TP53 Family Members and Human Cancers

Jean Bénard,1,2* Setha Douc-Rasy,2 and Jean-Charles Ahomadegbe1,3,4
1Unité de Génétique Tumorale, Service de Génétique, Département de Biologie Clinique, Institut Gustave Roussy, Villejuif, France; 2Centre National de la Recherche Scientifique UMR8126, Institut Gustave Roussy, Villejuif, France; 3UPRESS Pharmacologie et Nouveaux Traitements des Cancers, Institut Gustave Roussy, Villejuif, France; 4Faculté de Pharmacie, UPJV, Amiens, France

For the p53 Special Issue

Based on gene sequence homologies, a p53 (TP53) gene family become apparent with the addition of the most recently identified p63 (TP73L; formerly TP63) and p73 (TP73) genes to the already known p53. The p53 gene encodes for a unique protein eliciting well-known tumor suppressor gene (TSG) properties that mediate cellular response to DNA damage, e.g., cell cycle arrest or apoptosis. In contrast, both homologues specify an array of isoforms different in their N- and C-terminal domains. Transactivating isoforms, such as TAp63/p73, show TSG properties similar to p53, while isoforms lacking N-terminal transactivating domain such as DNp63/p73, induce a functional block against p53 as well as TAp63/p73 activities. Both p63/p73 types of isoforms are involved in development: p63 is critical for epithelial stem cell renewal and epithelial homeostasis, and p73 is involved in neurogenesis and natural immune response. These facts support interdependent functions for the p53 family members, which appear linked together in a complex and tight regulation network to fulfill cellular functions related to DNA damage and tissue homeostasis maintenance. The lack of p63/p73 mutations in human cancers rule out a typical TSG role for either of the p53 homologues. Nonetheless, p63 and p73 genes seem strongly involved in malignancy acquisition and maintenance process because of: 1) their tissue identities, and 2) their close interplay activities within the p53 family members, and primarily through the negative regulatory role played by ANp63/p73 isoforms for cell death control and differentiation. Hum Mutat 21:182–191, 2003. © 2003 Wiley-Liss, Inc.

**KEY WORDS:** p53; TP53; p63; TP73L; p73; TP73; tumor suppressor; splicing; transactivation; apoptosis; differentiation; DNA damage; development; tumorigenesis; cancers

**DATABASES:**
TP53 – OMIM: 191170; GenBank: NM_000546 (mRNA)
http://p53.curie.fr/ (p53 Web Site at Institut Curie)
www.iarc.fr/P53/index.html (IARC p53 Mutation Database)
TP73L – OMIM: 603273; GenBank: NM_003722 (mRNA)
TP73 – OMIM: 601990; GenBank: NM_005427 (mRNA)

**INTRODUCTION**

The discovery in 1997 of the p73 gene (TP73; MIM# 601990) at the 1p36 locus, a location known to be subject to recurrent loss of heterozygosity in various human cancers [Kaghad et al., 1997], led to the critical issue of whether this first-identified p53 homologue functions as the archetypal tumor suppressor gene (TSG) p53 (TP53; MIM# 191170). One year later, the p53 gene family expanded with the cloning of a second homologue, the p63 gene (TP73L; MIM# 603273) [Yang et al., 1998], located at 3q27-tet. This chromosomal region is not subject to loss but to gain in cancers, and therefore suggests some oncogenic functions for p63. These apparently paradoxical findings caused head scratching and skepticism in an oncology research community in search of a role for p53 homologues in tumorigenesis. Moreover, in stark contrast to p53, which is mutated in about 50% of human cancers, it was soon revealed that p73 and p63 genes were not inactivated by mutations in a large number of cancers from various tissues.

All together, these findings raised a hot question: Do p53 homologues elicit functions similar to p53? These issues have been in part elucidated for each p53 homologue from the evidence of an array of isoforms

*Correspondence to: Jean Bénard, Unité de Génétique Tumorale, Service de Génétique, Département de Biologie Clinique, Institut Gustave Roussy, Villejuif, France.

DOI 10.1002/humu.10172
Published online in Wiley InterScience (www.interscience.wiley.com).
expressed in tissues, some harboring transactivation domain (TAD), a hallmark for transcription factors with TSG activity, others lacking TAD, e.g., \( \text{DN} \)-isoforms, with antagonistic biological properties. Therefore, unlike their namesake, which stands as a canonical TSG, each \( \text{p53} \) homologue can play either a TSG or an oncogenic role with respect to its specific isoforms.

In this review we will first present what is currently known about the two genes and their role during developmental and adult states, both at the cell and tissue levels. Thereafter, we will examine their possible roles in pre-malignancies and cancers.

**COMPARATIVE ORGANIZATION AND TRANSCRIPTION REGULATION IN THE TP53 FAMILY**

Akin to their \( \text{p53} \) leader, \( \text{p63} \) and \( \text{p73} \) exhibit the three typical domains of a transcription factor across various species: namely an acidic, amino-terminal transactivation (TA) domain; a central core DNA-binding domain (DBD); and a carboxy-terminal oligomerization domain (OD) [Kaghad et al., 1997]. Both \( \text{p53} \) “siblings” exert \( \text{p53} \)-like activities: They can bind to \( \text{p53} \) DNA consensus target sites, transactivate \( \text{p53} \) responsive genes, and induce cell growth arrest or apoptosis [Kaghad et al., 1997; Jost et al., 1997; Yang and McKeon, 2000]. The 20kb \( \text{p53} \) is organized in 11 exons, whereas \( \text{p63} \) and \( \text{p73} \) genes are both over 60 kb and comprise 15 and 14 exons respectively. Unlike the unique and alternative, but infrequently used, splicing of the \( \text{p53} \) gene, both \( \text{p63} \) and \( \text{p73} \) consistently give rise to an array of multiple protein isoforms due to differential mRNA splicing [Kaghad et al., 1997; Jost et al., 1997; Yang and McKeon, 2000]. The 20kb \( \text{p53} \) is organized in 11 exons, whereas \( \text{p63} \) and \( \text{p73} \) genes are both over 60 kb and comprise 15 and 14 exons respectively. Unlike the unique and alternative, but infrequently used, splicing of the \( \text{p53} \) gene, both \( \text{p63} \) and \( \text{p73} \) consistently give rise to an array of multiple protein isoforms due to differential mRNA splicing [Kaghad et al., 1997; Jost et al., 1997; Yang and McKeon, 2000]. The 20kb \( \text{p53} \) is organized in 11 exons, whereas \( \text{p63} \) and \( \text{p73} \) genes are both over 60 kb and comprise 15 and 14 exons respectively. Unlike the unique and alternative, but infrequently used, splicing of the \( \text{p53} \) gene, both \( \text{p63} \) and \( \text{p73} \) consistently give rise to an array of multiple protein isoforms due to differential mRNA splicing [Kaghad et al., 1997; Jost et al., 1997; Yang and McKeon, 2000].

![Figure 1](https://www.interscience.wiley.com)

**Figure 1.** Comparative gene structures and functional organization of the \( \text{p53} \) family members. A: For each gene, the transactivation (TAD), proline-rich (PXXP), DNA binding (DBD), oligomerization (OD), sterile a motif (SAM), and post SAM basic domains are represented. B: Genomic organization of the \( \text{p73} \) gene. The proximal promoter (P1) yields \( \beta, \gamma, \delta, \epsilon, \zeta \) TAp73 isoforms while the distal promoter (P2) located in intron 3 gives rise to AN isoforms. C: In addition to the \( \alpha \) isoform, COOH terminal splicing leads to \( \beta, \gamma, \delta, \epsilon, \zeta \) p73 isoforms for both TA and ANp73 molecules species. [Color figure can be viewed in the online issue at www.interscience.wiley.com.]
complexity of the 2 gene loci. Most of alternative splicing occurs at the 3’ end and involves more specifically exons 10 to 13, hence yielding transcripts that encode protein isoforms with various C-terminal structures. So far, among the high number of spliced p63 and p73 isoforms found at the RNA level, three of them are consistently found at the protein level, namely α (full structure of both genes), β (splicing of exon 13), and γ (splicing of exons 10 to 12 for p73 and 15 for p63) [Yang et al., 2002]. In contrast, the p53 gene encodes one major transcript yielding a unique protein with transcriptional activity.

Another striking finding regarding p63 and p73 gene expression regulation has been the occurrence of two different promoters, P1 and P2, that yield two distinct classes of proteins [Yang et al., 1998, 2000]. The P1 promoter leads isoforms showing TA domain with p53 homology. The P2 promoter, located within intron 3 and over 30-kb downstream, gives rise to N-terminal-truncated (AN) isoforms with biological properties opposite to those of p63/p73 TA isoforms, and lacking TA domain. The (AN) isoforms were identified first in mouse, then in human [Ishimoto et al., 2001]. Specific sequences of P1 and P2 bring about distinct biological properties. E2F-1 binding sites are present in both promoters, but p53-binding sites are present only in the P2 promoter [Yang et al., 2000]. Very recently, two crucial studies have indicated that: 1) TAp73 directly activates the transcription of endogenous ANp73 by binding to the two p73-specific target elements located on P2 [Nakagawa et al., 2002]; and 2) p53 induces ANp73 both at the mRNA and protein levels, as a result of a p53 direct activation of the P2 promoter [Kartasheva et al., 2002]. ANp73α-isoform readily associates with TAp73α/β and p53, as assessed by immunoprecipitation assay, and inhibits their transactivation activities. On the contrary, TAp63 shows only a marginal AN p73 transcription. Importantly, the negative feedback regulation of TAp73 and p53 by their ANp73 target provides for a novel autoregulatory system modulating cell survival and death. At the post-translational level, AN-isoforms are consistently identified in various tissues expressing p53-homologues, suggesting that AN-isoform elicits a protein of higher stability than the TA isoforms transiently expressed and subject to rapid degradation.

Yet another remarkable distinctive property of the p53 homologues concerns their degradation process by MDM2. The turnover of p53, a short-lived protein, is regulated by ubiquitination through MDM2 binding, leading to p53 degradation by proteasome, and thereby limiting p53 accumulation [Haupt et al., 1997]. Similar to p53, the p73α and β proteins bind to MDM2 through their N-terminal but this interaction leads to transcription and apoptosis inactivation, and does not result in p73 degradation by proteasome [Balint et al., 1999; Zeng et al., 1999]. So far, p73 ubiquitination has not been demonstrated, but a p73α modified by a covalent linking with SUMO-1 (small ubiquitin-related modifier) was found to be more rapidly degraded by the proteasome than the unmodified p73 [Minty et al., 2000]. Unlike p53 and p73, p63 does not bind to MDM2 [Dohn et al., 2001], revealing yet another difference between p53 family members.

Therefore one can distinguish two negative feedback loops controlling p53 and TAp73, namely the mdm2 and ANp73 loops, keeping the cell death trigger under tight control.

A RANGE OF P63 AND P73 ISOFORMS WITH DISTINCT BIOLOGICAL ACTIVITIES

The combination of C-terminal diversity and acidic N-terminal regulation, including two distinct promoters, produces at least six major transcripts and subsequent protein isoforms (α,β,γ) for each gene [De Laurenzi et al., 1998; Schmale and Bamberger, 1997]: TAp63/p73 p53-like isoforms and ANp63/p73 p53-antagonist isoforms (Fig. 1). This unusual gene regulation suggests a “two genes in one” concept for p63/p73 since TA and AN-isoforms act respectively as tumor suppressors and oncogenes [Stiewe et al., 2002; Stiewe and Pützer, 2002; Grob et al., 2002].

It is well known that p53 TSG properties arise from its binding to specific DNA sequences and from transactivation of target genes that specify cell cycle control proteins (p21, MDM2, GADD 45, 14-3-3-σ, BTG2) and apoptosis (PIG3, Bax). Active p53 was also shown to play a key role in B lymphocyte, muscle, spermatogenesis, and neuronal differentiation [Rotter et al., 1994; Sidell and Koeffler, 1998] as well as in retinoic acid-mediated terminal differentiation of embryonic carcinoma cells [Curtin et al., 2001]. p53 is also involved in keratinocytic differentiation, although it does not determine this process because, in p53 knock-out mice, keratinocytic differentiation may be activated by Ca++ treatment.

Another important difference between p53 family members concerns their interactions with viral oncoproteins. Indeed if E6 protein, SV40 large T antigen, and AdE1B 55 kd protein bind to and inactivate p53 during cell transformation, they do not interact with either p63 or p73 [Irwin and Kaelin, 2001].

When over-expressed in human cells, TA p63/p73 proteins also bind to p53 DNA target sequences, transactivate p53-responsive genes, and thereby induce cell cycle arrest, differentiation, and apoptosis in a p53-like manner [Kaghad et al., 1997; Jost et al., 1997; Yang et al., 1998] running through p53 most characteristic biological effects. However, transcriptional activity fluctuates with p63/p73 splice variants and must be considered. Indeed, TAp63/p73α isoforms display a less active transcriptional activity than TAp63/p73β isoforms in p53 assays [Yang et al., 1998;
Schmale and Bamberger, 1997] indicative of a negative regulatory effect of the α isoforms from the sterile α motif (SAM) (Fig. 1) [Thanos and Bowie, 1999]. Similarly the COOH-terminal region located in the post SAM domain can have an auto-inhibitory effect on the TAp63/p73 transactivation [Ozaki et al., 1999].

Noteworthy, the above-described biological activation has been demonstrated using forced expression of TA isoforms of the p53 homologues, likely to mask differences between the various p53-family members. Not to mention that, so far, no binding of endogenous p63/p73 to their putative target gene promoters has been demonstrated in vivo by chromatin precipitation. Nonetheless, differences may exist between human p53 family members regarding their potency to induce activation of p53 target genes. Indeed, if p53 leads to strong p21 and MDM2 induction, p73 was shown to induce a major and strong 14-3-3 up-regulation [Zhu et al., 1998].

In response to various stresses resulting in DNA damage, p53 is activated by phosphorylation to perform its cell-cycle arrest and apoptotic tasks. Similarly, in response to DNA-damaging agents such as cis-platin and γ-irradiation, p73 up-regulation results in apoptotic response albeit using a pathway distinct from that of p53. p73 is phosphorylated by c-abl, the non-tyrosine kinase receptor, which is itself phosphorylated by the ATM (ataxia telangiectasia-mutated) protein [Gong et al., 1999; Shaul, 2000]. Therefore, a p73 repair pathway for DNA damage exists, which is p53-independent (Fig. 2).

So far regarding p63 response to DNA damage, important information has been gained from UV-treated human keratinocytes: TAp63 isoforms are up-regulated while ΔNp63 isoforms are dramatically down-regulated in these p63 expressing cells [Liefer et al., 2000]. As a matter of fact, the ΔNp63 down-regulation parallels p53 stabilization, which, in turn, acts as the apoptotic executor of UV-damaged keratinocytes. These findings provide evidence for a full interplay between the two main antagonistic p63 isoforms and p53 for DNA-damage response (Fig. 2).

To sum up, a large body of evidence favors specific upstream and downstream target gene regulation for

**FIGURE 2.** Schematic p53 family members pathways. Besides specific developmental and physiological functions, p63 and p73 participate to p53 genomic guardian function. Upon genotoxic stresses induced by ultraviolet (UV), γ irradiation (IR) or cisplatin, the two homologues interplay with p53 to achieve growth arrest and apoptosis functions. It is now established that p53 as well as TAp73 induce a direct activation of ΔN-p73 creating thus a feedback loop to control negatively these functions. [Color figure can be viewed in the online issue at www.interscience.wiley.com.]
each p53 family member, and for their respective isoforms, playing a role in non-redundant functions. Undoubtedly, RNA interference strategies specifically designed against these isoforms will provide definitive clues with regard to biological activities of each p53-family member.

THE TP53 HOMOLOGUES AS DEVELOPMENT PROTAGONISTS

The respective deficient mice have provided further insights concerning the physiological role of p53, p73, and p63. p53 null-mice develop spontaneous tumors at high frequency without exhibiting a specific phenotype, in particular during their development [Donehower et al., 1992]. If active p53 does not appear to be involved in physiological apoptosis occurring throughout embryogenesis, it becomes crucial for DNA-damage-induced apoptosis [Lowe et al., 1993]. In stark contrast, both p73 and p63 null-mice show specific developmental defects but no spontaneous tumors at all [Yang et al., 1999, 2000].

Studies with p63-null mice have revealed the occurrence of TA and DN isoforms in the stem cell compartment of stratified epithelia, thereby also offering a very useful guide for our understanding of p53-homologues regulation and functions [Yang et al., 1998, 1999]. p63 knockout-mice are born alive but display severe deformations of limbs as well as of epithelia including skin, breast, urothelia, and prostate [Mills et al., 1999; Yang et al., 1999]. The p63 --/- mouse skin does not undergo normal development because it is lacking stratification and differentiation markers. These mice also lack mammary glands, hair follicles, and teeth. Such murine phenotypic disorders were confirmed by p63 mutations in various human syndromes involving limb and ectodermal development. Indeed p63 mutations have been related to EEC syndrome, a set of autosomal dominant disorders (ectrodactyly, ectodermal dysplasia, and cleft lip/palate), as well as to sporadic split, hand-split foot malformations [Yang et al., 1999; Celli et al., 1999; van Bokhoven et al., 2001]. In fact, epithelial loss in p63-deficient mice reflects the inability of the multi-layered regenerative epithelia stem cells to undergo asymmetric division. Indeed, in contrast to the physiological asymmetrical division (one stem cell providing one stem cell to repopulate the stem compartment, and one basal cell for differentiation), all p63 --/- basal cells undergo terminal differentiation, leading to a complete depletion of the stem cell compartment. In mice [Yang et al., 1998] and human [Faridoni-Laurens et al., 2001] epithelia, p63 is highly expressed in stem cells.

Neuronal defects have been the first phenotypic traits observed in p73-deficient mice, including high intracranial pressure and hippocampus dysgenesis [Yang et al., 2000]. Importantly, it was shown that ΔNp73 isoform is predominantly expressed in murine developing brain and sympathetic neurons, hence explaining neuronal defects from lack of ΔNp73. As a matter of fact, ΔNp73 isoform was shown to inhibit neuronal apoptosis from NGF withdrawal by blocking the p53 pro-apoptotic function [Pozniak et al., 2000]. These seminal findings designate p73 and ΔNp73 isoforms as determinants of cellular differentiation and apoptosis in neuronal tissues. Moreover, in developing Cajal-Retzius cells of the human neocortex, very recently, a close association was shown between p73 expression and reelin, a glycoprotein involved in neuronal migration [Meyer et al., 2002]. Also interesting in this respect, a recent study indicated that human cytomegalovirus CMV, which causes severe neuronal defects in utero, induces dramatic apoptosis inhibition and thus abnormal nerve cell survival through accumulation of ΔNp73a [Allart et al., 2002]. Besides these neurological and cognitive impairments, p73 null-mice display severe defects in pheromone-based social behavior, reproduction, and infection control. Noteworthy, the complex range of physiological deficiencies correlates with high p73 levels expressed in corresponding tissues of p73-proficient mice. These phenotypic traits suggest that the p73 functions sum up a very basic signaling system in vertebrates, i.e., both sensor and controller functions of intra and extra cellular signals, which are integrated in cell homeostasis [Yang et al., 2002].

P63 AND P73 GENES AND TUMORIGENESIS

Although, in contrast to p53 null-mice, p63/p73 null-mice do not develop any spontaneous tumors, the complex network and regulation system formed by the main p53-homologue isoform proteins and p53 itself must be considered in the context of tumorigenesis. Two levels of interaction between p53 members can be distinguished: physical interactions and pathway regulations.

Human tumor-derived p53 mutants can engage with different p73 and p63 isoforms in a physical association. In fact, interaction with mutant p53 impairs, in vitro and in vivo, p73 and p63 sequence-specific DNA binding, consequently inhibiting their transcriptional activity [Strano et al., 2002]. Moreover, in cells carrying endogenous p53 mutants, p53 homologues are unable to recruit some of their target gene promoters [Strano et al., 2002]. Therefore, p63 and p73 may contribute to the biological properties of specific mutants by promoting tumorigenesis and by conferring selective survival advantage to cancer cells.

The p53 homologues are also integrated in various p53-independent pathways conducting the completion of various functions, in particular apoptosis as previously stressed. However, and strikingly, in response to DNA damage, p53-dependent apoptosis relies upon the functionality of both p63 and p73:
When using E1A p53-dependent apoptotic fibroblast system, the lack of p63 and p73 clearly results in the cell’s loss of functional p53 necessary to undergo apoptosis [Flores et al., 2002]. Another line of evidence favoring the interplay between p53 and its homologues for tumorigenesis is the fact that DNp73 proteins are effective inhibitors of p53 and TAp73 functions [Stiewe et al., 2002]. Consistent with this fact, endogenous DNp73 protects cells from p53-dependent apoptosis. The same phenomenon may be proposed for DNp63 in squamous cells. These functions provide a plausible explanation for high p73 and p63 levels in human cancer cells even in the absence of p63/p73 mutations.

Although completed soon after the p73 and p63 clonings and without any regulation knowledge of these novel genes, translational studies in cell lines and fresh tumors of various cancers support the above proposed mechanisms. Now let’s consider the developments of recent research saga. Since p73 gene was shown to reside at 1p36-3, a locus found to be subject to recurrent loss of heterozygosity in many cancers (neuroblastoma, breast, colorectal cancers, melanoma) and to belong to the smallest overlapping region [Kaghad et al., 1997], p73 has been proposed as the long sought after TSG candidate at 1p36 [Versteeg et al., 1995]. A thorough search of intragenic sequence variations in the p73 gene resulted in an unswerving lack of mutations. In a sampling of more than 1,400 human tumors including solid (breast, lung, colorectal, esophageal, hepatocellular, renal, prostate, melanoma, neuroblastoma, and brain tumors) as well as hematological malignancies, only nine cases (0.6%) showed missense mutations (Table 1) [Stiewe and Pützer, 2002]. The rare mutations found (P405R and P425L) had no significant effect on p73 transcriptional activity and growth suppressing ability [Naka et al., 2001]. Given that p73 was located in a region so far described as imprinted, the lack of mutation in the remaining allele raised the possibility that the loss of transcribed allele would render tumor cells totally p73-deficient. Although some studies pointed out monoallelic expression, particularly in inflammatory breast cancers [Ahomadegbe et al., 2000], most showed p73 biallelic expression or allele switching, which invalidates the latter working hypothesis. p73 expression seems very low and variable from tissue to tissue: This fact and the poor quality of the first antibodies raised against p73 prevented western-blot use and favored RT-PCR analyses. p73 transcript levels were regularly measured higher in a significant percentage of cancers than in normal tissues [Zaika et al., 1999; Stiewe and Pützer, 2002]. However, when polymerase reactions were carried out using cDNA binding domain as substrate, the semi-quantitative data obtained did not permit an answer to the question of whether specific TAp73 or DNp73 isoform was overexpressed and which one could play a role in tumorigenesis. p73 up-regulation in some tumors can result from mitogenic oncogenes as shown in experimental models [Zaika et al., 2001] that lead to massive apoptosis. Attempts have been made to relate cancer’s p73 status and disease prognosis: Studies of large-size patient groups with hepatocellular carcinomas [Tannapfel et al., 1999], colorectal carcinoma [Sun, 2002], and breast cancer [Domínguez et al., 2001] produced a poor prognosis statistical trend for tumors with high p73 level. However, again, no evidence pointed to either isoform species whose transcript level increased: Was it TA or

### Table 1. Meta-Analysis of Mutation, Loss of Heterozygosity, Imprinting and Methylation of p73 in Human Cancers (after Stiewe and Pützer [2002])

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Mutation</th>
<th>LOH</th>
<th>Imprinting/methylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder cancer</td>
<td>0/23</td>
<td>29/194</td>
<td>B: 35/46</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>1/145</td>
<td>12/32</td>
<td>B: 37/53</td>
</tr>
<tr>
<td>CNS-tumors</td>
<td>1/77</td>
<td>32/107</td>
<td>B: 30/36</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>0/125</td>
<td>16/108</td>
<td>B: 4/10</td>
</tr>
<tr>
<td>Esophageal cancer</td>
<td>0/48</td>
<td>11/39</td>
<td>methylated: 11/35</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>0/82</td>
<td>7/8</td>
<td>B: 7/8</td>
</tr>
<tr>
<td>Head and neck cancers</td>
<td>3/58</td>
<td>3/27</td>
<td>B: 16/25</td>
</tr>
<tr>
<td>Hematological malignancies</td>
<td>0/91</td>
<td>33/141</td>
<td>B: 27/27</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>1/70</td>
<td>21/71</td>
<td>B: 8/12</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>1/114</td>
<td>21/104</td>
<td>S: 3/21</td>
</tr>
<tr>
<td>Melanoma</td>
<td>0/68</td>
<td>46/246</td>
<td>B: 2/12</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>2/302</td>
<td>46/246</td>
<td>B: 18/25</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>0/63</td>
<td>33/141</td>
<td>B: 27/27</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>0/133</td>
<td>2/38</td>
<td>B: 3/21</td>
</tr>
<tr>
<td>Renal cancer</td>
<td>0/27</td>
<td>8/12</td>
<td>B: 35/46</td>
</tr>
<tr>
<td>Total</td>
<td>9/1426</td>
<td>213/1049</td>
<td>B: 35/46</td>
</tr>
</tbody>
</table>

LOH, loss of heterozygosity; B, biallelic; S, switch.
ΔNp73: Besides the physiological transcript ΔNp73, an N-terminal splice variant lacking exon 2 could be also found in tissues. Its role in malignancy of various tissues is under investigation (Fig. 1) [O’Nions et al., 2001; Douc-Rasy et al., 2002] (Ahomadegbe and Bénard, in preparation).

So far, two tumor models highlight a direct role for p73 and p63 in tumorigenesis, neuroblastoma (NB) and squamous carcinoma cells. Remarkably NB constitutes a first tumor model in which p73 status has been thoroughly studied and it provides a significant clinical impact [Melino et al., 2002]. In NB, p53 is not mutated but inactivated by cytoplasmic retention [Moll et al., 1995], making p73 a good candidate for NB tumorigenesis, given the recurrent loss of heterozygosity at the p73 locus in these tumors. Early research provided evidence for lack of p73 mutations in fresh NB, ruling out this gene for a typical TSG role [Nakagawara et al., 1999; Zaika et al., 1999; Barrois et al., 2001]. In fact, neuroblastic tumors (NTs), occurring in early childhood, display a wide spectrum of differentiation. Recurrent deletions involving the p73 locus are frequently observed in undifferentiated NTs. To address the question of possible p73 implication in neuroblastic differentiation, this gene-expression status was investigated in a panel of differentiated and undifferentiated tumors. Although no mutations were found, p73 transcript profiles differed between undifferentiated and differentiated tumors. The frequency of the transcripts lacking exon 2 (species 1–3) appeared to be higher in undifferentiated than in differentiating and differentiated NTs [Douc-Rasy et al., 2002]. In contrast, products from the use of an alternate promoter (ΔNp73) were present in all NTs. In addition, only ΔNp73, but no full-length proteins, were detected by immunoblot, suggesting a greater stability of N-truncated isoforms [Douc-Rasy et al., 2002]. To determine the role of ΔNp73 in NB, the pattern of this gene's in vivo expression has been analyzed and the prognostic significance of its expression evaluated. Recent results clearly indicate that ΔNp73 expression is linked to a reduced apoptosis in NB tumor tissue. Expression of this variant in NB patients significantly correlates with reduced survival and progression-free survival: It is a predictor of poor outcome and constitutes a prognostic factor independent of age, primary tumor site, stage, and MYCN amplification [Casciano et al., 2002a]. At a mechanistic level, it is noteworthy that P2 promoter functionality in NB cells and tumor tissues is, at least in part, regulated by epigenetic mechanisms [Casciano et al., 2002b].

In direct correlation with its role in epithelia development and homeostatic maintenance, and consistent with its locus at 3q27-29, a chromosomal region submitted to gain, p63 levels significantly increased in squamous cell carcinoma [Hibi et al., 2000]. Clearly, it is the ΔNp63 isoform level that was found to be significantly increased [Hibi et al., 2000; Crook et al., 2000; Park et al., 2000]. In agreement with the ΔNp63 oncogenic role, premalignant and malignant lesions of the cervix and endometrium show the highest p63 level in differentiated squamous cells [Park et al., 2000]. Additional data regarding p73 involvement in cancers came recently from the biopsy analysis from 50 patients with head and neck squamous cell carcinoma (HNSCC). It showed a down regulation of p73 expression in 30% of the HNSCC associated with high p63 expression in basal layers as assessed by immunohistochemistry [Faridomi-Laurers et al., 2001]. Microdissection of HNS epithelium and HNSCC, currently in progress, is likely to reveal a ΔNp63 overexpression concommitent to this p73 down regulation.

Another finding of interest is ΔNp63 isoform overexpression resulting from p63 amplification in these tumors. This overexpression mediates a decrease in phosphorylation levels of β-catenin, which in turn induces β-catenin nuclear accumulation and activates β-catenin signaling pathway. So, ΔNp63 isoforms act as positive regulators of the β-catenin signaling pathway, hence providing a basis for their oncogenic properties [Patturajan et al., 2002]. From all these studies it emerges that p63, p73, and p53 play a role in concert in the differentiation and carcinogenesis of HNS epithelium.

In conclusion, the discovery of the p53 homologues has defined the p53 family. Clearly, p53 is not a lonely genome guardian counteracting genotoxic insults: It operates with the mutual assistance of p73 and p63 within a complex network including distinct but complementary pathways. Lack of p63/73 mutations in human cancers definitively rule out a typical TSG role for either p53 homologues. Nonetheless, it is strongly suggested that p63 and p73 genes may be involved in acquisition and maintenance of malignancy given: 1) their tissues identities, as well as 2) the close interplay activities within the p53-family members, primarily through the negative regulatory role played by ΔNp63/p73 isoforms for cell death control and differentiation. The state of the p53 field has increased in complexity tremendously over the last 5 years. New clues may permit researchers to accurately typify tumors according to alterations of their respective p53 and p63 homologue pathways. Undoubtedly, this p53 family genomic signature in every tumor will become a prerequisite for designing new therapeutic approaches for cancers.

ACKNOWLEDGMENTS

The authors thank Dr. Daniel CAPUT (Sanofi Recherches, Labèges, France) for invaluable discussions about their research and Sanofi Recherches for financial support. The manuscript was edited by English Booster Ltd, www.englishbooster.com.
REFERENCES


