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Drug Resistance Updates xxx (2007) xxx–xxx

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Review

# One, two, three—p53, p63, p73 and chemosensitivity

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Received 6 December 2006; received in revised form 2 January 2007; accepted 3 January 2007

**Abstract**

Molecular links between apoptosis, tumorigenesis and drug resistance provide starting points for new therapeutic approaches and for a targeted cancer therapy. The discovery of the p53-related genes p63 and p73 raised the possibility that they may be cancer-associated genes and as a consequence that p53 is not the only component in predicting prognosis and response to chemotherapy, but instead the status of a network that contains p53, p73 and p63. This review focuses on the status and interrelationship of the p53 family members in human cancer as critical elements for tumor progression and response to therapy. Literature up to December 2006 is reviewed.

p63 and p73 – as well as p53 – each use multiple promoters and alternative splicing to generate an array of isoforms, including full-length isoforms with a transactivation (TA-) domain homologous to that of full-length p53, and amino-terminally truncated ( $\Delta$ N-) isoforms. Whereas the full-length TA isoforms of p63 and p73 can activate downstream target genes and induce apoptosis, the  $\Delta$ N isoforms which lack the transactivation domain can act as dominant inhibitors of the full-length forms of p53, p63 and p73, inhibiting transactivation of target genes and induction of apoptosis. Deregulated dominant negative p63 and p73 isoforms play an oncogenic role in human cancer and contribute to chemoresistance.

Thus, therapeutic modulation of TAp63/ $\Delta$ Np63, TAp73/ $\Delta$ Np73 and mutant p53 levels might be used to target the large percentage of human tumors that harbor p53 mutations and/or overexpress  $\Delta$ Np63 or  $\Delta$ Np73. Interfering with the expression or function of  $\Delta$ Np63 and/or  $\Delta$ Np73 and/or mutant p53 in tumor cells may render such tumors more responsive to therapy and reduce their aggressiveness and metastatic capacity.

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**Keywords:** Apoptosis; Chemosensitivity; p53; p63; p73; Drug resistance

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## 1. Introduction

Tumor progression usually involves blockage of normally regulated cell cycle control and apoptosis mediated by tumor suppressor genes. Inactivation of tumor suppressor genes or activation of protooncogenes can lead to a lack of proper control, especially under stress, leading to clonal outgrowth and tumor progression. These oncogenic events are evolving as important determinants in the response of human tumors to commonly used DNA damaging treatments (Johnstone et al., 2002).

The transcription factor p53 regulates many target genes that induce cell cycle arrest, apoptosis, senescence, DNA repair or alter metabolism, in response to diverse stresses (including DNA damage, overexpressed oncogenes and various metabolic limitations) (Vousden, 2000; Harms et al., 2004; Harris and Levine, 2005; Mashima and Tsuruo, 2005; Green and Chipuk, 2006). In addition, p53 has also been shown to induce apoptosis through non-transcriptional, cytoplasmic processes (Mihara et al., 2003).

The recent identification of the p53–p63–p73 axis has opened a new chapter in cancer research (Zaika and El Rifai, 2006). p63 and p73 play important roles in normal development, but are also clearly implicated in human tumorigenesis and tumor response to therapy. This review focuses on recent developments in our understanding of the role of p53-homologs in tumorigenesis. We need to describe the dynamic changes that occur in the network of interactions between the multiple isoforms of p73 and p63, as well as wild-type and mutant p53 in tumor tissues to provide the foundation for new therapeutic approaches.

Indeed, there is mounting evidence that p63 and p73 play an important role in human cancer (Flores et al., 2002; Casciano et al., 2002; Melino et al., 2003, 2004; Westfall and Pietenpol, 2004; Moll and Slade, 2004; Wu et al., 2005; Mills, 2006; Murray-Zmijewski et al., 2006), although their precise roles in tumorigenesis remain to be clarified.

p63 and p73 give rise to proteins that have p53-agonistic as well as p53-antagonistic functions and new functions. One reason for this diversity in p53/p63/p73 function lies in their gene structure (Figs. 1, 2 and 4). Hence, p63 and p73 share some p53 functions, such as induction of cell cycle arrest and apoptosis (Osada et al., 1998; Vousden, 2000; Melino et al., 2003, 2004; Moll and Slade, 2004). However, there

are many functional differences between p53, p63 and p73. Studies of p53-, p63- and p73-deficient mice established that the expression of p63 and p73 is more important for mouse development than the expression of p53. In addition, the loss of p73 does not predispose mice to cancer (Yang et al., 1999, 2000, 2002).

Knockout p63 mice are not viable and show severe structural deficiencies, such as the complete absence of skin (Candi et al., 2005), lack of limbs as well as other epithelial structures (Yang et al., 1998, 1999, 2002; Mills et al., 1999) and severe craniofacial dysplasia (Yang et al., 1999; Mills et al., 1999; Celli et al., 1999). In humans, heterozygous germ-line mutations of p63 cause the autosomal dominant disorders ectrodactyly, ectodermal dysplasia, facial clefts (EEC) (Celli et al., 1999) and ankyloblepharon, ectodermal dysplasia, clefting (AEC) (Fomenkov et al., 2003). The reason for these deficiencies lies in the lack of stem cells that are required for the development and differentiation of such complex epithelial structures (Yang et al., 1999, 2002). p63 is the only gene known to be of essential relevance for the survival of epithelial stem cells (Pellegrini et al., 2001).

There is also intense debate on whether and how p63 and p73 interact with p53 in apoptosis and tumor suppression. The p53/p63/p73 family members are capable of interacting in many ways that involve direct or indirect protein interactions, regulation of common target genes and regulation of each other's promoters. The p53 family members and their isoforms can bind differentially to promoters and it may well be that the ratio of the isoforms is an important cell fate determinant deciding about normal cell cycling, senescence and the onset of tumor formation.

The involvement of p63 and p73 in p53-mediated apoptosis is controversial (Benchimol, 2004). An example of cooperativity among the three p53 family members has been reported in E1A-expressing mouse embryo fibroblasts and in primary neuronal cultures (Flores et al., 2002). In these cellular systems p63 and p73 expression is required to induce p53-dependent apoptosis in response to doxorubicin, cisplatin and  $\gamma$ -radiation. However, results of another study indicate, that at least in thymocytes, p53-dependent apoptosis occurs independently of p63 and p73 (Senoo et al., 2004). The dispensability of p63 and p73 for T cell death does not preclude interactions among p53 family members, which are supported by evolutionary, genetic and biochemical considerations (Benchimol, 2004).

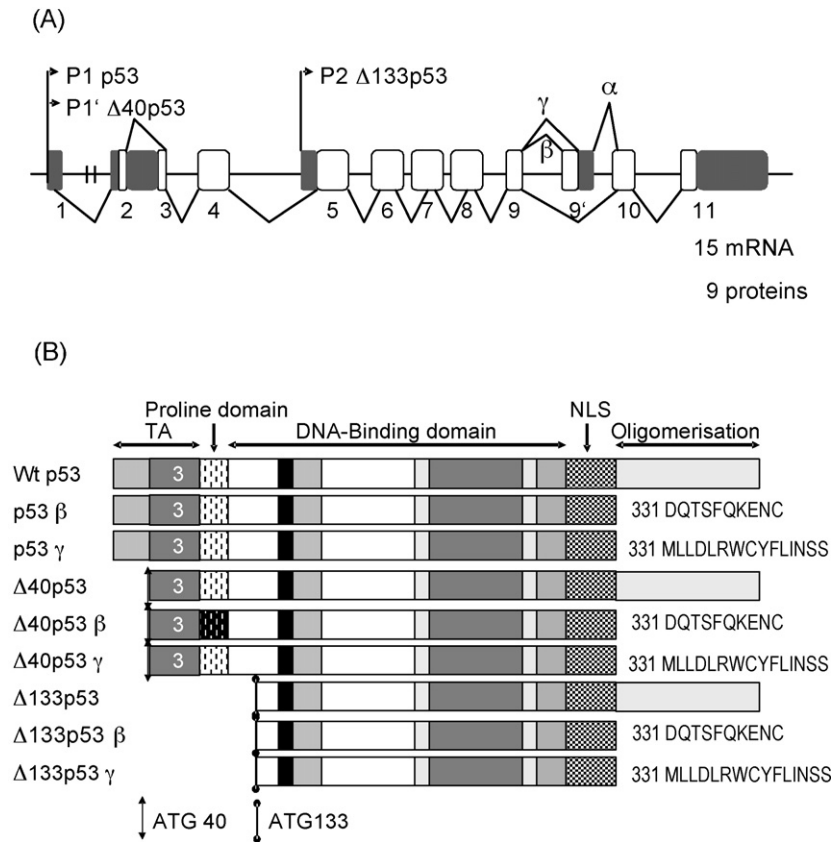


Fig. 1. Human p53. (A) Structure of the p53 gene: the alternative promoters (P1, P1' and P2) and the alternative splicing variants ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) are shown. (B) p53 protein isoforms: p53, p53 $\beta$  and p53 $\gamma$  proteins encoded from the P1 or P1' promoter contain the conserved N-terminal domain of transactivation.  $\Delta 40$ p53 protein isoforms encoded from P1 or P1' have lost the conserved N-terminal domain of transactivation but still contain part of the transactivation domain.  $\Delta 133$ p53 isoforms encoded from P2 lack the entire transactivation domain and part of the DNA binding domain. Translation is initiated at ATG-133 (modified according to Murray-Zmijewski et al., 2006).

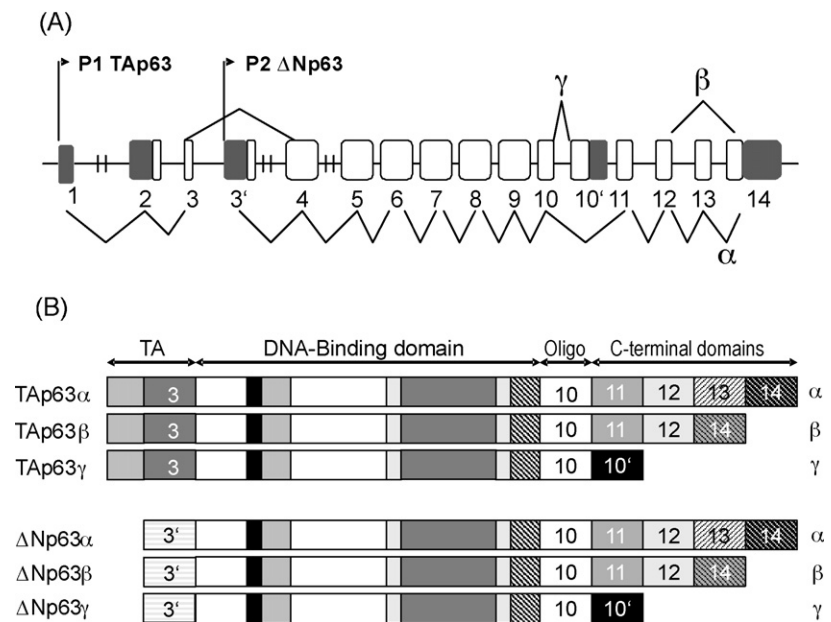


Fig. 2. Human p63. (A) Structure of the p63 gene: the alternative promoters (P1 and P2) and the alternative splicing variants ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) are shown. (B) TAp63 protein isoforms p63 $\alpha$ , p63 $\beta$  and p63 $\gamma$  encoded from P1 contain the N-terminal transactivation domain.  $\Delta$ Np63 proteins  $\Delta$ Np63 $\alpha$ ,  $\Delta$ Np63 $\beta$  and  $\Delta$ Np63 $\gamma$  encoded from P2 are amino-truncated proteins. Numbers indicate the exons encoding p63 protein isoforms (modified according to Murray-Zmijewski et al., 2006).

## 2. p53

The human *p53* gene is composed of 19,200 bp, spanning over 11 exons on chromosome 17p13.1 (Fig. 1). Recent results revealed that the human *p53* gene has indeed a dual gene structure similar to *p73* and *p63* genes (Bourdon et al., 2005). *p53* gene transcription can be initiated from two distinct sites upstream of exon 1 and from an internal promoter located in intron 4. The alternative promoter leads to the expression of an amino-terminally truncated p53 protein initiated at codon 133 ( $\Delta 133p53$ ). The intron 9 can be alternatively spliced to generate three isoforms, p53, p53 $\beta$  and p53 $\gamma$ . The p53 $\beta$  and p53 $\gamma$  isoforms lack the oligomerization domain. Thus, the human *p53* gene can encode at least nine different p53 protein isoforms, p53, p53 $\beta$ , p53 $\gamma$ ,  $\Delta 133p53$ ,  $\Delta 133p53\beta$  and  $\Delta 133p53\gamma$  due to alternative splicing of the intron 9 and usage of the alternative promoter in intron 4, and also  $\Delta 40p53$ ,  $\Delta 40p53\beta$ ,  $\Delta 40p53\gamma$  due to alternative splicing of the intron 9 and alternative initiation of translation or alternative splicing of the intron 2 (Ghosh et al., 2004; Bourdon et al., 2005; Murray-Zmijewski et al., 2006). p53 isoforms can exert distinct biochemical activities. p53 $\beta$  preferentially binds the p53-responsive promoters p21 and Bax rather than MDM2, while p53 binds preferentially to MDM2 and p21 rather than Bax promoters (Murray-Zmijewski et al., 2006). Co-transfection of p53 with p53 $\beta$  increases slightly p53-mediated apoptosis, while co-transfection of p53 with  $\Delta 133p53$  strongly inhibits p53-mediated apoptosis. This suggests that the ratio/balance of the different p53 isoforms may regulate cellular outcome in response to p53 activation (Bourdon et al., 2005). The clinical relevance of the newly described p53 isoforms remains to be investigated; it will be essential to analyze their expression in different human tumor entities.

p53 is a modular protein with an N-terminal transactivation domain (TA), a potential conformational element consisting of a proline-rich domain (PRD) adjacent to the TA, a DNA-binding domain (DBD), a tetramerization domain (4D) and a basic C-terminal domain (CTD). The primary amino-acid sequence of p53 contains many conserved serine, threonine and lysine residues that are of potential regulatory significance. In vitro transfection studies have suggested that p53 post-translational modifications at these conserved residues have a role in p53 stabilization and activation (Ashcroft et al., 1999; Brooks and Gu, 2003; Bode and Dong, 2004; Toledo and Wahl, 2006). In contrast, recent in vivo data show that the related proteins MDM2 and MDM4 (also known as MDMX) may even have more profound roles in p53 regulation (Francoz et al., 2006; Toledo et al., 2006).

### 2.1. p53 in cancer

TP53 is the prototype tumor suppressor gene in human cancer due to its pro-apoptotic and antiproliferative function in response to oncogenic stress (Vousden, 2000; Levine et al., 2004; Dimri, 2005; Harris and Levine, 2005; Green

and Chipuk, 2006). Depending on the severity of damage to the genome, p53 can activate genetic programs that halt cell proliferation transiently (G1 and G2 cell cycle arrest) or permanently (senescence), or eliminate the cell altogether (apoptosis). The p53 pathway is inactivated in the majority of human malignancies (Oren, 1992; Vousden and Lu, 2002; Harris and Levine, 2005) and increased levels of its negative regulators MDM2 and MDM4 downregulate p53 function in many of the rest (Toledo and Wahl, 2006). Thus, the p53 pathway is most likely disrupted also in a large fraction of wild-type p53-carrying tumors.

### 2.2. p53 and chemosensitivity

Understanding p53 regulation remains a crucial goal to design anticancer strategies based on this pathway. Reconstitution of the p53 tumor suppressor pathway is one of the most exciting novel concepts for improved cancer therapy. Adenoviral vectors are used to deliver intact *TP53* cDNA to tumor cells carrying mutant *TP53* or lacking *TP53*. This strategy has already been tested clinically and shown antitumor effect in a subset of patients (Clayman et al., 1998; Swisher et al., 1999; Nemunaitis et al., 2000; Bykov and Wiman, 2003; Swisher et al., 2003; Wiman, 2006; Yu, 2006; Roth, 2006). One molecule, RITA (reactivation of p53 and induction of tumor cell apoptosis), was described to induce the expression of p53 target genes and apoptosis in wild-type p53-expressing cell lines and could coordinate an antitumor response upon oncogene expression (Issaeva et al., 2004; Chipuk and Green, 2006). RITA was identified in a screen of the Diversity set from the National Cancer Institute and functions by abrogating the p53/MDM2 interaction via binding the N-terminus of p53. A family of small molecules aimed at targeting the p53 binding domain of MDM2, the Nutlins, were also able to induce robust p53-dependent apoptosis in tumor xenograft models (Vassilev et al., 2004). The Nutlins provide both in vitro and in vivo ‘proof of principle’ evidence that the p53/MDM2 interface is a key drug target (Vassilev, 2004, 2005). In a screen of a chemical library from the National Cancer Institute, Bykov et al. (2002, 2005b) identified a small molecule, PRIMA-1, that induces apoptosis preferentially in mutant p53-expressing human tumor cells. PRIMA-1 restores wild-type conformation and transcriptional transactivation to mutant p53 in vitro. MIRA-1 represents a novel family of small molecules that target mutant p53 (Bykov et al., 2005a). These compounds are structurally different from PRIMA-1 but have similar potency and mutant p53 selectivity in cellular assays (Wiman, 2006). The list of p53-based therapeutic strategies under preclinical development is growing and novel p53-based therapeutic strategies may also be combined with conventional cancer therapy. Wang et al. (2006) performed a chemical library screen by a strategy using bioluminescence imaging to identify small molecules that can induce a p53-responsive transcriptional activity and subsequent apoptosis in tumor cells deficient in p53. They obtained a number of small molecules, which stimulated a

strong p53 response not only in p53 wild-type tumor cells but also in p53-deficient cells, including p53-null and p53 mutant cells. These compounds activated p53 reporter activity and increased expression of p53 target genes such as p21 or TRAIL-R2 or TNF-related apoptosis-inducing ligand (TRAIL). Of note, some of the compounds activated a p53 response by increasing p73 expression, and knock-down of transactivating isoforms of p73 by small interfering RNA reduced their induction of p53-responsive transcriptional activity. These results demonstrate that it is possible to restore p53 responses in p53-deficient cancer cells, and that this may occur by targeting p53 family members such as p73. Further studies are required to determine whether p73 is the only target responsible for the observed antitumor effects in p53-null tumors.

### 3. p63

The human *p63* gene is composed of 15 exons, spanning over 270,000 bp on chromosome 3q27 (McKeon, 2004; Murray-Zmijewski et al., 2006). The discovery of an internal promoter within the p53 family was first made with *p63* (Yang et al., 1998). The human *p63* genes expresses at least three alternatively spliced C-terminal isoforms ( $\alpha$ ,  $\beta$ ,  $\gamma$ ), and can be transcribed from an alternative promoter located in the intron 3 (Fig. 2). The transactivating isoforms (TAp63) are generated by the activity of the promoter upstream of exon 1 while the alternative promoter in intron 3 leads to the expression of amino-terminally truncated isoforms ( $\Delta$ Np63) which lack the transactivation domain. In summary, the p63 gene expresses at least six mRNA variants which encode for six different p63 protein isoforms (TAp63 $\alpha$ , TAp63 $\beta$ , TAp63 $\gamma$ ,  $\Delta$ Np63 $\alpha$ ,  $\Delta$ Np63 $\beta$  and  $\Delta$ Np63 $\gamma$ ).

The TAp63 isoforms are able to bind DNA through p53-responsive elements and activate transcription of many p53 target genes. Thus, these p63 isoforms are described as ‘p53-like’. Recent studies indicate that p63 proteins can bind DNA through response elements (p63RE) slightly different to p53RE conferring responsiveness to p63 but not p53 proteins (Osada et al., 2005; Sasaki et al., 2005; Murray-Zmijewski et al., 2006). The  $\Delta$ Np63 isoforms can bind DNA through p53RE and can exert dominant negative effects over p53, p73 and p63 activities by either competing for DNA binding sites or by direct protein interaction (Flores et al., 2002; Bakkers et al., 2002; Benard et al., 2003; Westfall et al., 2003; Barbieri et al., 2005; Murray-Zmijewski et al., 2006).  $\Delta$ Np63 isoforms were also shown to directly activate specific gene targets not induced by TA isoforms (Dohn et al., 2001; Wu et al., 2003). By expressing TAp63 and  $\Delta$ Np63 isoforms, p63 has the ability to regulate a number of genes and possesses opposing regulatory effects depending on the form used.

#### 3.1. p63 and apoptosis

We have recently described the downstream mechanisms of TAp63 $\alpha$ -induced apoptosis in different cellular systems. In summary, TAp63 $\alpha$  activates genes exerting roles in different steps of the apoptosis program (Fig. 3). We demonstrated that TAp63 $\alpha$ , like p53, is involved in more than one apoptotic operation, being capable of transactivating genes encoding death receptors, for example, CD95, TNF-R1 and TRAIL-R1 and -R2 as well as genes encoding mitochondrial proteins, e.g. Bax, BCL2L11 and the genes encoding RAD9, DAP3 and APAF1 (Gressner et al., 2005). Thus, like it is the case for p53, p63 may simultaneously recruit several genes within the same cell, probably acting additively or synergistically,

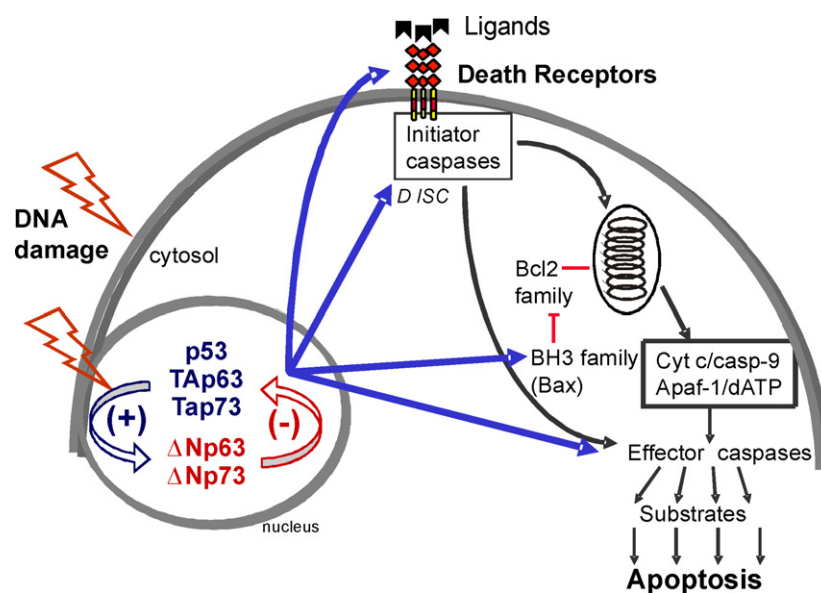


Fig. 3. Model of p53 family regulated apoptosis. Upon DNA damage, the p53 family members can activate both, the death receptor pathway and the mitochondrial apoptosis pathway.  $\Delta$ Np73 and  $\Delta$ Np63 are inhibitors of both pathways.

whereas others may be more cell type-restricted with regard to their requirement for p63-mediated apoptosis.

$\Delta$ Np63 negatively regulates apoptosis: HSP70, an anti-apoptotic stress response protein associated with malignancy, is induced by  $\Delta$ Np63 $\alpha$  but not by TAp63 $\alpha$  (Wu et al., 2003) consistent with the proposed oncogenic role of  $\Delta$ Np63 (Wu et al., 2005). By contrast, the pro-apoptotic gene IGFBP-3 is transcriptionally repressed by  $\Delta$ Np63 $\alpha$  (Barbieri et al., 2005).

### 3.2. p63 in cancer

The identification of the p53–p63–p73 network emphasizes particularly that there is a tight link between developmental processes and tumorigenesis (Zaika and El

Rifai, 2006). With regards to classical characteristics of a tumor suppressor, p63 contrasts markedly with p53: p63 is rarely mutated in human cancers. The majority of tumors maintain p63 expression, and in many cases p63 appears to be over expressed or the p63 locus is amplified, consistent with p63 performing a pro-proliferative or oncogenic role (Mills, 2006; Murray-Zmijewski et al., 2006).

A potential role for p63 in tumorigenesis is supported by the finding that p63 is a target of genomic amplification and/or over expression in >80% of primary head and neck squamous cell carcinomas (HNSCC) as well as other squamous epithelial malignancies (Hibi et al., 2000; Massion et al., 2003; Sniezek et al., 2004; DeYoung et al., 2006) (Table 1). A genome-wide micro-array screen of non-small cell lung cancer revealed that the 3q26-29 locus encompass-

Table 1  
p63 expression in human cancer

| Organ/tissue                                                                                                                  | Isoform expressed            | Comment                                                                                                           | References                                                                                                              |
|-------------------------------------------------------------------------------------------------------------------------------|------------------------------|-------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------|
| Esophagus: esophageal adenocarcinoma squamous cell carcinoma (ESCC), esophageal adenocarcinoma (EA), Barrett's esophagus (BE) | $\Delta$ Np63                | $\Delta$ Np63 expression in all squamous tissues                                                                  | Glickman et al. (2001)                                                                                                  |
|                                                                                                                               | Not determined               | Protein expression in squamous cell carcinoma                                                                     | Hara et al. (2004)                                                                                                      |
|                                                                                                                               | Not determined               | Nuclear expression increases with severity of neoplastic changes in BE                                            | Hall et al. (2000)                                                                                                      |
| Gastric cancer                                                                                                                | TAp63 and $\Delta$ Np63      | Protein and mRNA expression of TAp63 and $\Delta$ Np63                                                            | Geddert et al. (2003)                                                                                                   |
|                                                                                                                               | $\Delta$ Np63                | $\Delta$ Np63 mRNA expression                                                                                     | Hu et al. (2002)                                                                                                        |
|                                                                                                                               | TAp63 and Np63               | TAp63 and $\Delta$ Np63 overexpressed in ESCC but not in EA                                                       | Cui et al. (2005)                                                                                                       |
| Pancreatic cancer                                                                                                             | TAp63 and $\Delta$ Np63      | Tumor specific upregulation of TAp63 and $\Delta$ Np63                                                            | Tannapfel et al. (2001)                                                                                                 |
|                                                                                                                               | TAp63                        | Tumor specific upregulation of TAp63 $\gamma$                                                                     | Huang and Xie (2002)                                                                                                    |
| Bladder cancer                                                                                                                | Not determined               | Tumor specific upregulation                                                                                       | Ito et al. (2001)                                                                                                       |
|                                                                                                                               | Not determined               | Only in metastatic pancreatic adenocarcinoma                                                                      | Hornick et al. (2005)                                                                                                   |
| Urothelial cancer                                                                                                             | TAp63 and $\Delta$ Np63      | Expression of $\Delta$ Np63 only in tumor tissue                                                                  | Park et al. (2000), Urist et al. (2002)                                                                                 |
|                                                                                                                               | TAp63 and $\Delta$ Np63      | Impaired TAp63 and $\Delta$ Np63 expression is associated with tumor grade, tumor stage and lymph node metastasis | Koga et al. (2003a)                                                                                                     |
| Lung cancer                                                                                                                   | TAp63 and $\Delta$ Np63      | Protein expression                                                                                                | Koga et al. (2003b)                                                                                                     |
| Breast cancer                                                                                                                 | Not determined               | Squamous cell carcinoma                                                                                           | Reis-Filho et al. (2003), Tonon et al. (2005)                                                                           |
|                                                                                                                               | $\Delta$ Np63                | Overexpression of $\Delta$ Np63 in tumor tissue                                                                   | Valerie and Povirk (2003)                                                                                               |
|                                                                                                                               | TAp63                        | No p63 mutations                                                                                                  | Tani et al. (1999)                                                                                                      |
| Ovarian cancer                                                                                                                | TAp63 and $\Delta$ Np63      | Protein expression of TAp63 and $\Delta$ Np63                                                                     | Hibi et al. (2000), Yamaguchi et al. (2000), Wang et al. (2002a), Massion et al. (2003)                                 |
|                                                                                                                               | $\Delta$ Np63                | Overexpression of $\Delta$ Np63                                                                                   | Barbareschi et al. (2001), Wang et al. (2002b), Ribeiro-Silva et al. (2003), Reis-Filho et al. (2003)                   |
|                                                                                                                               | TAp63                        | Protein expression                                                                                                | Reis-Filho et al. (2003)                                                                                                |
| Head and neck cancer                                                                                                          | TAp63 and $\Delta$ Np63      | Expression of $\Delta$ Np63 is correlated with clinical response to chemotherapy                                  | Zangen et al. (2005)                                                                                                    |
|                                                                                                                               | TAp63 and $\Delta$ Np63      | Expression of TAp63 and $\Delta$ Np63 in Squamous cell carcinoma                                                  | Hibi et al. (2000), Yamaguchi et al. (2000), Crook et al. (2000), Choi et al. (2002)                                    |
| Prostate cancer                                                                                                               | $\Delta$ Np63                | Expression of $\Delta$ Np63                                                                                       | Weinstein et al. (2002), Davis et al. (2002), Iczkowski et al. (2003)                                                   |
| Cervix cancer                                                                                                                 | Not determined $\Delta$ Np63 | Squamous cell carcinoma protein expression                                                                        | Nishi et al. (1999), Cviko et al. (2000), Wang et al. (2001), Quade et al. (2001), Cho et al. (2003), Lin et al. (2006) |
| Uterine cancer                                                                                                                | Not determined               | Protein expression                                                                                                | Koga et al. (2003b)                                                                                                     |

Table 2  
Effect of chemotherapy on endogenous p63

| Treatment                            | Isoform                          | Organ/tissue/cell line                                   | Comments                                                                                          | References              |
|--------------------------------------|----------------------------------|----------------------------------------------------------|---------------------------------------------------------------------------------------------------|-------------------------|
| Bleomycin, doxorubicin, mitoxantrone | TAp63 $\alpha$ ↑                 | Hepatocellular carcinoma cell lines                      | Chemotherapy induces expression of TAp63 $\alpha$                                                 | Gressner et al. (2005)  |
| Cisplatin                            | $\Delta$ Np63 $\alpha$ ↓         | Squamous cell carcinoma of head and neck                 | Downregulation of $\Delta$ Np63 $\alpha$ and increase of TAp73 levels after DNA damage            | Rocco et al. (2006)     |
| Doxorubicin, etoposide               | TAp63 $\alpha$ ↑, $\Delta$ Np63→ | Hepatocellular carcinoma cell lines, primary hepatocytes | Increase of TAp63 $\alpha$ mRNA and protein, no change of $\Delta$ Np63 status after chemotherapy | Petitjean et al. (2005) |

ing p63 is frequently amplified in squamous cell carcinomas of the lung, suggesting that over expression of p63 facilitates tumorigenesis (Tonon et al., 2005).

A study of 245 esophageal tumors demonstrated that both TAp63 and  $\Delta$ Np63 isoforms are specifically upregulated at the transcript level in squamous cell carcinoma, and  $\Delta$ Np63 was the predominant isoform expressed at the protein level (Cui et al., 2005). Some tumor types have been reported to lose p63 expression, suggesting that p63 loss accelerates tumorigenesis (Urist et al., 2002; Koga et al., 2003a). This is supported by in vitro data which reveal that disruption of p63 in squamous cell lines resulted in upregulation of genes associated with increased capacity for invasion and metastasis in tumors (Barbieri et al., 2006). Table 1 gives an overview of p63 expression in different human tumor entities.

Differences between p63 and p53 were revealed by the phenotypes of mice deficient for these proteins: p63-deficient mice have several developmental abnormalities, whereas p53-deficient mice are viable and predispose to malignancy. Some p63 $\pm$  mice are cancer-prone (Flores et al., 2005) while other genetic background p63 $\pm$  mice show premature aging but no cancer (Keyes et al., 2005, 2006). Keyes et al. (2006) reported that p63 heterozygosity does not accelerate tumorigenesis, even when p53 is compromised. This is in contrast to an independent analysis of the role of p63 in spontaneous tumorigenesis by Flores et al. (2005), who concluded that p63 functions as a tumor suppressor. It should be noted that distinct *p63* models were used in these two studies (Mills, 2006). The two studies used mice heterozygous for distinct *p63* alleles (Yang et al., 1999; Mills et al., 1999), one of which retains the reading frame in the targeted allele (Yang et al., 1999). The generation of isoform-specific knockout mice may be a further step to solve this controversy.

Our understanding of the role of p63 in tumorigenesis is still preliminary. Future studies are needed to study the complex network of interactions between different p63 isoforms, in addition to determining how these proteins impact other members of the p53 protein family.

### 3.3. p63 and chemosensitivity

We have recently shown a relevant role for TAp63 $\alpha$  in chemosensitivity of hepatocellular carcinoma cell lines. Of clinical importance, we found that endogenous TAp63 $\alpha$  is induced by many chemotherapeutic agents (Table 2). Blocking endogenous TAp63 by an siRNA approach conferred protection against a variety of cytostatic drugs due to inhibition of apoptosis (Table 3). Our findings indicate that downregulation of endogenous TAp63 leads to chemoresistance of cancer cells (Gressner et al., 2005). To our knowledge this is the first study to link chemosensitivity to TAp63 $\alpha$  function. These data are consistent with observations that p63 participates in p53-mediated DNA damage responses (Flores et al., 2002; Petitjean et al., 2005). Our in vitro data have been confirmed by a recent report which shows that  $\Delta$ Np63 $\alpha$  expression is directly correlated with the clinical response to cisplatin in patients with head and neck tumors (Zangen et al., 2005). A critical role of p63 in cell death following DNA damage is further substantiated by a study in head and neck squamous cell carcinoma (HNSCC), cells which demonstrated that  $\Delta$ Np63 $\alpha$  is an essential survival factor in HNSCC. These data define a pathway in which  $\Delta$ Np63 $\alpha$  promotes survival in squamous epithelial malignancy by repressing a p73-dependent pro-apoptotic transcriptional program, suggesting that p63 levels and p73 status may be key determinants of therapeutic response in patients with HNSCC. Of clinical importance, in addition

Table 3  
Effect of p63 on chemosensitivity

| Treatment                                       | Isoform        | Chemosensitivity | Organ/tissue/cell line              | Comments                                                                                                       | References             |
|-------------------------------------------------|----------------|------------------|-------------------------------------|----------------------------------------------------------------------------------------------------------------|------------------------|
| Bleomycin, doxorubicin, mitoxantrone            | TAp63 $\alpha$ | ↓↓               | Hepatocellular carcinoma cell lines | Blocking TAp63 function by siRNA<br>TAp63 inhibits chemotherapy-induced apoptosis                              | Gressner et al. (2005) |
| Bleomycin, doxorubicin, cisplatin, mitoxantrone | TAp63 $\alpha$ | ↑↑               | Hepatocellular carcinoma cell lines | Adenoviral gene transfer of TAp63 $\alpha$ and chemotherapeutic agents synergize in the induction of apoptosis | Gressner et al. (2005) |

to the known role of p53, both p63 ( $\Delta Np63\alpha$ ) and p73 are critical mediators of cell death following chemotherapy in HNSCC (Rocco et al., 2006).

Thus, chemosensitivity is determined by a complex web of interactions between different p63 isoforms and p53 and p73 isoforms.

#### 4. p73

The human *p73* gene is composed of 15 exons spanning over 80,000 bp on chromosome 1p36.3 (Ozaki and Nakagawara, 2005; Murray-Zmijewski et al., 2006). The expression of the TP73 gene is complicated by the presence of at least seven alternatively spliced C-terminal isoforms (p73 $\alpha$ - $\eta$ ) (Kaghad et al., 1997; De Laurenzi et al., 1998; Melino et al., 2002; Moll and Slade, 2004) and of at least four alternatively spliced N-terminal isoforms initiated at different ATG (Murray-Zmijewski et al., 2006) (Fig. 4). Like *p63*, the *p73* gene can be transcribed from two distinct promoters, driving the expression of p53-like proteins containing the transactivation domain (TAp73), and inhibitory proteins lacking TA, called  $\Delta$ TAp73 (the collective name for four different p73 TA-deficient forms, mainly  $\Delta Np73$ ). The transactivating isoforms are generated by the activity of the promoter upstream of exon 1 while the alternative promoter in intron 3 leads to the expression of the amino-terminally truncated isoforms ( $\Delta Np73$ ). Altogether, the *p73* gene expresses at least 35mRNA variants, which can encode theoretically 29 different p73 protein isoforms. So far, 14 different p73 isoforms have been described (Murray-Zmijewski et al., 2006). The TAp73 isoforms are able to bind specifically to DNA through p53RE and activate transcription of target genes. Like p53, such activation can induce cell cycle arrest or apoptosis.  $\Delta Np73$  acts as a potent transdominant inhibitor of TAp63, TAp73 and wild-type p53 (Zaika et al., 2002; Nakagawa et al., 2002; Moll and Slade, 2004). Thus, the TP73 locus encodes both a tumor suppressor (TAp73) and a putative oncogene ( $\Delta Np73$ ) (Melino et al., 2002).

##### 4.1. *p73* and apoptosis

We have previously investigated the mechanisms by which TAp73 $\beta$  and dominant negative p73 ( $\Delta Np73$ ) regulate apoptosis. Our results support a two-pathway model for the TAp73-apoptotic response in hepatoma cells. TAp73 $\beta$  is involved in the activation of both, the extrinsic/death receptor-mediated apoptosis pathway as well as the intrinsic/mitochondria-mediated apoptosis pathway, pathways 1 and 2, respectively. Endogenous TAp73 was upregulated in response to DNA damage by chemotherapeutic drugs. On the contrary,  $\Delta Np73$  conferred resistance to chemotherapy. Inhibition of CD95 gene transactivation was one mechanism by which  $\Delta Np73$  functionally inactivated the tumor suppressor action of p53 and TAp73 $\beta$ . Concomitantly,  $\Delta Np73$  inhibited apoptosis emanating from mitochondria.

Thus,  $\Delta Np73$  expression in tumors selects against both the death receptor and the mitochondrial apoptosis activity of TAp73 $\beta$  (Melino et al., 2004; Terrinoni et al., 2004; Ramadan et al., 2005; Müller et al., 2005).

##### 4.2. *p73* in cancer

TP73, despite significant homology to p53, is not a classic Knudson-type tumor suppressor gene (Melino et al., 2002; Moll and Slade, 2004; Ramadan et al., 2005). TP73-deficient mice lack a tumor phenotype (Yang et al., 2000) and inactivating mutations in patients suffering from cancer are extremely rare (Moll and Slade, 2004). Though mutated rarely, the *p73* gene, does show a significant incidence of loss of heterozygosity in a number of different cancers.

The finding that a significant percentage of tumors specifically select for dominant negative p73 isoforms strongly argues for their oncogenic role during tumorigenesis (Zaika et al., 2002; Casciano et al., 2002; Dominguez et al., 2006a).  $\Delta Np73$  cooperates with oncogenic Ras in transforming primary mouse embryo fibroblasts (MEFs) in vitro and in inducing MEF-derived fibrosarcomas in nude mice in vivo (Petrenko et al., 2003).

Preferential upregulation of  $\Delta Np73$  human tumors might impose oncogenic activity that specifically interferes with the tumor suppressor function of wild-type p53, TAp63 and TAp73 disabling major apoptosis pathways.

Emerging evidence from the analysis of primary human tumors shows that deregulated  $\Delta Np73$  expression is rather frequent (Zaika et al., 2002; Casciano et al., 2002). In neuroblastoma, which is almost exclusively wild-type for p53, a correlation of  $\Delta Np73$  status with the clinical outcome was seen (Casciano et al., 2002). Childhood acute lymphoblastic leukaemia (ALL) was found to be associated with a high expression of  $\Delta$ TAp73, which may contribute to cellular resistance to DNA-damaging drugs in children at initial diagnosis of T-ALL (Meier et al., 2006).

Many of the (early) p73 overexpression studies in human cancers determined total p73 levels, because the antibodies used could not distinguish between TA and  $\Delta Np73$  (Table 4). Using highly specific antibodies (Sayan et al., 2005) we could show for the first time that it is the  $\Delta Np73$  isoform, which is upregulated in hepatocellular carcinoma (Fig. 5). Importantly, the upregulation of  $\Delta Np73$  in hepatocellular carcinoma was correlated with a poor prognosis. This is an important and clinically relevant finding which suggests the use of  $\Delta Np73$  status as a prognostic marker for patients with hepatocellular carcinoma (Müller et al., 2005). Table 4 summarizes currently available data on p73 and  $\Delta Np73$  expression in human cancer.

##### 4.3. *p73* and chemosensitivity

There have been several reports which have demonstrated that p73 is essential for apoptosis induced by many cytotoxic agents (Tables 5 and 6) and that inactivation of p73 by

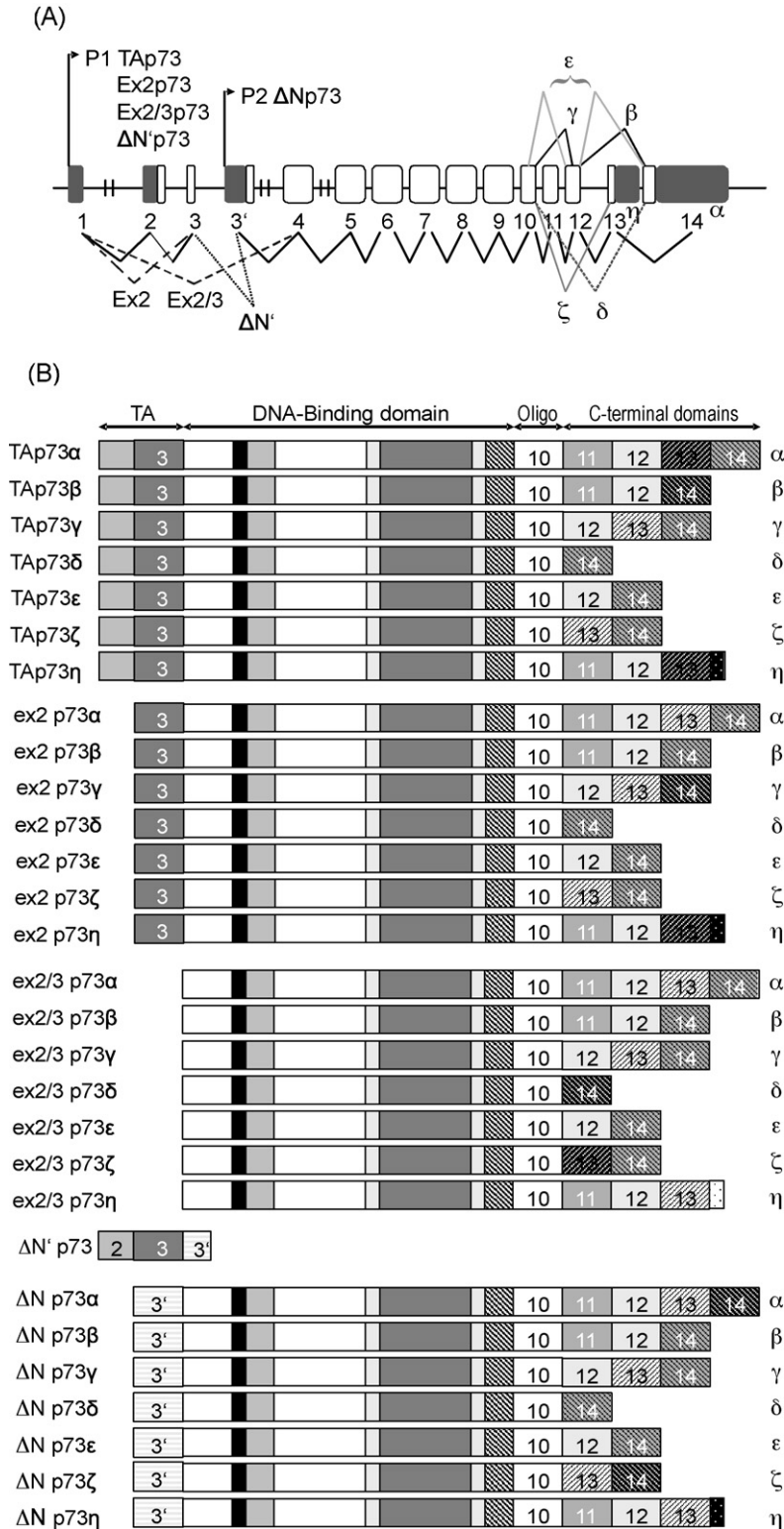


Fig. 4. Human p73. (A) Structure of the p73 gene: the alternative promoters (P1 and P2) and the alternative splicing variants (α, β, γ, δ, ε, ζ, η) are shown. (B) TAp73 proteins encoded from P1 contain the conserved N-terminal domain of transactivation. Ex2p73 proteins are due to alternative splicing of exons 2 and 3. They have entirely lost the transactivation domain. ΔNp73 proteins encoded from P2 are amino-truncated proteins containing an N-terminal domain different from TA proteins. Numbers indicate the exons encoding p73 protein isoforms (modified according to Murray-Zmijewski et al., 2006).

Table 4  
p73 expression in human cancer

| Organ/tissue                                                            | Isoform                 | Comment                                                                                               | References                                                               |
|-------------------------------------------------------------------------|-------------------------|-------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------|
| Esophagus: esophageal adenocarcinoma squamous cell carcinoma (ESCC)     | Not determined          | Tumor-specific upregulation of p73                                                                    | Masuda et al. (2003)                                                     |
|                                                                         | TAp73                   | TAp73 $\alpha$ > TAp73 $\beta$                                                                        | Nimura et al. (1998)                                                     |
| esophageal adenocarcinoma adenocarcinoma (EA), Barrett's esophagus (BE) | Not determined          | mRNA and protein expression                                                                           | Cai et al. (2000)                                                        |
|                                                                         | TAp73 and $\Delta$ Np73 | Overexpression of TAp73 and $\Delta$ Np73 in ESCC and EA                                              | Cui et al. (2005)                                                        |
| Gastric cancer                                                          | p73                     | Tumor specific upregulation of p73                                                                    | Kang et al. (2000), Tannapfel et al. (2001), Huang and Xie (2002)        |
| Pancreatic cancer                                                       | TAp73                   | Tumor specific upregulation of TAp73                                                                  | Ito et al. (2001)                                                        |
| Colorectal cancer                                                       | p73                     | Expression of p73                                                                                     | Sun (2002), Liu et al. (2001), Guan et al. (2003), Pfeifer et al. (2005) |
|                                                                         | TAp73 and $\Delta$ Np73 | Expression of $\Delta$ Np73 is correlated with poor prognosis                                         | Dominguez et al. (2006a,b)                                               |
| Hepatocellular carcinoma (HCC)                                          | TAp73 and $\Delta$ Np73 | Tumor specific expression of TAp73 and $\Delta$ Np73                                                  | Stiewe et al. (2003)                                                     |
|                                                                         | TAp73                   | TAp73 $\alpha$ > TAp73 $\beta$ No difference in TAp73 mRNA expression between tumor and normal tissue | Mihara et al. (1999)                                                     |
|                                                                         | Not determined          | T > N                                                                                                 | Fukushima et al. (2001), Pan et al. (2002)                               |
|                                                                         | TAp73                   | Tumor specific upregulation of TAp73mRNA                                                              | Herath et al. (2000)                                                     |
| Lung cancer                                                             | TAp73                   | Tumor specific upregulation of TAp73                                                                  | Tannapfel et al. (1999), Sayan et al. (2001), Qin et al. (2005)          |
|                                                                         | TAp73 and $\Delta$ Np73 | Expression of $\Delta$ Np73 is correlated with a poor prognosis                                       | Müller et al. (2005)                                                     |
|                                                                         | Not determined          | AT/AT polymorphism in p73 is linked to disease                                                        | Li et al. (2004)                                                         |
|                                                                         | $\Delta$ Np73           | Expression of $\Delta$ Np73 is correlated with poor prognosis                                         | Reis-Filho et al. (2003)                                                 |
| Breast cancer                                                           | p73                     | Tumor specific expression of TAp73                                                                    | Mai et al. (1998), Tokuchi et al. (1999)                                 |
|                                                                         | $\Delta$ Np73           | Expression of $\Delta$ Np73 is correlated with poor prognosis                                         | Dominguez et al. (2006a)                                                 |
| Ovarian cancer                                                          | p73                     | Expression of wt p73                                                                                  | Zaika et al. (1999)                                                      |
|                                                                         | TAp73 and $\Delta$ Np73 | Expression of $\Delta$ Np73 and $\Delta$ Np73 are correlated with poor prognosis                      | Concin et al. (2004, 2005)                                               |
| Head and neck cancer                                                    | TAp73                   | Expression of wt p73                                                                                  | Ng et al. (2000)                                                         |
|                                                                         | p73                     | Tumor specific expression of p73                                                                      | Chen et al. (2000)                                                       |
| Leukemia                                                                | $\Delta$ Np73           | Expression of $\Delta$ Np73 is correlated with poor prognosis in neuroblastoma                        | Casciano et al. (2002)                                                   |
|                                                                         | Not determined          | Acute lymphoblastic leukemia, chronic lymphoblastic leukemia                                          | Sahu and Das (2005)                                                      |
| Gliial tumors                                                           | TAp73                   | Acute myeloblastic leukemia, tumor specific expression of TAp73 $\epsilon$                            | Peters et al. (1999), Tschan et al. (2000)                               |
|                                                                         | $\Delta$ Np73           | Expression of $\Delta$ Np73 is correlated with poor prognosis                                         | Rizzo et al. (2004), Bueso-Ramos et al. (2005), Meier et al. (2006)      |
| Gliial tumors                                                           | TAp73 and $\Delta$ Np73 | Expression of TAp73/ $\Delta$ Np73 is correlated with tumor grade                                     | Wagner et al. (2006)                                                     |

T: tumor; N: normal tissue.

a dominant negative mutation or RNA interference leads to resistance of cells to apoptosis induced by genotoxic agents (Yuan et al., 1999; White and Prives, 1999; Gong et al., 1999; Agami et al., 1999; Costanzo et al., 2002; Bergamaschi et al., 2003; Irwin et al., 2003; Ben Yehoyada et al., 2003; Gonzalez et al., 2003; Müller et al., 2005; Vayssade et al., 2005). Endogenous TAp73 is activated in response to a variety of chemotherapeutic drugs and  $\gamma$ -irradiation in a path-

way that depends on the non-receptor tyrosine kinase c-Abl (Gong et al., 1999; Agami et al., 1999). In addition, a recent study indicated that also c-Abl-independent p73 stabilization pathways could account for gemcitabine-induced apoptosis (Thottassery et al., 2006). Furthermore, certain human tumor-derived p53 mutants are able to bind and inhibit TAp73 function (Di Como et al., 1999; Strano et al., 2000; Gaiddon et al., 2001; Bergamaschi et al., 2003; Monti et al., 2003; Concin

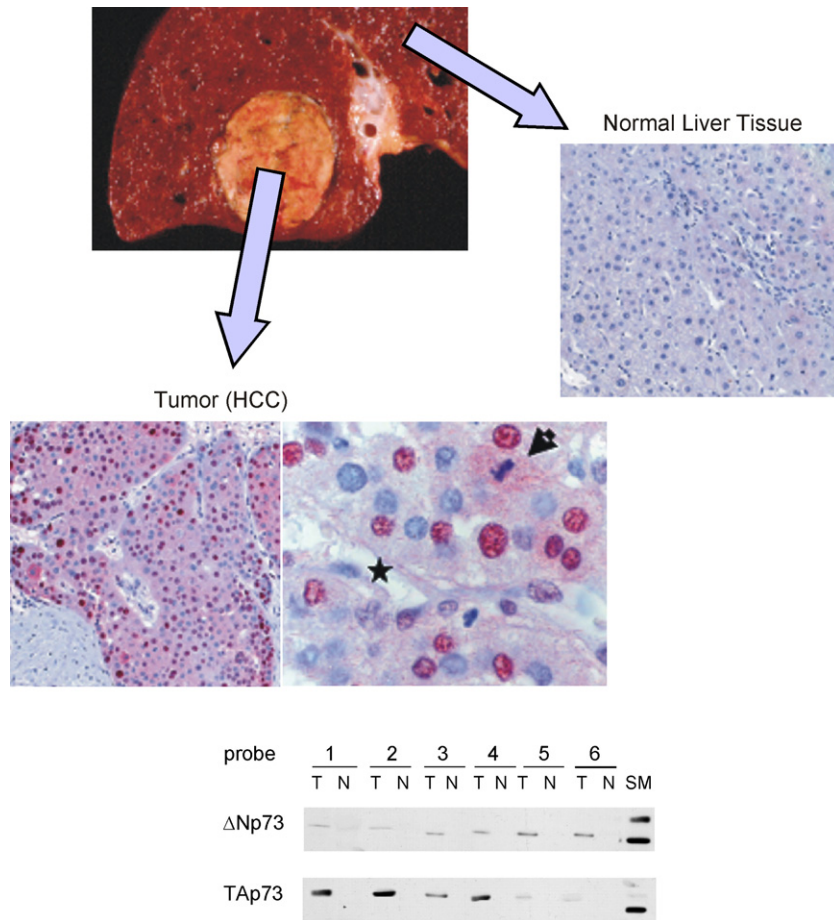


Fig. 5. Immunohistochemical demonstration of ΔNp73 protein in hepatocellular carcinoma (HCC) but not in non-neoplastic liver tissue (original magnification ×20). The ΔNp73 staining signal is localized within tumor cell nuclei (original magnification ×20). Positive tumor cell nuclei with a perinuclear staining during mitosis (arrowhead): ΔNp73 immunoreactivity occurs also in the cytoplasm. Sinus endothelial cells are ΔNp73 negative (asterisk) (original magnification ×100).

The Western blot analysis shows the distinct expression of ΔNp73 and TAp73 in tumor tissue of six HCCs ('T') but not in non-neoplastic liver tissue ('N').

Table 5  
Effect of chemotherapy on endogenous p73

| Treatment                                                          | Isoform           | Organ/tissue/cell line                                                                | Comments                                                                         | References                                                                                                        |
|--------------------------------------------------------------------|-------------------|---------------------------------------------------------------------------------------|----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------|
| Adriamycin, cisplatin, taxol, etoposide, doxorubicin, camptothecin | TAp73α↑↑, TAp73β↑ | Different tumor cell lines (colon cancer, squamous carcinoma cell lines, Saos2, a.o.) | Expression of TAp73α/β in response to chemotherapy                               | Gong et al. (1999), Costanzo et al. (2002), Bergamaschi et al. (2003), Irwin et al. (2003), Moll and Slade (2004) |
| Bleomycin, doxorubicin, mitoxantrone                               | TAp73↑, ΔNp73↑    | Hepatocellular carcinoma cell lines                                                   | Chemotherapy induces expression of TAp73 and ΔNp73                               | Müller et al. (2005)                                                                                              |
| Cisplatin                                                          | TAp73α↑           | Neuroblastoma and astrocytoma cell lines                                              | Accumulation of TAp73α in response to cytostatic drug                            | Terrasson et al. (2005), Hosoda et al. (2005)                                                                     |
| Cisplatin, taxol                                                   | TAp73β↑           | Saos2, Cos7 cells                                                                     | Drug induces TAp73β                                                              | Lin et al. (2004)                                                                                                 |
| Cisplatin                                                          | TAp73α↑           | Primary pancreatic acinar cells                                                       | Accumulation of TAp73α                                                           | Sphyris et al. (2004)                                                                                             |
| Cisplatin                                                          | TAp73             | Colorectal cancer cell lines                                                          | PMS2 stabilizes TAp73 in response to DNA damage                                  | Shimodaira et al. (2003)                                                                                          |
| Gemcitabine T-ara-C                                                | TAp73↑            | Colorectal cancer cell lines                                                          | Increase of TAp73 and apoptosis via c-Abl-independent pathways                   | Thottassery et al. (2006)                                                                                         |
| Adriamycin                                                         | TAp73↑            | Breast cancer cell lines                                                              | Drug activates TAp73 and induces apoptosis                                       | Vayssade et al. (2005)                                                                                            |
| Cisplatin                                                          | TAp73α↑, TAp73β↑  | HeLa cells                                                                            | TAp73α and TAp73β lead to an increase in caspase 1 gene transcriptional activity | Jain et al. (2005)                                                                                                |

Table 6  
Effect of p73 on chemosensitivity

| Treatment                                          | Isoform                        | Chemosensitivity       | Organ/tissue/cell line                              | Comments                                                                                | References              |
|----------------------------------------------------|--------------------------------|------------------------|-----------------------------------------------------|-----------------------------------------------------------------------------------------|-------------------------|
| Adriamycin, cisplatin and adenoviral gene transfer | TAp73 $\beta$                  | $\uparrow\uparrow$     | Human malignant melanoma cell lines                 | Overexpression of TAp73 $\beta$ enhances chemosensitivity                               | Tuve et al. (2006)      |
| Adriamycin, cisplatin                              | TAp73 $\alpha$                 | $\uparrow\uparrow$     | Human lung adenocarcinoma cell lines                | Overexpression of TAp73 $\beta$ enhances chemosensitivity                               | He et al. (2006)        |
| Bleomycin                                          | TAp73 $\beta$                  | $\uparrow\uparrow$     | Hepatocellular carcinoma cell lines and Saos2 cells | Overexpression of TAp73 $\beta$ sensitizes carcinoma cells to chemotherapy              | Müller et al. (2005)    |
| Bleomycin                                          | $\Delta$ Np73                  | $\downarrow\downarrow$ | Hepatocellular carcinoma cell lines                 | Overexpression of $\Delta$ Np73 leads to chemoresistance                                | Müller et al. (2005)    |
| Platinum-based chemotherapy                        | $\Delta$ N'p73                 | $\downarrow\downarrow$ | Ovarian cancer                                      | High levels of $\Delta$ N'p73 lead to chemoresistance                                   | Concin et al. (2005)    |
| Cisplatin                                          | $\Delta$ Np73 $\alpha$         | $\downarrow\downarrow$ | Neuroblastoma and astrocytoma cell lines            | $\Delta$ Np73 $\alpha$ inhibits Fas-mediated apoptosis                                  | Terrasson et al. (2005) |
| Cisplatin                                          | $\Delta$ Np73 $\alpha$         | $\downarrow\downarrow$ | Neuroblastoma and astrocytoma cell lines            | Human cytomegalovirus induces drug resistance by accumulation of $\Delta$ Np73 $\alpha$ | Allart et al. (2002)    |
| Adriamycin                                         | TAp73 $\alpha$                 | $\uparrow\uparrow$     | Breast cancer cell lines                            | TAp73 $\alpha$ -dependent induction of 14-3-3 $\beta$ increases chemosensitivity        | Sang et al. (2006)      |
| Daunorubicin                                       | $\Delta$ Np73ex2               | $\downarrow\downarrow$ | Childhood T-ALL                                     | $\Delta$ Np73ex2 leads to chemoresistance                                               | Meier et al. (2006)     |
| Fludarabine                                        | TAp73 $\alpha$                 | $\uparrow\uparrow$     | CLL                                                 | CD154-expressing cells induce TAp73 $\alpha$ and induces chemosensitivity               | Dicker et al. (2006)    |
| 5-Aza-CdR epirubicine etoposide                    | TAp73 $\alpha$ , TAp73 $\beta$ | $\uparrow\uparrow$     | Epithelial cell lines 293 and HeLa                  | Expression of TAp73 $\alpha/\beta$ after DNA damage                                     | Schmelz et al. (2005)   |

et al., 2005). It has been shown that these p53 mutants, at least partly, confer cellular resistance through abrogation of TAp73 function (Irwin et al., 2003). A polymorphism encoding either arginine (72R) or proline (72P) at codon 72 of p53 influences inhibition of p73 by a range of p53 mutants identified in squamous cancers. Patients whose cancers express p53 mutants with mutation in the 72arginine (72R) variant have a worse response to therapy than those expressing p53 mutants encoding proline at position 72 (72P). Expression of 72R mutants that efficiently inhibit p73 was associated with a particular poor outcome to therapy (Bergamaschi et al., 2003). Thus, mutant p53 or N-terminally truncated p73 may cause resistance to anticancer agents through inhibition of wild-type p53 and TAp73 (Bergamaschi et al., 2003; Gasco and Crook, 2003; Strano and Blandino, 2003; Irwin, 2004; Müller et al., 2005; Meier et al., 2006).

### 5. Degradation pathways of p63 and p73: future potential drug targets?

Recent work has allowed the identification of the degradation pathway of p63 and p73. This involves the ubiquitin-proteasome degradation pathway, and in particular the E3 ligase Itch. Interestingly, the physical interaction

involves an area that is absent in p53, and consequently Itch is specific for p63/p73 and does not bind and degrade p53 (Fig. 6).

Itch belongs to the HECT domain family of ubiquitin E3 ligases. Itch mutant mice (Itchy) develop a fatal disease characterized by inflammation of several epithelia, including the skin, and hyperplasia of epithelial and hemopoietic cells (Perry et al., 1998). At the cellular level, Itch promotes the ubiquitination of several proteins involved in the regulation of cell death, such as p63 (Rossi et al., 2006) and p73 (Rossi et al., 2005). Itch-dependent ubiquitination of c-Jun in lymphoid cells is triggered by the activation of the JNK pathway, which results in suppression of cytokine production and could potentially explain the immunological defects observed in the Itchy mice. Interestingly, we have also shown that Itch is downregulated in tumor cell lines upon treatment with chemotherapeutic drugs, and this correlates with the induction of p73.

The promyelocytic leukemia protein PML also regulates the stability of p63 (Bernassola et al., 2005) and p73 (Bernassola et al., 2004) (Fig. 7), but this seems to be part of a strictly regulated sequence of events involving also a kinase-dependent phosphorylation (Gong et al., 1999).

The identification of a specific degradation pathway is of vital importance for p63/p73 biology as the rele-

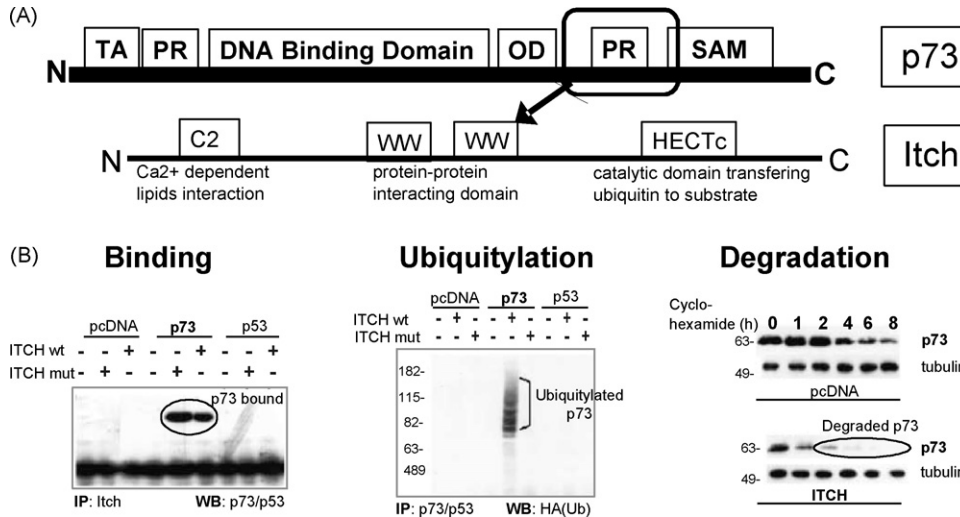


Fig. 6. The E3 ligase ITCH regulates p73 stability. (A) The PR domain of p73 physically interacts with the WW domain of the E3 ligase Itch. Itch is an ubiquitin E3 ligase, part of the Nedd4 family, containing an HECT domain. Itch protein includes several domains: the C2 domain is a calcium-dependent region able to interact also with lipids, the WW domain is a protein-protein interacting region, able to bind PY motif, such as the PR region of p73, the HECT domain is the catalytic domain transferring ubiquitin to the specific substrate, in this case p73. Itch degrades p73, p63 and also c-Jun, Jun-B, Notch and Flip, therefore suggesting a relevant role in cancer development and chemosensitivity. A natural mouse knockout of Itch is available, the 18H aguty mice, that shows specific immune defects and atrophin-related defects. (B) Itch specifically binds (left panel), ubiquitylates (central panel) and degrades (right panel) p73. These data are extracted from Rossi et al. (2005); relevant controls are omitted to facilitate the presentation.

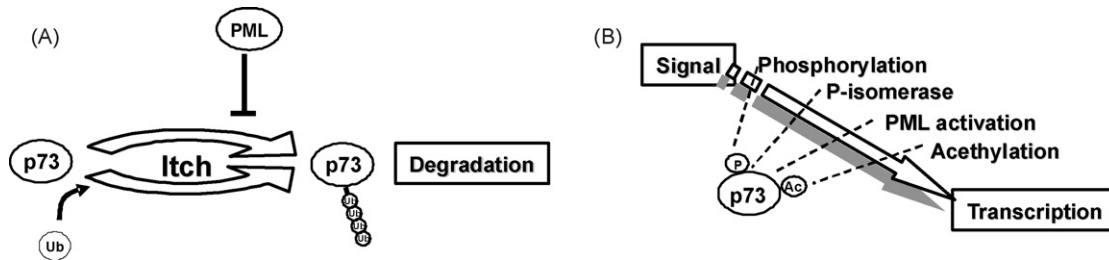


Fig. 7. Regulation of p73 stability and transcriptional activation. (A) The p73 protein is degraded via the ubiquitin-proteasome degradation pathway, regulated by the E3 ligase Itch. Additional E3 ligases are also involved, even though their identification and specificity is still under evaluation. The PML protein is able to attenuate protein degradation by diverting the protein to the transcription pathway. (B) The transcription pathway of p73 follows a very strict regulation involving several steps: (i) phosphorylation by a specific kinase such as MAP p38 and c-Abl, (ii) prolyl-isomerization by the enzyme Pin-1, (iii) recruitment and activation by PML into the PML-nuclear body and (iv) acetylation by the CBP/p300 complex. The protein that is phosphorylated, pro-isomerised and acetylated is transcriptionally competent. During this pathway the protein seems less sensitive to the degradation process.

vant E3 ligase may be an ideal pharmacological target for a potential clinical therapy. This is even more important for transcription factors (like p53/p63/p73) that are traditionally very bad pharmaceutical targets. Indeed, as detailed above, MDM2 inhibitors have been developed to stabilize the protein and function of p53. Similarly, Itch inhibitors could have an effect on p63/p73 stability in cancer.

**6. Conclusions**

- The identification and characterization of the p53–p63–p73 network provides evidence of a tight link between developmental processes and tumorigenesis.
- The p53 family genes produce multiple isoforms that vary in composition of the NH<sub>2</sub>- and C-termini.

- The ΔN isoforms can oppose the transactivation capabilities of the full-length proteins.
- p63 and p73 are important for normal development and differentiation, but are also implicated in human carcinogenesis.
- The finding that a significant percentage of tumors select for dominant negative p63 and/or p73 isoforms argues for their oncogenic role.
- Like p53, TAp63 and TAp73 activate genes exerting roles in different steps of the apoptosis program.
- Accordingly, chemosensitivity is determined by the interactions between the different isoforms of the p53 family members.
- Defining the exact repertoire of isoforms expressed in a given cell type/tumor entity is needed to determine their precise roles in tumor suppression/tumorigenesis and development.

- Studies are also needed to determine which p53 family isoforms are activated in response to specific stresses (DNA damage or others).
- Furthermore, the novel p53 isoforms require studies on the mechanisms involved in promoter choice and alternative splicing.
- Distinct post-translational modifications and interactions with cofactors further modulate the transcriptional activity of the p53 family members in response to particular stress signals.
- The identification of a specific degradation pathway is of clinical importance for p63/p73 biology as the relevant E3 ligase Itch may be an ideal pharmacological target.
- We believe that manipulation of the apoptotic pathways with the p53 family members as central targets show long-term promise in treating human cancer.

### Acknowledgements

This work was supported by grants of the Tumorzentrum Heidelberg/Mannheim to M.M. and P.H.K., the Deutsche Krebshilfe to P.H.K. and the Forschungsschwerpunktprogramm Baden-Württemberg to M.M.

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