Review

What We Currently Know about the Structure and Function of the p53 Homologue – p73 Protein: Facts, Hypotheses and Expectations

(p53 / p73 / cell cycle / apoptosis / tumour biology)

P. ČEŠKOVÁ[1], D. VALÍK[2], B. VOJTĚŠEK[1]

[1]Department of Experimental Oncology and [2]Department of Laboratory Medicine, Masaryk Memorial Cancer Institute, Brno, Czech Republic

During the last 23 years, investigation of the *p53* gene and its respective protein product became a major focus in cancer research. It has been established that the p53 protein plays an important role in preventing tumour development and that a majority of cancers show evidence of loss of the p53 function (Hollstein et al., 1994). Alterations of the *p53* tumour suppressor gene and its encoded protein are the most frequently encountered genetic events in human malignancies (Hollstein et al., 1991). More than a half of the human tumours contain somatic mutations in *p53* that inactivate its function. Furthermore, germline mutations in *p53* are responsible for the majority of cases of the inherited cancer family syndrome known as Li-Fraumeni syndrome (Malkin et al., 1990). The p53 is a well-known tumour suppressor affecting – through regulation of expression of different target genes – important cellular processes like cell cycle progression, apoptosis and senescence. These activities were linked to the ability of the p53 protein to act as a powerful transcription factor that binds to as many as 300 different promoter elements in the genome, thus profoundly altering patterns of specific gene expression (Zhao et al., 2000; Lane and Lain, 2002). The transcriptional activity of the p53 protein is activated in response to cellular stress such as DNA damage or oncogene activation and is very highly regulated. All attempts to find p53-related genes had been unsuccessful until 1997. The realization that *p53* in fact belongs to a family of related genes came almost 20 years after the discovery of *p53* (Yang and McKeon, 2000) when in 1997 Kaghad et al. (Kaghad et al., 1997) discovered cDNA encoding the p73 protein in the hybridization screen of a COS-cell cDNA library using degenerative oligonucleotide primers for different genes. During subsequent screening they found existence of cDNAs encoding p73α and p73β, which are splice variants of *p73* differing at their C-termini. The *TP73* gene maps to the 1p36.2-3 chromosome region (tumour suppressor gene locus the existence of which was suspected for a long time), in which deletions frequently appear in neuroblastoma, colon cancer, melanoma and breast cancer (Schwab et al., 1996). Based on all new findings it has been hypothesized that p73 could be a new tumour suppressor protein. In 1998, another p53 homologue – p63 protein (Yang et al., 1998) was described, which maps to the 3q27-29 chromosome region. These facts stimulated further p73 and p63 protein studies, whose number is rising exponentially.

Functional similarities and differences

Within the short period since cloning of *TP73*, a number of investigators have reported on the structure, function and regulation of the p73 protein. Though showing many similarities, substantial differences can be found between the p53 and p73 proteins. In contrast to the p53-deficient mice, which exhibit increased susceptibility to spontaneous tumorigenesis, mice functionally deficient for all p73 isoforms exhibit profound defects including hippocampal dysgenesis, hydrocephalus, chronic infections and inflammation together with affection of pheromone sensory pathways. This findings indicate the role of the p73 protein in neurogenesis, sensory pathways and homeostatic control (Yang et

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Corresponding author: Bořivoj Vojtěšek, Department of Experimental Oncology, Masaryk Memorial Cancer Institute, Žlutý kopec 7, 656 53 Brno, Czech Republic. E-mail: vojtesek@mou.cz

Abbreviations: ALL – acute lymphatic leukaemia, ESCC – oesophageal squamous cell carcinomas, DBD – DNA-binding domain, NK – natural killer, OD – oligomerization domain, OSCC – oral squamous cell carcinomas, SAM – sterile alpha motif, SH2 – Src homology 2, SH3 – Src homology 3, TAD – transactivation domain.

al., 2000). Extensive searching for the p73 status in primary tumours also brought the surprising result that *TP73* mutations are quite rare, unlike mutations in *p53*, which are present in more than 50% of tumours. In almost 909 tumours studied, only five missense mutations were found, whereas no deletions or truncation mutants have been identified (Levrero et al., 2000; Stiewe and Putzer, 2002). Two mutations (P405R and P425L) were found in neuroblastoma (Ichimiya et al., 1999), one mutation (R269Q) was found in breast cancer (Han et al., 1999), one mutation (P405R) was found in lung cancer (Ikawa et al., 1999) and one mutation (N204S) was found in CNS-tumours (Lomas et al., 2001). The suggested explanation was that genomic imprinting and promoter methylation, albeit in a tissue-dependent manner, may be responsible for these events. The gene *TP73* is biallelically expressed in all foetal tissues except brain, where differential expression of the two parental alleles was observed (Hu et al., 2000). Bladder, prostate, ovarian tumours, neuroblastomas and oesophageal squamous cell carcinomas (ESCC) do not show any differences in comparison to normal tissue in mostly biallelic expression (Yokomizo et al., 1999a; Yokomizo et al., 1999b; Cai et al., 2000; Chen et al., 2000; Liu et al., 2000). More recent studies show that monoallelic expression of the *TP73* gene is rare (Nomoto et al., 1998; Yokomizo et al., 1999a; Yokomizo et al., 1999b; Yokozaki et al., 1999; Zaika et al., 1999) and occurs in normal renal (Mai et al., 1998) and gastric tissue (Kang et al., 2000) where, on the contrary, loss of imprinting is associated with carcinoma development. Studies on haematological malignancies showed existence of associations between negligible or limited expression of the *TP73* gene and gene hypermethylation at the CpG island in the 5'untranslated region of the p73 mRNA (Kawano et al., 1999). Hypermethylation is considered to cause inactivation of the *TP73* gene in both T- and B-cell acute lymphatic leukaemia (T- and B-ALLs) (Liu et al., 2001), Burkitt's lymphomas (Corn et al., 1999), natural killer (NK) cell lymphoma, and could be associated with tumour progression of human oral squamous cell carcinomas (OSCC) (Araki et al., 2002) and development of oligodendroglial tumours as well (Dong et al., 2001). Surprisingly, studies from different laboratories suggest that altered expression of the wild-type *TP73* gene rather than its loss of expression is involved in tumorigenesis (Kovalev et al., 1998; Zaika et al., 1999), because it was shown that various tumours express higher levels of p73 than tissues from which they originated.

The gene *TP73*

The human *p53* gene has a single promoter, which encodes for a single protein of 393 amino acids, while the structure of the *TP73* gene is complex. The *TP73* gene is alternatively transcribed from two different promoters P1 in the 5'UTR upstream of a non-coding exon 1, and P2 located within the 23 kb spanning intron 3. P1 and P2 promoters give rise to two different proteins: i) TA-p73 with transactivation domain (TA) and ii) ΔN-p73 that lacks the TA domain (Fig. 1).

The *TP73* gene involves 14 exons. Different splicing of these exons is responsible for formation of many p73 C- and N-terminal isoforms. In addition to the mRNA which encodes the full-length α form and the alternatively spliced transcript lacking exon 13 (β form), other splice variants from the C-terminal region, lacking exon 11 (γ form), splicing exons 11, 12 and 13 (δ form), exons 11 and 13 (ε form) and exons 11 and 12 (ξ form) are also produced (De Laurenzi et al., 1998; De Laurenzi et al., 1999). Recently, new C-terminal isoform p73η was discovered (Ishimoto et al., 2002). Together with C-terminal isoforms of p73, the N-terminal isoforms p73Δexon2 (Kaghad et al., 1997), other ΔN-p73 and p73Δexon2/3 isoforms (Grob et al., 2001; Ishimoto et al., 2002; Stiewe and Putzer, 2002) were described in human cells. To make the issue more complex, Grob et al. (2001) found two ATG – start codons in cDNA for the ΔN-p73 isoform, which yield two different ΔN-p73 proteins: ΔN-p73-ATG_1 and ΔN-p73-ATG_2 (Grob et al., 2001). All p73 isoforms are demonstrated in the Fig. 1.

Structure of the p73 protein

The p53 and p73 proteins share significant homology both at the genomic and at the protein level. Each of these proteins contains the acidic amino terminal transactivation domain (TAD), the central core specific DNA-binding domain (DBD) and the carboxy-terminal oligomerization domain (OD). Unlike the p53 protein, the p73 protein contains long C-termini. The highest level of homology is observed in the DBD (63% identity between p53 and p73), which suggests that the two proteins can bind to the same DNA sequence and be responsible for transactivation from the same set of promoters. In addition, p73 shows 29% identity with the TAD and 38% identity with OD (Kaghad et al., 1997).

DNA-protein interaction

In many independent experiments it was shown that p73 proteins can activate the promoters of several p53-responsive genes in co-transfection experiments. Even though the DNA-binding domain of p73 interacts with the consensus p53-responsive sequences and activates the promoters of several p53-responsive genes (including *$p21^{WAF1}$*, *bax*, *mdm2*, *cyclin-G*, *GADD45* and *IGFBP3* (Jost et al., 1997; Kaghad et al., 1997; De Laurenzi et al., 1998; Dobbelstein and Roth, 1998; Yang et al., 1998; Lee and La Thangue, 1999; Takada et al., 1999; Zeng et al., 1999)), some results indicate that many target genes respond differently to p53 or p73 proteins. Also, the splice variants of *p73* differ in their

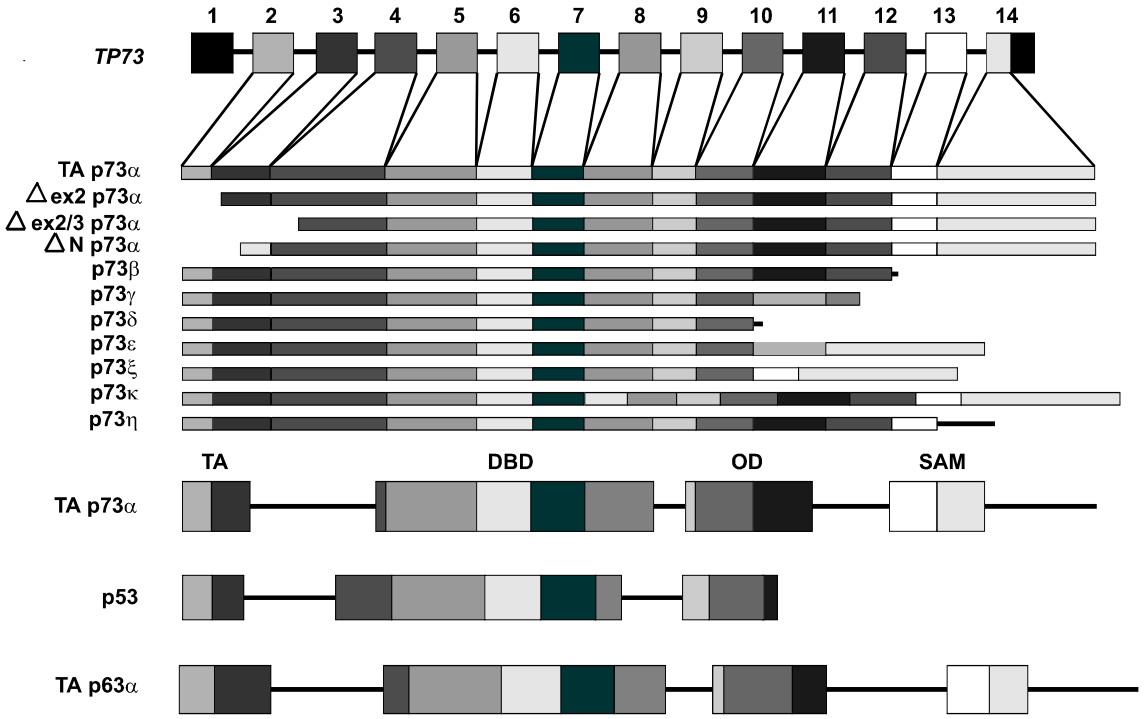

Fig. 1. The *TP73* gene structure, different splicing products and comparison with p53 and p63 proteins.
The p53 and p73 proteins share very significant homology both at the genomic and protein levels. The p73α protein contains the acidic amino terminal TAD, the central core DBD and the carboxy terminal OD. Unlike the p53 protein, the p73 protein contains long C-termini with the SAM domain. The protein p63, which belongs to the p53 family, is also shown. Different splicing of the p73 gene is responsible for formation of seven p73 C-terminal isoforms and four N-terminal isoforms. Splicing of different exons in p73 protein isoforms is denoted. Two different promoters give rise to two different proteins with and without the TAD. N-terminal truncated variants are incapable of transcriptional activation.

transcriptional activity on p53-responsive promoters. It is reported in general that C-terminal deletion mutants of p73α showed significantly higher levels of transcriptional activity than wild-type p73α. From this result it is apparent that the C-terminal structure of p73α represses the transcriptional activity of the p73 protein (Ueda et al., 2001).

Another factor regulating transcriptional activity of the p73α protein is its transactivation domain. N-terminal truncated variants lacking the transactivation domain are incapable of transcriptional activation and serve as antagonists of TA family members, including p53, and in fact they may exert the oncogenic rather than tumour-suppresor effect (Ishimoto et al., 2002).

Protein-protein interaction

Specific protein-protein interactions are often mediated by functionally and structurally defined families of the so-called ”small protein modules” (Pawson et al., 2002). Typically, protein-protein interaction domains are independently folding modules of 35–150 amino acids, which can be expressed in isolation from their host proteins while retaining their intrinsic ability to bind their physiological partners. Interaction domains are often used repeatedly in numerous different proteins to mediate a particular type of molecular recognition. Protein-protein interaction domains can be divided into separate families that are interrelated either by sequence or ligand-binding properties. Thus, a large number of cytoplasmic proteins contain one or two Src homology 2 (SH2) domains that directly recognize phosphotyrosine-containing motifs. A large group of interaction domains (Src homology 3 (SH3), WW) bind to proline-rich motifs; these complexes are less dependent on post-translational modifications, and therefore more stable than the phospho-dependent interactions involving SH2 domains. In addition, a number of modules form homo- or heterotypic domain-domain interactions.

Oligomerization domain

The p53 oligomerization domain is a protein-protein interaction domain which permits the oligomerization of the p53 protein to its tetrameric, active conformation (Fig. 2). Based on the homology with p63 and p73 oligomerization domains it is supposed that active p63 and p73 conformation is also tetrameric. This hypothesis is encouraged by glutaraldehyde cross-linking experiments with separate p63 and p73 oligomerization

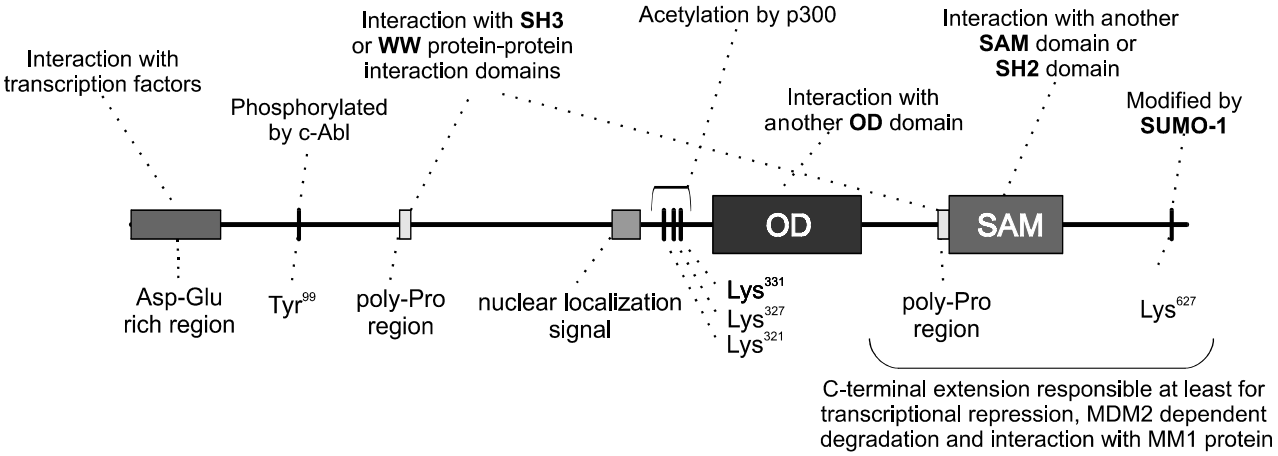

Fig. 2. Protein–protein interactions in the p73 protein.
Functionally important domains shown in the linear p73 structure involved in protein–protein interactions.

domains (Davison et al., 1999). Surprisingly, no heterotypic tetramer formation between wt p53 and p73 or wt p53 and p63 proteins was observed, and interactions between p63 and p73 proteins are only weak (Kojima et al., 2001). On the other hand, it was reported that human tumour-derived p53 mutants can associate with p73 isoforms under physiological conditions and that for the associations with the mutated p53 protein, an oligomerization domain together with a DNA binding domain is required (Strano et al., 2000). Mutant p53 binding to the oligomerization domain can interfere with the formation of p73 homo-oligomers and mutant p53 binding to the DNA-binding domain of p73 can interfere with its binding to DNA. These findings suppose that the mutant form of the p53 protein can inactivate the p73 functions.

Sterile alpha motif (SAM) domain

This domain is predicted to occur at C-terminal p63α, p73α and squid p53 protein extensions which are not found in human p53. The SAM domain, which is approximately 70 amino acids long, was first described in 1995 (Ponting, 1995) to be involved in yeast sexual differentiation and Drosophila polyhomeotic proteins. Also, this domain was later found in such diverse organisms as fungi, protozoa and animals and, surprisingly, the proteins having the SAM domain are all compatible in developmental regulation. The SAM domain can be described as a protein-protein interaction domain which is able to bind other SAM domains or, via phosphorylation of a conserved tyrosine, SH2 domains (Schultz et al., 1997). Despite the fact that the sequence identity between the p73 protein SAM domain and other SAM domains (only four SAM domain structures have now been reported) is quite low (15-17%), the helices and intervening loops of the p73 SAM-like domain align significantly. However, no evidence of homo- or hetero-oligomerization of the SAM-like domains of p73 and p63 proteins was found. It implies that if the p73/p63 SAM-like domain is involved in protein-protein interactions, the binding partner proteins must be either less structurally related SAM domains or the binding is modulated through non-SAM domain proteins (Chi et al., 1999).

Proline-rich region

It was shown that a proline-rich region, which maps to the close proximity of the SAM domain and is specific for p73α and p73β isoforms, interacts with the SH3 and WW domains. The interaction was demonstrated for the p73α proline-rich region and SH3 domain of c-Abl. c-Abl is a non-receptor tyrosine kinase activated by agents that damage DNA. Its interaction with the p73α protein was further shown to be necessary to the p73α-dependent apoptotic function as discussed below (Agami et al., 1999). Kim et al. (2001) described interaction of the p73β isoform (region 321–376) with the SH3 domain of amphiphysin IIb-1 thought to have yet unspecified function in endocytosis (Kim et al., 2001).

Interaction between the p73 C-terminal proline-rich region and WW domain was shown for the YAP protein; YAP is a ubiquitously expressed phosphoprotein interacting with the SH3 domain of the proto-oncogene protein c-Yes, a non-receptor tyrosine kinase that belongs to the Src family (Strano et al., 2001).

Other interaction sites located on p73α C-terminus

An as yet uncharacterized site for interaction with the MM1 protein (a nuclear c-Myc-binding protein) was identified at the extreme C-terminal region of p73α. It was also found that c-Myc was physically associated with p73α and significantly impaired the transcriptional activity of p73α on *bax* and $p21^{waf1}$ promoters (Nakagawa et al., 2002).

The C-terminal Lys ^{627} is the major SUMO-1 (ubiquitin-like tagging molecule) covalent modified residue in a p73α isoform. Experimentally, SUMO-1-modified p73α is more rapidly degraded by the proteasome than unmodified p73α. Although SUMO-1 modification is not required for p73 degradation *in vivo*, we can consider that SUMO-1 modification is not a major factor influencing p73α degradation by the proteasome, but can only potentiate proteosomal degradation of p73α. SUMO-1 modification does not affect the transcriptional activity of p73α, but may alter the subcellular localization of p73α or may modulate the interaction of p73α with other proteins that interact with SUMO-1 (Minty et al., 2000).

Acetylation sites

Three conserved lysines occurring within the p73α protein at positions 321, 327 and 331 are acetylated by the acetyltransferase p300 in response to apoptosis-inducing concentrations of the DNA-damaging drug doxorubicin. The finding that non-acetylatable mutant p73α exogenously expressed in p53 null cells is deficient in inducing apoptosis, but activates *p21* transcription as efficiently as wild-type p73α (Costanzo et al., 2002), indicates that acetylation following DNA damage potentiates p73-dependent apoptotic function by increasing its ability to activate transcription of proapoptotic target genes.

Interaction with MDM2 protein

Additional C-terminal 243 amino acids found in the p73α isoform against the p53 protein probably play an important role in its interaction with the MDM2 protein. MDM2 is the product of a p53-inducible gene, which displays an E3 ubiquitin ligase activity towards the p53 protein (Kubbutat and Vousden, 1998). The p73 proteins are homologous with p53 in the N-terminal MDM2 binding region. The binding of p73α and p73β to MDM2 mediated through this N-terminal region has been demonstrated by several independent studies (Balint et al., 1999; Ongkeko et al., 1999; Zeng et al., 1999). Binding of MDM2 to p53 targets this protein for degradation, while binding of MDM2 to p73 stabilizes p73. One possible explanation for this finding is that the C-terminus of p53, which is necessary for efficient MDM2-mediated degradation of p53, is not conserved in p73, and additional 243 amino acids found in the p73α isoform can inhibit the proteosomal degradation. The observation that inhibition of proteasome increases endogenous p73α levels suggests that p73α stability may be regulated by another protein, which functions in a manner similar to MDM2. One potential candidate is the MDMX protein (Shvarts et al., 1996), which would be expected to interact with p73α through the MDM2 binding domain at its N-terminus. The interaction between p73α and MDM2 inhibits the ability of p73α to activate transcription, but less efficiently than in the case of the p53 protein. One potentially important consequence of the interaction between MDM2 and p73α could be the ability to prevent degradation of p53 through titration of MDM2, when a high level of p73α could result in the p53 stabilization (Balint et al., 1999). It can be postulated that activation of p73α expression by any mechanism could lead to both p73α-dependent cell growth inhibition and potentially activate the p53-dependent response, too. Stimuli that can induce p73α will be of great interest.

The role of p73 in apoptosis

The protein p53 has been recognized as central to the induction of apoptosis in response to DNA damage, a function that is critical for tumour suppression and the response of tumour cells to chemotherapeutic agents. Overexpression of the *p73* can activate typical p53-responsive genes and also induce apoptosis like p53. In addition, activation of the p73 has been implicated in apoptotic cell death induced by aberrant cell proliferation and some forms of DNA damage. Flores et al. showed that the combined absence of p63 and p73 proteins affected induction of p53-dependent apoptosis in response to DNA damage in E1A-expressing cells and in the developing mouse CNS. They explained this observation by the inability of the p53 protein to bind promoters of apoptosis-associated target genes and to upregulate their transcription in *p63*$^{-/-}$ and *p73*$^{-/-}$ E1A-expressing cells. These data support the notion that there may be two classes of p53-family target genes: i) class one includes genes such as *mdm2* and *p21*, which p53 regulates independently of the presence and/or absence of p63 and p73; ii) class two that includes genes such as *PARP*, *bax* and *NOXA*, which p53 can regulate only in co-operation with p63 or p73.

Recent reports have shown that the p73 protein accumulates and is phosphorylated at Tyr^{99} in response to DNA-damaging agents (reviewed by Levrero et al., 1999). It is well known that the accumulation and tyrosine phosphorylation of p73 are dependent on the activation of the nuclear c-Abl tyrosine kinase (Levrero et al., 1999; Shaul, 2000) and, interestingly, different types of DNA-damage inducers appear to affect the p73 protein in different ways. The kinetics of c-Abl activation and p73 stabilization also correlates with the apoptotic response of the cells studied. Tyrosine phosphorylation after ionizing radiation occurs well before cells undergo apoptosis, whereas stabilization of p73 in response to *cis*-platin is maximal when cells begin to die. Evidence is available that p73 can contribute to DNA damage-induced apoptosis by a link between the mismatch repair system and the induction of the p73 protein. Gong et al. (1999) showed that induction of the p73 protein by *cis*-platin was dependent on mismatch repair, while induction of the p53 by *cis*-platin was not mismatch repair-dependent. These

data suggest that a pathway linking mismatch repair to c-Abl tyrosine kinase and to p73 exists.

Other stimuli that do not activate c-Abl, such as UV (Liu et al., 1996; Baskaran et al., 1997), do not affect p73 and do not induce apoptosis. All these data suggest that c-Abl can either stabilize or phosphorylate the p73 protein depending on the DNA-damage inducer and the cell type used and activate p73-dependent apoptosis.

The p73 function

The p53 tumour suppressor has been under intense study by the research community for more than 20 years. Identification of two close structural homologues, p63 and p73, had several important impacts on what we think about p53 and simplified our approaches towards revealing biological functions of p63 and p73. Current available information suggests that members of the p53 family have overlapping but also distinct biological functions. The basis for their biological effects stems in the fact that different members of this gene family are regulated by separate mechanisms. An example of such regulations of both proteins p53 and p73 is their induction by *cis*-platin, where each protein is induced by a distinct mechanism through various signalling pathways activated by *cis*-platin (Gong et al., 1999; Levrero et al., 1999). The p73 protein is an important component of the cellular response to DNA damage and may play a critical role in tumour suppression and chemosensitivity.

The p73 protein is not a classic Knudson-type tumour suppressor gene like p53 and its role in tumorigenesis is still unclear. However, the existence of a ΔTA-isoform that acts antagonistically with possible oncogenic activity and essential functional cross-talk among all family members may argue for both tumour-suppressive and oncogenic roles. The C-terminal region of the p73α protein seems to be important for many specific protein-protein interactions, which take part in different regulation processes. Each of the p73 isoforms has different potential for protein-protein and DNA-protein interactions. Together with p63 isoforms and through their oligomerization domains they may also interact with each other and with the p53 protein, forming a complex network finely tuning the actual physiological cell state and its response to different kinds of stress. Differences in the degree of transactivation by the p73 isotypes suggest that the C-terminal region of p73α may modulate the transcriptional activity of these proteins and therefore may contribute to the distinctive biological properties of this protein relative to p53.

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