p73 acquires oncogenic activity in tumor cells (Figure 2).

Conclusions and perspectives

Starting with the identification of p73 as the first homologue of the prototype tumor suppressor p53 the prominent question has always been, whether p73 has a similar function in human malignancies as p53. The few years since p73 was brought into the p53 family have yielded impressive progress, but also important questions that need to be answered. Unfortunately, initial studies on p73 have come up with quite contradictory results. In particular, the data from ectopic p73 expression in tissue culture, together with the structural homology of p73 to p53 on the one hand, are not easily reconcilable with genetic, viral, and primary tumor data of p73, on the other hand. Is p73 a tumor suppressor gene that does not conform to Knudson's two-hit hypothesis or does p73 have oncogenic activity in the end? This duality in function appears to parallel the complexity of the *TP73* gene locus with its 'two genes in one' structure. Whereas the full-length TA-p73 protein exhibits growth-inhibitory, tumor suppressive functions, the anti-apoptotic ΔTA-isoforms act antagonistically with possible oncogenic activity. Last but not least, a further level of diversity is generated by the complexity of differential splicing at the p73 C-terminus. For future research it has to be kept in mind that there is not one single p73 protein but many different oligomeric p73 proteins with variable subunit composition. Despite the recent advances in understanding the unique roles of p73 isoforms, there are many outstanding questions. What are the unique functions of the TA and ΔTA isoforms? How is p73-mediated gene expression regulated by the interaction with (mutant) p53 and p73 splicing variants in the cell? What are the patterns of p73 isoform expression during both normal development and tumorigenesis? Understanding

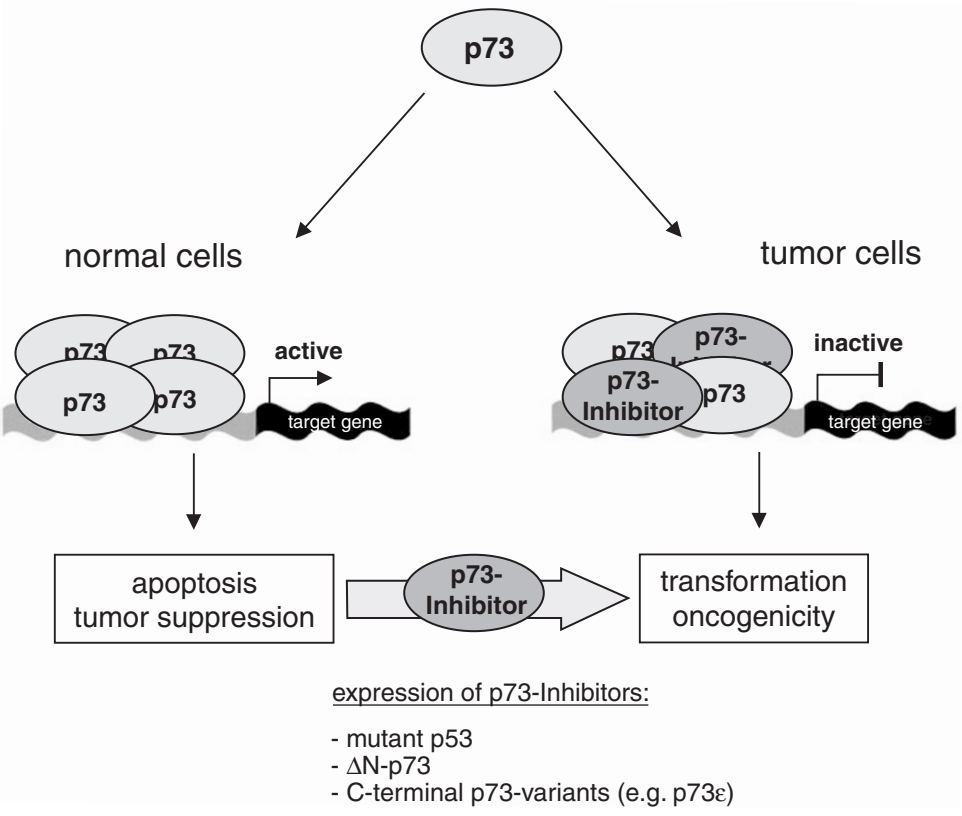

Figure 2 Model of p73 function in human malignancies. Expression of p73-inhibitors (mutant p53, ΔTA-p73 and C-terminal p73 variants) converts pro-apoptotic, tumor suppressive p73 tetramers into growth-promoting, oncogenic p73 complexes

the complexity of these interactions may not only allow to delineate the function of p73 in human tumorigenesis but may also enable the development of new cancer therapeutics. Overall, it becomes increasingly clear, that p73 – no matter whether tumor suppressor or oncogene – is not just a relative of p53, but has developed an identity on its own.

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