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COMMENTARY

The p53/p63/p73 family of transcription factors: overlapping and distinct functions

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SUMMARY

The *p53* gene is the most frequently mutated gene in human cancer. The identification of two homologues, p63 and p73, revealed that p53 is a member of a family of related transcription factors. Given that they share amino acid sequence identity reaching 63% in the DNA-binding domain, p53, p63 and p73 should have redundant functions in the regulation of gene expression. Indeed, p73 can activate p53-regulated genes and suppress growth or induce apoptosis. Moreover, *p53* and *p73* are both induced by DNA damage – albeit through distinct mechanisms. Other evidence, however, suggests that p63 and p73 are important for regulation of normal development. An extended C-terminal region, not found in p53, is alternatively spliced in p63 and p73. Within this C-terminal extension is a sterile alpha motif (SAM) previously found in other proteins that regulate development. The *p63*-deficient mice showed developmental abnormalities. Interestingly, the human *p63* gene is mutated in children who have the disease Ectrodactyly, Ectodermal dysplasia and facial Clefts (EEC) syndrome, and the disease phenotype is similar to the one of *p63*-deficient mice. The *p63* and *p73* genes are rarely mutated in human cancer, although *p73* loss is observed in neuroblastoma and a subtype of T-cell lymphoma. p53, p63 and p73 appear to have overlapping and distinct functions: p53 regulates the stress response to suppress tumors; p63 is essential for ectoderm development; and p73 might regulate both the stress response and development. Because p53 and p73 are linked to different upstream pathways, this family of transcription factors might regulate a common set of genes in response to different extracellular signals and developmental cues.

Key words: p53, p73, p63, c-Abl, Apoptosis, DNA damage, Differentiation, Development

INTRODUCTION

The *p53* gene is a tumor suppressor gene and the most frequent site of genetic alterations found in human cancers (Hollstein et al., 1991, 1996). Transgenic mice expressing mutant *p53* or *p53*$^{-/-}$ mice are very prone to both spontaneous and induced tumors (Donehower et al., 1992). The p53 protein is a sequence-specific transcription factor that regulates the expression of genes involved in cell cycle arrest or apoptosis in response to genotoxic damage or cell stress (see for review Ko and Prives, 1996; Levine, 1997; Choisy-Rossi and Yonish-Rouach, 1998; el-Deiry, 1998; Prives and Hall, 1999; Oren, 1999). Induction of growth arrest or cell death upon activation of p53 prevents the replication of damaged DNA and the division of genetically altered cells. Therefore, p53 is thought to play an important role in maintaining the integrity of the genome (Lane, 1992); this activity is central to its role as a tumor suppressor and is thought to determine the response of tumor cells to anti-cancer drugs that trigger apoptosis by inducing DNA damage. Indeed, inactivation of p53 due to deletion, mutations or to the interaction with cellular and viral proteins is recognized as the key step in the development of half of all human cancers.

In the past two years, two genes, *p63* and *p73* (reviewed by Arrowsmith, 1999; Levrero et al., 1999), have been found to encode several proteins whose structure and functions are similar, but not identical, to those of p53 (Kaghad et al., 1997; Yang et al., 1998). The identification of a p53 superfamily of transcription factors, potentially redundant in their ability to trigger similar cellular responses (i.e. cell cycle arrest and apoptosis), has challenged us to understand the basis for their similarities and differences in terms of physical and genetic interactions with one another, their regulation and their mechanisms of activation. Here we summarize the current information on the *p63* and the *p73* genes, focusing on the differences among the three members and their role in tumor suppression, the response to DNA damage, and differentiation.

STRUCTURE AND EXPRESSION OF *p73* AND *p63* GENES

Structure of the *p73* promoter

The human *p73* promoter has recently been cloned and partially characterized (Ding et al., 1999; A. Costanzo et al. and V. De Laurenzi et al., unpublished). It does not exhibit extended homology to the *p53* promoter and, unlike the latter, has a TATA-like box. Transient transfection experiments using 5′-deletion mutants localized the region critical for basal activity of the *p73* promoter between nucleotides −119 and +19 (relative to the start of exon I). This region contains putative SP1, AP2, Egr-1, Egr-2 and Egr-3 sites, and several stretches of CpG dinucleotides. The region located between positions −119 and −2714 contains additional putative SP1 and AP-2 sites, as well as three E2F sites, five NFAT sites, one c-Myb site, two N-Myc sites and a homeobox. Downstream of the short noncoding exon I, a long first intron (~30 kb) precedes exon II, which contains the putative ATG for p73 proteins. The putative transcription-factors-recognition sites, upstream of the basal promoter might be important for inducible expression of *p73*. In particular, the E2F sites, if functional, might provide the molecular basis for the recent observation that E2F-1 induces *p73* expression and that disruption of p73 function reduces E2F-1-dependent apoptosis in a *p53*-null cell line (Lohrum and Vousden, 1999). However, the roles, if any, of the different putative sites in the regulation of *p73* transcription remain to be defined.

Alternative splicing of the new *p53*-family members

Unlike *p53* both *p63* and *p73* give rise to differentially spliced mRNAs, which are translated into several different proteins. Most of the splicing occurs at the 3′ end (i.e. in the part of the sequence that is not present in *p53*) and creates proteins that have different C-termini (Fig. 1). At least six different p73 proteins (α-ζ) are generated in normal cells (Kaghad et al., 1997, De Laurenzi et al., 1998, 1999; Ueda et al., 1999). The β and δ isoforms are truncated forms of full-length p73-α: the alternative reading frame created by splicing generates a stop codon after a few codons. The δ isoform lacks the majority of the C-terminal region, becoming the most similar to p53. The ζ isoform has an internal deletion, lacking residues 400-496 of the α isoform. The γ isoform contains a long alternative reading frame that leads to the formation of a different, 75-residue C terminus. The ε isoform has a C-terminal region composed of parts of the γ and α reading frames (Fig. 1). Additional isoforms have been detected by RT-PCR both in cancer cells (see below) and in normal cells, but have not yet been cloned.

Similarly to p73, p63 gives rise to three different splice variants that differ in their C-termini: a full-length α form; a β form that is truncated after exon XII; and a γ form that lacks exons XII-XIV and uses an additional exon XV. Each of these isoforms may (TA forms) or may not (ΔN forms) contain the transactivation domain (TAD), depending on whether transcription of the precursor mRNA starts from exon I (TA forms) or from exon III′ (ΔN forms). The ΔN isoforms of p63 do not activate transcription but instead can act dominant negatively and inhibit transactivation by TA p63 proteins and p53 (Yang et al., 1998). At present, N-terminal-deletion variants of p73 have not been reported. Nevertheless, the possibility that such variants exist cannot be excluded.

Structural motifs in the p53 family

The three members of the p53 family share very significant homology both at the genomic and at the protein level. Each contains a TAD, a DNA-binding domain (DBD) and an oligomerization domain (OD; Figs 1 and 2). In addition, p63 and p73, but not p53, contain long C-termini. The determination of the three-dimensional solution structure of the p73 C terminus has shown that this region contains a sterile alpha motif (SAM), which is a protein-protein interaction domain (Chi et al., 1999). Sequence comparison and computer modeling show that this structure is also present in p63 (Fig. 2A). The SAM domain is also found in squid p53 and the rat KET protein. The SAM domain is a globular domain composed of four α-helices and a small 3_{10}-helix (Fig. 2B). The majority of SAM-domain-containing proteins are involved in the regulation of development; this supports the hypothesis that p63 and p73 play roles in differentiation. Unlike other SAM domains, those present in p73 and p63 do not mediate homo- or hetero-dimerization (Chi et al., 1999), which implies that they are important for interactions with other, as-yet-unidentified proteins.

The highest level of homology is reached in the DBD (63% identity between p53 and p73, and 60% identity between p53 and p63), which suggests that the three proteins can bind to the same DNA sequences and transactivate the same promoters (Fig. 2C). Despite the potential for all three family members to bind to and activate transcription from the different p53-responsive promoters, the fact that there is a certain degree of selectivity is emerging from many recent reports (see below).

The high conservation of the OD (Fig. 2D) suggests that members of this family form hetero-oligomers as well as homo-oligomers. However, yeast two-hybrid assays and co-immunoprecipitation experiments have shown that the OD of each family member tends to form homo-oligomers rather than hetero-oligomers (Kaghad et al., 1997; De Laurenzi et al., 1998; Davison et al., 1999; Di Como et al., 1999). In contrast to the observation that wild-type p53 does not co-immunoprecipitate with p73, some p53 dominant negative mutants (p53-R175H and p53-R248H) can bind to p73 and inactivate its transactivation function (Di Como et al., 1999). Whether mixed oligomers do exist in vivo is still an open question that requires further investigation.

GENES RESPONSIVE TO REGULATION BY p63 AND p73

Several genes that are apparently regulated by p53 have been described, and it is widely believed that these p53-regulated genes mediate most p53 effects (Gottlieb and Oren, 1998; el-Deiry, 1998). Studies aimed at the identification of p53 targets have been performed in different cell types, often from different species, and have used a wide variety of stimuli and agents to activate p53. This has made the interpretation of the different induction patterns observed quite difficult. Cellular promoters that respond to p53 include those of genes encoding proteins associated with growth control and cell cycle checkpoints (e.g. $p21^{Waf1/Cip1}$, Gadd45, Wip1, Mdm2, EGFR, PCNA, cyclin D1, cyclin G, TGF-α and 14-3-3σ), DNA repair (Gadd45, PCNA and $p21^{Waf1/Cip1}$) and apoptosis (Bax, Bcl-XL, Fas, IGFBP3, PAG6O8 and DR5; Amundson et al., 1998).

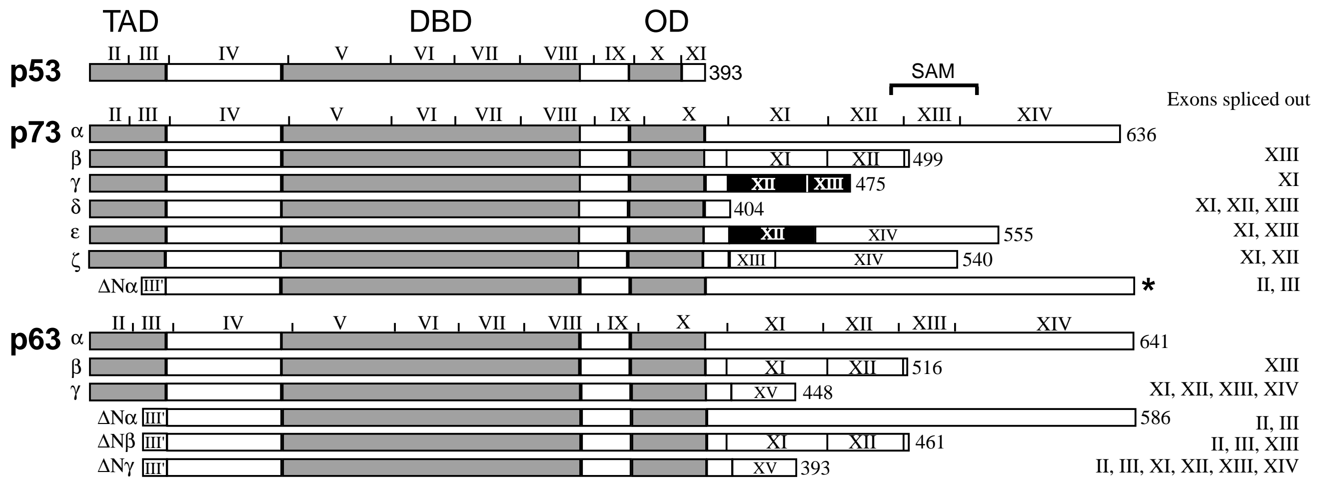


Fig. 1. Schematic representation of the exon structure of p53, p63 and p73. The transactivation domain (TAD), DNA-binding domain (DBD) and the oligomerization domain (OD) are indicated in grey; the sterile alpha motif (SAM) domain is also indicated. The number of the exons is indicated in Roman letters above each full length protein. Exons read out of frame with the α isoform are in black. Exons spliced out in each isoform are indicated on the right. The ΔNp63 and ΔNp73 variants lacking the TAD, which are translated from an alternative ATG are indicated in dotted grey. (*) ΔNp73 was described only in mouse.

Recently, Yu et al. (1999) identified several new potential transcriptional targets of p53. They observed a marked heterogeneity in the extent, timing and p53 dependence. Even in tumor cells derived from the same stem cell type, the transcriptional responses to p53 vary considerably, according to the cell type and the stimulus that is applied (Yu et al., 1999). Only a few genes were induced in all cell types (class I), whereas some were induced in a subset of cell lines but not in others (class II). Yu et al. also observed important differences in the timing of induction of different genes, in their induction by chemotherapeutic drugs and, finally, in their inducibility by p73. Indeed, although the DNA-binding domains of p63 and p73 interact with the consensus p53-responsive sequences, and both p63 and p73 activate the promoters of several p53-responsive genes in cotransfection experiments (including *p21$^{Waf1/Cip1}$*, *bax*, *mdm2*, *cyclin-G*, *GADD45*, *IGFBP3* (A) and *IGFBP3*; Kaghad et al., 1997; Jost et al., 1997; De Laurenzi et al., 1998; Dobbelstein and Roth, 1998; Osada et al., 1998; Takada et al., 1999; Yang et al., 1998; Lee and La Thangue, 1999; Zheng et al., 1999), growing evidence indicates that many target genes respond differently to the different family members.

Zhu et al. (1998), using cell lines that inducibly overproduce either p53, p73-α or p73-β, have detected quantitative differences between the effects of p53 and p73 isoforms on the induction of endogenous genes such as *p21$^{Waf1/Cip1}$*, *mdm2* and *GADD45* and have identified genes that are selectively induced by p53 but not by p73. Yu et al. have made similar observations and, using a serial analysis of gene expression (SAGE), have found that only six out of 36 p53-induced transcripts are significantly induced by p73-α. Note that the quantitative differences reported by Zhu et al. (1998) and Yu et al. (1999) in their system were not reproducibly observed by Lee and La Thangue (1999) in another experimental system. In conclusion, current data indicate the existence of transcription specificity among the p53-family members. It remains to be determined whether, and how, different stimuli selectively recruit one or more members of the p53 family to achieve specialized transcription responses in specific cellular contexts.

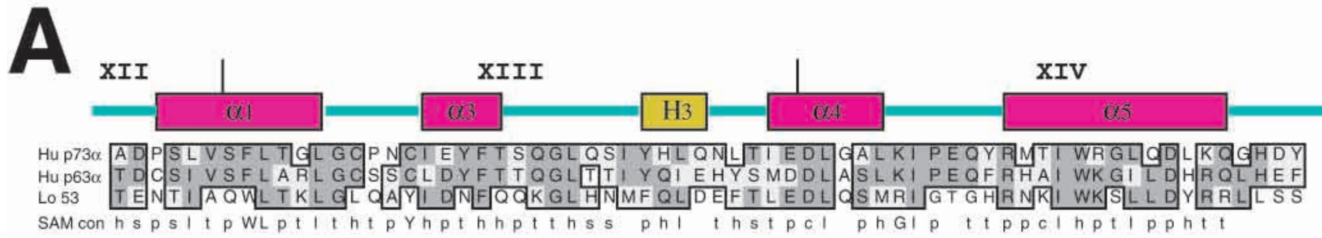
DIVERGING PATHWAYS FOR THE p53-FAMILY MEMBERS: INACTIVATION IN CANCER CELLS AND REGULATION BY PROTEIN-PROTEIN INTERACTIONS

The role of p73 in the apoptosis response to DNA damage

The central role of p53 in the cellular response to DNA damage from both endogenous and exogenous sources is well established (Gottlieb and Oren, 1998; Ko and Prives, 1996; Levine, 1997; Amundson et al., 1998; Giaccia and Kastan, 1998): activation of p53 causes induction of p21$^{Waf1/Cip1}$, which inhibits CDK-cyclin activity and thereby causes cell cycle arrest. This allows DNA repair to occur before replication or mitosis. In some cell types p53 activation leads to the elimination of irreparably damaged cells by apoptosis. The final outcome of p53 activation (growth arrest vs apoptosis) depends on many factors and is influenced by the pattern of the downstream effector genes transactivated by p53. In order to perform its cellular functions of growth arrest and/or apoptosis, p53 must rapidly accumulate in response to DNA damage. This rapid regulation is achieved by post-translational modifications that include both phosphorylation, acetylation and addition of SUMO polypeptides at specific sites (Giaccia and Kastan, 1998; G. De Sal and M. Levrero, personal communication).

Recent reports have shown that the p73 protein accumulates and is tyrosine phosphorylated in response to DNA-damaging agents (reviewed by Levrero et al., 1999). Both the accumulation and the tyrosine phosphorylation of p73 are dependent on the activation of the nuclear c-Abl tyrosine kinase (Levrero et al., 1999; Shaul, 2000). Interestingly, different types of DNA-damage inducers appear to affect p73 in different ways.

Cisplatin, which crosslinks DNA (Gong et al., 1999), and taxol, an agent that stabilizes microtubules and prevents completion of mitosis (A. Costanzo et al., unpublished) induce p73 accumulation and increase its half-life. In human colon cancer cells and in mouse embryo fibroblasts (MEFs), the


A
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XIII
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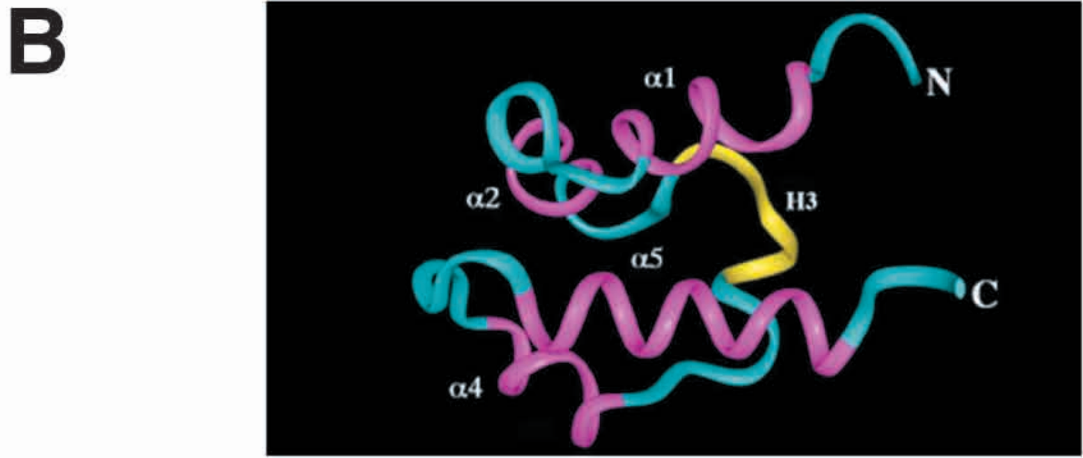
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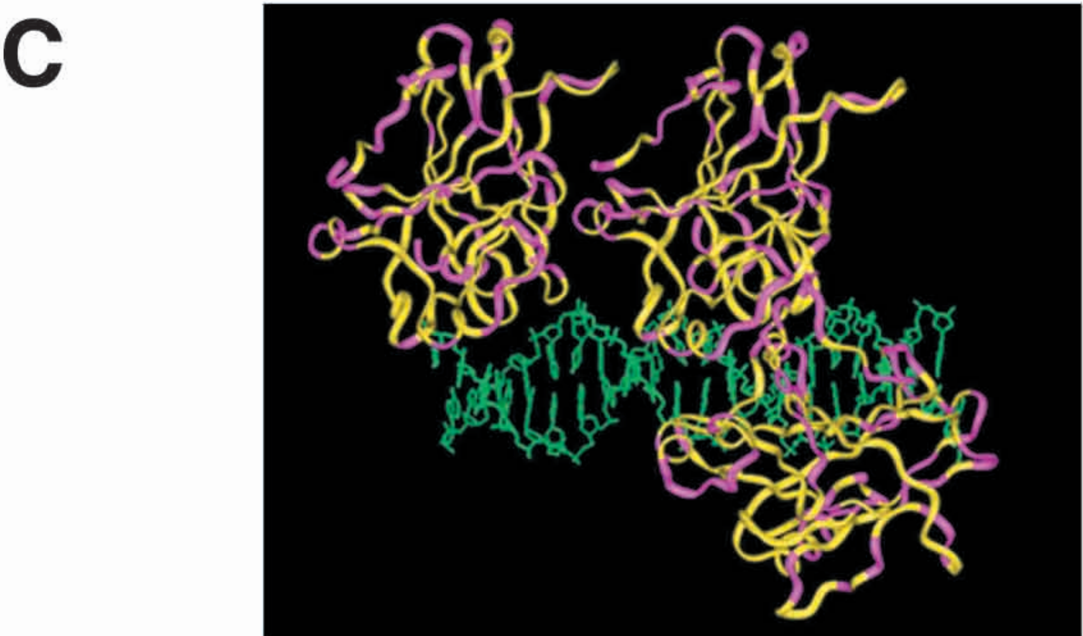
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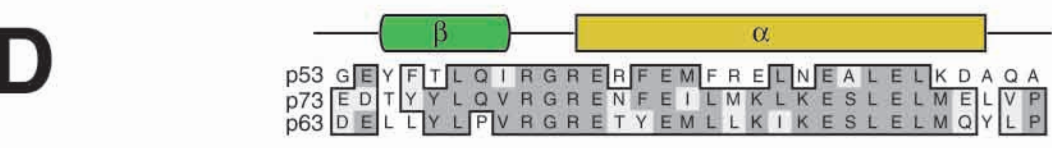
D
β
α
p53 G E Y F T L Q I R G R E R F E M F R E L N E A L E L K D A Q A
p73 E D T Y Y L Q V R G R E N F E I L M K L K E S L E L M E L V P
p63 D E L L Y L P V R G R E T Y E M L L K I K E S L E L M Q Y L P

mismatch-repair protein MLH1 and the tyrosine kinase c-Abl are required for cisplatin to induce p73, whereas taxol requires c-Abl but not MLH1 (A. Costanzo and J. Gong, unpublished). The c-Abl tyrosine kinase can prolong the half-lives of p73-α, p73-β and p73-δ in transient coexpression experiments (Gong et al., 1999; J. Gong et al., unpublished). By contrast with cisplatin (Gong et al., 1999) and taxol (A. Costanzo et al., unpublished), ultraviolet light (UV), actinomycin D, ionizing irradiation (IR), and methylmethane sulfonate (MMS) do not induce the accumulation of p73 in several cell types. Therefore, the cell must discriminate between different DNA-damage inducers, and p73 accumulation occurs only in response to specific stimuli.

The p73 protein can be phosphorylated by the c-Abl tyrosine kinase in vitro or under transient coexpression conditions (Agami et al., 1999; Yuan et al., 1999). Tyrosine phosphorylation of p73 is observed in MCF7 cells treated with a high dose of IR (20 Gy) two hours after treatment (Agami et al., 1999; Yuan et al., 1999; Shaul, 2000); This indicates that IR induces tyrosine phosphorylation of p73 through activation of c-Abl. Activation of c-Abl and tyrosine phosphorylation of p73 after IR treatment require a functional ATM kinase (Yuan et al., 1999). The kinetics of activation of c-Abl by IR are rapid, occurring within 30 minutes of irradiation, whereas the kinetics of c-Abl activation by cisplatin or taxol are much slower, occurring after 6-18 hours (Liu et al., 1996; Baskaran et al., 1997; Gong et al., 1999; M. Falco and A. Costanzo, unpublished). Indeed, Agami et al. (1999) observed tyrosine phosphorylation of p73 but no change in steady-state levels of p73 2 hours after IR of MCF-7 cells; however, cisplatin or taxol caused a sustained activation of c-Abl and stabilization of p73. The kinetics of c-Abl activation and p73 stabilization also correlate with the apoptotic response of cells. Stabilization of p73 in response to cisplatin or taxol is maximal when cells start to die, whereas tyrosine phosphorylation after IR occurs well before cells undergo apoptosis. Further studies must assess whether these observations are relevant to the regulation of p73-dependent apoptosis. Note that ionizing radiation is a potent inducer of apoptosis in some cell types (e.g. thymocytes) but not in others (e.g. fibroblasts). 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 in all cell types. In conclusion, the available evidence suggests that c-Abl can either stabilize or phosphorylate p73, depending on the DNA-damage inducer and the cell type studied.

Additional evidence to link activation of p73 by c-Abl to apoptosis comes from overproduction experiments. Exogenously expressed p73-α or p73-β can induce apoptosis in p53-deficient cells but does not kill c-Abl-deficient 3T3 cells efficiently (Gong et al., 1999; Agami et al., 1999; Yuan et al., 1999). The apoptotic function can be rescued by co-expression

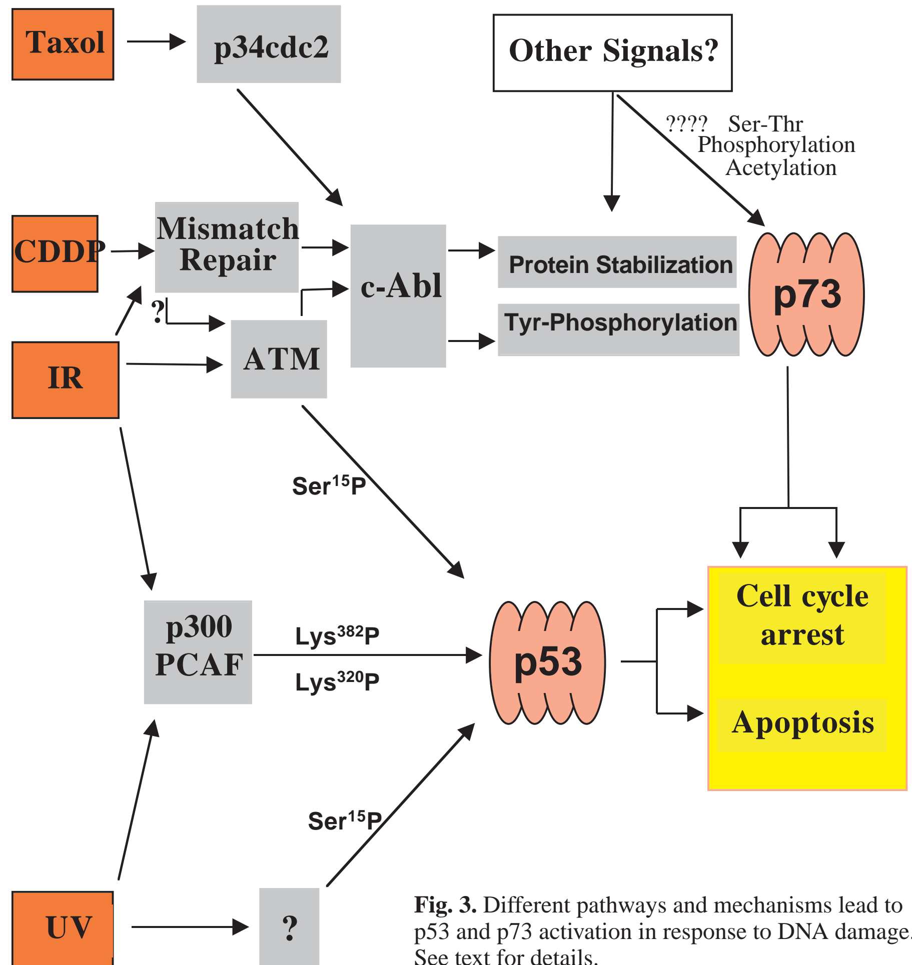

Fig. 3. Different pathways and mechanisms lead to p53 and p73 activation in response to DNA damage. See text for details.

Fig. 2. Structural domains of p73. (A) Alignment of the sterile alpha motifs (SAMs) of p73, p63 and squid (*Loligo*) p53. The exons are indicated in Roman numerals. The five helical regions are indicated: four are α-helices; one is a 3_{10}-helix (H). The SAM consensus sequence for the p53 family is also indicated [h, hydrophobic; l, aliphatic; p, polar; c, charged; s, small; t, turnlike (tiny or polar)]. The hydrophobic residues that form the hydrophobic core of p73 (487-554) align with hydrophobic residues in a consensus sequence that was derived from a multiple alignment of >40 members of the SAM superfamily (Schultz, 1997; Arrowsmith 1999). The pattern of surface residues of p73 (487-554) is most similar to that of p63 and unrelated to that of the Eph receptors. Therefore, the p73/p63 C-terminal domain might constitute a unique subclass of human SAM-like domain (Arrowsmith, 1999). (B) A backbone representation of the 3-D structure of the SAM region of human p73α; this structure was resolved by NMR by Chi et al. (1999). (C) Computerized homology modeling of the DBD of p73 based on the p53/DNA complex crystal structure (Cho et al., 1994). Identical residues are in red, indicating an extremely close structure and DNA interaction. In particular, the residues that control the protein-DNA interactions are identical. Three molecules are shown binding to DNA. (D) Alignment of the OD region indicating conservation both of the α-helices (α) and of the β-sheet (β). More importantly, the connection between the β and α regions is identical (RGRE) which indicates that they have an identical tertiary orientation. Dark shading indicates conserved residues; light shading indicates residues of similar chemical character.

Table 1. Mutation analysis for *p73* mutations in cancer

Tumor	Mutations*	LOH	References
Neuroblastoma	2/209 (P405R, P425L)	30/89	Kovalev et al., 1998; Ichiyama et al., 1999; Ejeskar et al., 1999; Han et al., 1999
Lung cancer	0/65		Nomoto et al., 1998 Mai et al., 1998
Oligodendroglioma	0/20		Mai et al., 1998
Colorectal cancer	0/125	8/46	Sunahara et al., 1998 Han et al., 1999
Esophageal cancer	0/48	2/25	Nimura et al., 1998
Prostate cancer	0/133	2/38	Takahashi et al., 1998 Yokomizo et al., 1999
Melanoma	0/17		Kroiss et al., 1998 Tsao et al., 1999
Hepatocellular cancer	0/48	5/25	Mihara et al., 1999
Brest cancer	1/142 (R269Q)	6/46	Zaika et al., 1999 Shishikura et al., 1999 Han et al., 1999
Bladder cancer	0/23		Yokomizo et al., 1999
Gastric cancer	0/43	12/32	Han et al., 1999 Yokozaki et al., 1999
TOTAL	3/873	65/301	

*The amino acid substitution is indicated. Frequency is calculated from the sum of the indicated references.

of wild-type c-Abl (Gong et al., 1999; Agami et al., 1999; Yuan et al., 1999). This indicates that accumulation of p73 above a threshold and/or tyrosine phosphorylation of p73 is required for induction of apoptosis by p73 in these assays. The strongest evidence that p73 can contribute to DNA-damage-induced apoptosis in vivo is provided by the link between the mismatch-repair system, the induction of p73 and the sensitivity of cells to cisplatin (Gong et al., 1999). Induction of p73, but not p53, by cisplatin is dependent on mismatch repair not only in the colon cancer cell line HCT116 but also in mouse embryo fibroblasts (Gong et al., 1999). Although resistance of mismatch-repair-deficient cells to cisplatin correlates with their inability to activate the c-Abl/p73 apoptosis function, experiments performed in cells with a defined genetic background that both p53 and p73 contribute to the apoptotic response to cisplatin (Gong et al., 1999; Fig. 3).

The *p73* gene in human cancers

The high homology shared by p73 and p53 and the observation that p73 maps to chromosome 1p36.1, a region frequently deleted in several tumors, including neuroblastoma, colorectal cancer and breast cancer (Kaghad et al., 1997; reviewed by Ikawa et al., 1999), initially suggested that p73 is a tumor suppressor. A high frequency of loss of heterozygosity for p73 has been observed in neuroblastoma (Ichimiya et al., 1999; Ejeskar et al., 1999), lung cancer (Nomoto et al., 1998), gastric cancer (Yokozaki 1999,), and ovarian cancer (Nimura et al., 1998). In almost 900 tumors studied, only three missense mutations were found, and no deletions or truncation mutants exist (see Table 1). Similarly, in the few mutation studies of *p63*, only three mutations were found in 80 non-small cell lung carcinomas (Sunahara et al., 1998; Osada et al., 1998) and none in 85 breast cancers studied (Osada et al., 1998). If we consider that *p53* is mutated in ~50% of human cancers, these data define strikingly different behaviors of p63 and p73.

Kaghad et al. (1997) have suggested that p73 is an imprinted gene expressed only by one allele, preferentially of maternal origin; the loss of a single copy would therefore be sufficient for loss of function and predispose the cell to cancer development. More-recent studies seem to show that monoallelic expression of this gene is rare (Nomoto et al., 1998; Yokomizo et al., 1999; Zaika et al., 1999; Yokozaki et al., 1999), and the matter remains controversial. The scenario is even more complicated in that various tumors express higher levels of p73 than does the tissue from which they originated. These tumors include neuroblastoma (Kovalev, 1998), lung cancer (Mai et al., 1998), colorectal cancer (Sunahara et al., 1998), breast cancer (Zaika et al., 1999), bladder cancer (Yokomizo et al., 1999), hepatocellular carcinoma (Tannapfel et al., 1999a) and liver cholangiocarcinoma (Tannapfel et al., 1999b). In the case of hepatocellular carcinoma, patients that have p73-positive tumors have a poorer prognosis, compared with the patients that have p73-negative tumors (Tannapfel et al., 1999a). These results are difficult to reconcile with our current understanding of p73 function. Nevertheless, they suggest that altered expression of the *p73* gene rather than a loss of function is involved in tumorigenesis.

Interestingly, we and others have detected abnormal splicing variants of *p73* in different human cancers and cancer-derived cell lines (V. De Laurenzi et al., unpublished observations; Zaika et al., 1999; Casciano et al., 1999). Casciano et al. have described a *p73* variant mRNA that lacks exon II and is expressed in neuroblastomas; if translated in vivo, the protein would lack the transactivating domain. Interestingly, the pattern of splicing variants found was different in different subareas of the same neuroblastoma tumor; this suggests that p73 heterogeneity reflects biological heterogeneity of the tumor. These results indicate that instability in the splicing of *p73* exons occurs in cancer cells, but its role is unclear. Whether some of these variants function in vivo as dominant negative molecules or interfere with the transcription machinery is not known.

Interactions between p73 and viral oncoproteins

Oncoproteins encoded by certain DNA tumor viruses inhibit the function of p53 (see for review Ko and Prives, 1996; Levine, 1997; Choisy-Rossi and Yonish-Rouach, 1998; el-Deiry, 1998; Prives and Hall, 1999; Oren, 1999). The adenovirus E1B 55-kDa protein and the SV40 T antigen bind to p53 and sequester it into an inactive complex. The human papillomavirus E6 protein interacts with p53 and promotes its ubiquitin-dependent degradation. Several studies have examined the interaction of p73 with these viral proteins. The adenovirus E1B 55-kDa protein does not interact with p73 (α or β; Higashino et al., 1998; Marin et al., 1998; Steegenga et al., 1999) and, as expected, inhibits the transactivating function of p53 but not p73. The SV40 T-antigen does not interact with p73-α or p73-β and does not inhibit the transactivation function of p73 (α or β; Marin et al., 1998; Dobbelstein and Roth, 1998). E6 has no effect on the stability of p73-α or p73-β (Marin et al., 1998; Prabhu et al., 1998), and overproduction of the p73-β protein in E6-expressing cells leads to growth inhibition and apoptosis (Prabhu et al., 1998). Therefore, three viral oncoproteins known to inhibit p53 (i.e. HPV-16 E6, SV40 T-antigen and E1B 55-kDa) do not inhibit p73.

The finding that the adenovirus E4orf6 protein inhibits the transactivation and the apoptotic functions of p73 (α or β;

Steegenga et al., 1999; Higashino et al., 1998) suggests that oncogenic viruses inactivate p73 by using other viral proteins. E4orf6 binds to a C-terminal region common to p73 α and β (Higashino et al., 1998). Given that it binds to a region of p53 encompassed by residues 318-360 (Dobner et al., 1996), it might bind to the equivalent region of p73 (Higashino et al., 1998). Moreover, the p73 protein, like p53, is upregulated upon adenovirus infection, which suggests that inhibition of cellular p73 is important for adenovirus infection (Steegenga et al., 1999). The E1A and E1B 55-kDa proteins, but not the small E1B protein or E6orf4, increase the steady-state levels of both endogenous p73 (Steegenga et al., 1999) and p53. E1A-induced accumulation of p53 protein is mediated by induction of p19Arf, which interferes with the Mdm2-mediated degradation of p53 (Tao and Levine, 1999; Zhang and Xiong, 1999). The E1B 55-kDa protein inhibits the transactivating functions of p53 and down regulates Mdm2. However, Mdm2 does not affect the stability of p73 (see below), and the induction of p73 protein by E1A and E1B 55-kDa is probably mediated by other, as-yet-unknown, mechanisms. The inactivation of p73 by E4orf6 is likely to be important for suppression of the death of viral-infected cells in which E1A and E1B 55-kDa have induced the accumulation of p73. Although currently available data are consistent with a role for p73 in the cellular response to adenovirus infection, elucidation of the interplay between p73 and adenoviruses or other DNA tumor viruses require further investigation.

Regulation of p73 and p53 by cellular proteins

Several recent reports have examined the regulation of p73-α and p73-β by the Mdm2 and p300/CBP families of cellular proteins, which are known to regulate p53 protein stability and transcriptional activity (reviewed by Lohrum and Vousden 1999). Similarities and differences between p53 and p73 have been found, and the emerging picture is one in which parallel and independently regulated pathways regulate p53 and p73.

Mdm2/MdmX

The p53 protein accumulates dramatically in response to different cellular stresses, including DNA damage, hypoxia and depletion of the ribonucleotide triphosphate pools (see Levine, 1997, Giaccia and Kastan, 1998). p53 levels are regulated mainly at the level of protein stability. p53 is both neutralized and targeted for degradation by the products of the murine *Mdm2* gene and its human homologue *Hdm2* (Lohrum and Vousden, 1999; Oren, 1999), which itself is a p53-target gene. The p73 proteins α and β bind to Mdm2 through their N-terminal region, which is highly homologous to the p53 N-terminal, Mdm2-binding region (Balint et al., 1999; Ongkeko et al., 1999; Zeng et al., 1999), and alternative splicing of the p73 C terminus should not affect the binding of p73 isoforms to Mdm2. The p73 protein also binds to MdmX, a protein related to Mdm2 (Ongkeko et al., 1999). The interaction with Mdm2 or MdmX leads to the inactivation of transcription and apoptosis functions of p73-α and p73-β (Zeng et al., 1999) but does not result in the rapid degradation of p73 protein by the proteasome (Balint et al., 1999; Lee and La Thangue, 1999; Ongkeko et al., 1999; Zeng et al., 1999). Mdm2 stimulates ubiquitination of p53 and its degradation by the proteosome. p73 stability is also controlled by the proteosome, and treatment of cells with inhibitors of the proteosome (LLnL and lactocystin) results in the accumulation of p73 protein (Balint et al., 1999; Lee and La Thangue, 1999). However, whereas the accumulation of p53, induced by DNA damage, requires disruption of the Mdm2-p53 interaction, Mdm2 does not shorten the half-life of p73. Disruption of the Mdm2-p73 interaction is needed for stimulation of transcription and apoptosis by p73 but does not affect the stability of p73.

Accumulation of p73 protein in response to certain DNA-damaging stimuli relies on the activity of the c-Abl tyrosine kinase, which prolongs the half-life of p73 (Gong et al., 1999). Whether c-Abl can disrupt the interaction between Mdm2 and p73 is not known. The interaction between p53 and Mdm2 can be disrupted through phosphorylation of p53 at Ser15 (Prives and Hall, 1999), a residue that is not conserved in p73. Considering that c-Abl tyrosine kinase activates the apoptotic function of p73 (α and β) but not that of p53 (Gong et al., 1999), it appears that the accumulation of p53 and p73 in response to certain stimuli occurs through parallel independent pathways. The accumulation of p53 is regulated by the inhibition of its degradation (Mdm2 removal), whereas the accumulation of p73 requires the activation of a stabilizing protein (the c-Abl kinase). The role of Mdm2 in the regulation of p73 function under physiological conditions remains to be determined.

p300/CBP

The interaction of p53 with the transcription adaptors/co-activators p300 and CBP regulates the ability of p53 to bind to its cognate DNA sequences and to activate transcription. Unlike p53, which binds to the C terminus of p300, p73 binds to the N-terminal CH1 domain through its N-terminal region (residues 1-56; Lee and Thangue, 1999; Zeng et al., 1999, 2000). The interaction with p300/CBP is required for p73 to activate transcription and induce apoptosis, and Mdm2 inhibits the transactivating function of p73 by excluding p300/CBP (Zeng et al., 1999). Whether p73 is acetylated by p300/CBP and how p73 functions are regulated by the acetyltransferase activity of p300/CBP remain to be clarified.

INVOLVEMENT OF p63 AND p73 IN DIFFERENTIATION AND DEVELOPMENT

The homology shared by p53, p63 and p73 has suggested that the products of this gene family share similar functions as transcription factors. However, not only might the spectrum of target genes activated by the different members of the family be different (Zhu et al., 1998; Yu et al., 1999) but their biological functions are also likely to be distinct. It is still quite unclear whether the role of p73 in the induction of cell cycle arrest or apoptosis is physiological. Induction of p73 surely contributes to but does not determine the apoptosis response to cisplatin (Gong et al., 1999), and the induction of p73 under conditions in which there are mitotic spindle defects (A. Costanzo et al., unpublished) does not appear to contribute significantly to apoptosis (J. Gong et al., unpublished observations). Moreover, despite the original hypothesis that *p73* is a tumor suppressor gene that is lost in neuroblastomas, *p73* mutations are not found in human cancers, and the same seems to be true for *p63*. All these considerations and several recent findings suggest that both p63 and p73 do not play roles

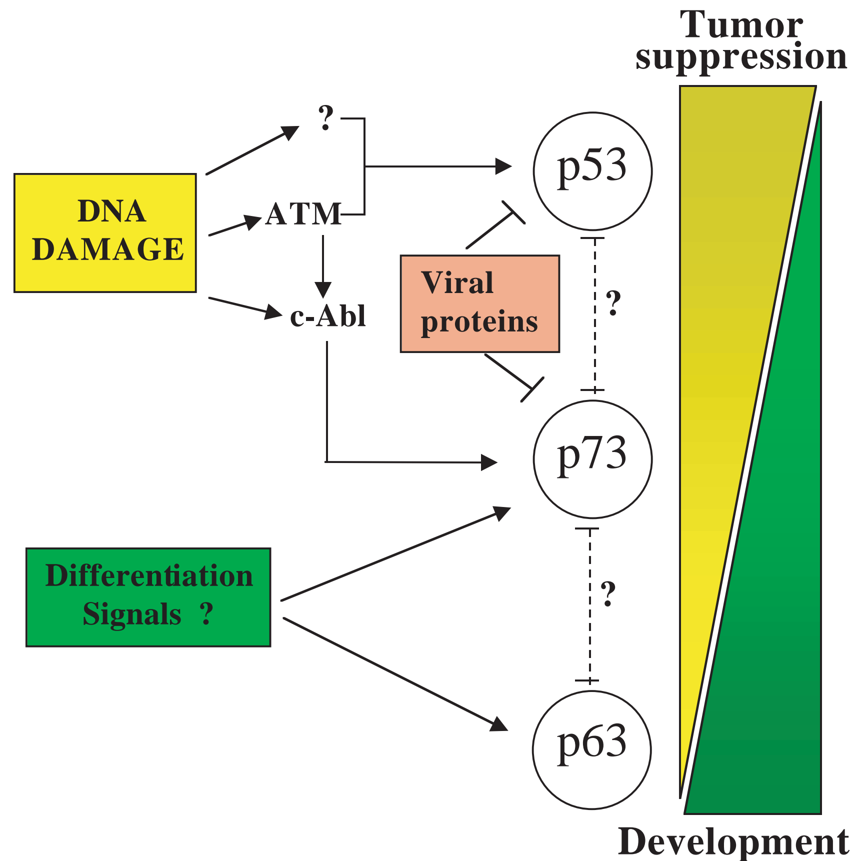

Fig. 4. The p53-family of transcription factors have overlapping as well as distinct biological functions. p53 and p73 are activated by DNA damage through different signaling pathways to induce either growth arrest or apoptosis. However the role of p73 as a tumor suppressor is still to be proven. Both p63 and p73 are recruited during development and differentiation but the stimuli involved are not known.

similar to those of p53 in the development of cancer.

An important line of evidence supporting the proposal that p53-family members have distinct biological roles has come from genetic analyses in mice. The *p63*-deficient mutant mice differentiate abnormally but do not show any defect related to spontaneous or X-ray-induced apoptosis during embryogenesis. In the *p63*-deficient mice, the apical ectodermal ridge essential to limb development is defective, and the mice have truncated limbs. In addition, *p63*$^{-/-}$ mice have no hair follicles, no teeth, no mammary, lachrymal or salivary glands (Mills et al., 1999; Yang et al., 1999). Thus, the phenotype of *p63*-deficient mice suggests that the primary biological function of p63 proteins is to regulate development. Recently, mutations of *p63* have been detected in nine children affected by ectrodactyly, ectodermal dysplasia and facial clefts (EEC syndrome; Celli et al., 1999). Celli et al. found that heterozygous germline mutations or frameshifts in *p63* cause the disease; the mutated proteins cannot transactivate, acting as dominant negatives. We have recently observed that the ectopic expression of *p63* in normal human epidermal keratinocytes upregulates markers specific for keratinocyte differentiation (i.e. loricrin, involucrin and transglutaminase 1; V. De Laurenzi et al., unpublished). The phenotype of *p63*$^{-/-}$ mice (as well as the data from EEC patients) is strikingly different from what is observed in the *p53*-null mice. The latter develop normally but develop multiple types of tumor with a much higher frequency than their wild-type counterparts. In addition cells derived from the *p53*-null mice have defective cell cycle checkpoints and defective apoptotic responses to DNA damage (Donehower et al., 1992, 1995).

Similarly to *p63*$^{-/-}$ animals, *p73*$^{-/-}$ mice show developmental defects (Yang et al., 2000). Indeed, *p73*$^{-/-}$ mice show congenital hydrocephalus, hippocampal dysgenesis, due to disappearance of Cajal-Retzius neurons, and defects of pheromone detection that lead to lack of interest in sexually mature females. In addition to these severe neurological defects, *p73*$^{-/-}$ mice show a generalized pan-mucositis, with consequent microbiological infections, which are characterized by massive neutrophil infiltration at the affected sites. The pathogenesis of this massive inflammation, however, is not clear yet, since no major defects of the lymphoid and granulocyte populations are present in these mice; this raises the possibility that the inflammation and infections are related to the epithelial barrier function – as in cystic fibrosis. Interestingly, *p73*$^{-/-}$ mice did not show evident development of spontaneous tumors up to 15 months; however, considering the mild oncogenic potential of *p53*$^{-/-}$ mice, it is still early to draw in vivo conclusions on the involvement of this gene in cancer (Yang et al., 2000).

In agreement with the neurological defects found in the *p73*$^{-/-}$ mice, we have found that the ectopic expression of *p73* in undifferentiated neuronal cells (neuroblastoma) induces neurite outgrowth and expression of neuronal differentiation markers (De Laurenzi et al., 2000). Interestingly, induction of differentiation by retinoids in the same neuroblastoma cells is accompanied by accumulation of p73, which is due to an increase in *p73* transcription. p73 transactivates the *NCAM* and *p21* promoters, thus upregulating the steady-state levels of NCAM, p21 and neurofilaments. Moreover, transfection of dominant negative p73 in neuroblastoma cells abrogates the transactivation of the *NCAM* promoter induced by retinoids, a well known trigger of neuronal differentiation in these cells (De Laurenzi et al., 2000). Similarly, exogenously expressed p73 induces expression of the same differentiation markers as p63 in normal human keratinocytes. Indeed, the loricrin, involucrin and transglutaminase 1 promoters can be activated by p73 as well as p63 (V. De Laurenzi et al., unpublished).

Finally, the levels of both *p63* (Ikawa et al., 1999) and *p73* (F. Cimino and M. Levrero, unpublished observations) transcripts increase in C2C12 skeletal muscle cells induced to differentiate in vitro. These preliminary observations need to be confirmed and expanded but clearly indicate that p73 and p63 play roles in differentiation. Future work will elucidate what physiological signals recruit p63 and p73 to the developmental programme.

In conclusion, current available information suggests that the members of the p53 family of transcription factors have overlapping as well as distinct biological functions. Both p53 and p73 are induced by cisplatin but different signaling pathways seems to be used. A picture is emerging in which p53, p73 and p63 are part of a gradient of functions ranging from tumor suppression to development. The existence of distinct regulatory mechanisms selectively recruited by different stimuli provides the basis for their distinct biological effects (Fig. 4).

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REFERENCES

Agami, R., Blandino, G., Oren, M. and Shaul, Y. (1999). Interaction of c-Abl and p73 and their collaboration to induce apoptosis. *Nature* **399**, 809-813.

Amundson, S. A., Myers, T. G. and Fornace, A. J. Jr (1998). Roles for p53 in growth arrest and apoptosis: putting on the brakes after genotoxic stress. *Oncogene* **17**, 3287-3299.

Arrowsmith, C. H. (1999). Structure and function in the p53 family. *Cell Death Differ.* **6**, 1169-1173.

Balint, E., Bates, S. and Vousden, K. H. (1999). Mdm2 binds p73 alpha without targeting degradation. *Oncogene* **18**, 3923-3929.

Baskaran, R., Wood, L. D., Whitaker, L. L., Canman, C. E., Morgan, S. E., Xu, Y., Barlow, C., Baltimore, D., Wynshaw-Boris, A., Kastan, M. B. and Wang, J. Y. (1997). Ataxia telangiectasia mutant protein activates c-Abl tyrosine kinase in response to ionizing radiation. *Nature* **387**, 516-519.

Casciano, I., Ponzoni, M., Cunsolo, C., Tonini, G. and Romani, M. (1999). Different p73 splicing variants are expressed in distinct tumour areas of a multifocal neuroblastoma. *Cell Death Differ.* **6**, 391-393.

Celli, J., Duijf, P., Hamel, B. C. J., Bamshad, M., Kramer, B., Smits, A. P. T., Newbury-Ecob, R., Hennekam, R. C. M., Van Buggenhout, G., Van Haeringen, A., Woods, C. G., Van Essen, A. J., De Waal, R., Vriend, G., Haber, D. A., Yang, A., McKeon, F., Brunner, H. G. and Van Bokhoven, H. (1999). Heterozygous germline mutations in the p53 homolog p63 are the cause of EEC syndrome. *Cell* **99**, 143-153.

Chi, S.-W., Ayeda, A. and Arrowsmith, C. H. (1999). Solution structure of a conserved C-terminal domain of p73 with structural homology to the SAM domain. *EMBO J.* **18**, 4438-4445.

Cho, Y., Gorina, S., Jeffrey, P. D. and Pavletich, N. P. (1994). Crystal structure of a p53 tumor suppressor-DNA complex: understanding tumorigenic mutations. *Science* **265**, 346-355.

Choisy-Rossi, C. and Yonish-Rouach, E. (1998). Apoptosis and the cell cycle: the p53 connection. *Cell Death Differ.* **5**, 129-131.

Davison, T. S., Vagner, C., Kaghad, M., Ayed, A., Caput, D. and Arrowsmith, C. H. (1999). p73 and p63 are homotetramers capable of weak heterotypic interactions with each other but not with p53. *J. Biol. Chem.* **274**, 18709-18714.

De Laurenzi, V., Costanzo, A., Barcaroli, D., Terrinoni, A., Falco, M., Annicchiarico-Petruzzelli, M., Levrero, M. and Melino, G. (1998). Two new p73 splice variants, gamma and delta, with different transcriptional activity. *J. Exp. Med.* **188**, 1763-1768.

De Laurenzi, V., Catani, M. V., Costanzo, A., Terrinoni, A., Corazzari, M., Levrero, M., Knight, R. A. and Melino, G. (1999). Additional complexity in p73: induction by mitogens in lymphoid cells and identification of two new splicing variants epsilon and zeta. *Cell Death Differ.* **6**, 389-390.

De Laurenzi, V., Raschella', G., Barcaroli, D., Annicchiarico-Petruzzelli, M., Ranalli, M., Catani, M. V., Tanno, B., Costanzo, A., Levrero, M. and Melino, G. (2000). Induction of neuronal differentiation by p73, in a neuroblastoma cell line. *J. Biol. Chem.* (in press).

Di Como, C. J., Gaiddon, C. and Prives, C. (1999). p73 function is inhibited by tumor-derived p53 mutants in mammalian cells. *Mol. Cell. Biol.* **19**, 1438-1449.

Ding, Y., Inoue, T., Kamiyama, J., Tamura, Y., Ohtani-Fujita, N., Igata, E. and Sakai, T. (1999). Molecular cloning and functional characterization of the upstream promoter region of the human p73 gene. *DNA Res.* **6**, 347-351.

Dobbelstein, M. and Roth, J. (1998). The large T antigen of simian virus 40 binds and inactivates p53 but not p73. *J. Gen. Virol.* **79**, 3079-3083.

Dobner, T., Horikoshi, N., Rubenwolf, S. and Shenk, T. (1996). Blockage by adenovirus E4orf6 of transcriptional activation by the p53 tumor suppressor. *Science* **272**, 1470-1473.

Donehower, L. A., Harvey, M., Slagle, B. L., McArthur, M. J., Montgomery, C. A. Jr, Butel, J. S., Bradley. A. (1992). Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature* **356**, 215-221

Donehower, L. A., Godley, L. A., Aldaz, C. M., Pyle, R., Shi, Y. P., Pinkel, D., Gray, J., Bradley, A., Medina, D. and Varmus, H. E. (1995). Deficiency of p53 accelerates mammary tumorigenesis in Wnt-1 transgenic mice and promotes chromosomal instability. *Genes Dev.* **9**, 882-895.

Ejeskar, K., Sjoberg, R. M., Kogner, P. and Martinsson, T. (1999). Variable expression and absence of mutations in p73 in primary neuroblastoma tumors argues against a role in neuroblastoma development. *Int. J. Mol. Med.* **3**, 585-589.

el-Deiry, W. S. (1998). Regulation of p53 downstream genes. *Semin. Cancer Biol.* **8**, 345-357.

Ferreira, A. and Kosik, K. S. (1996). Accelerated neuronal differentiation induced by p53 suppression. *J. Cell Sci.* **109**, 1509-1516.

Giaccia, A. J. and Kastan, M. B. (1998). The complexity of p53 modulation: emerging patterns from divergent signals. *Genes Dev.* **12**, 2973-2983.

Gong, J. G., Costanzo, A., Yang, H. Q., Melino, G., Kaelin, W. G., Levrero, M. and Wang, J. Y. J. (1999). The tyrosine kinase c-Abl regulates p73 in apoptotic response to cisplatin-induced DNA damage. *Nature* **39**, 806-809.

Gottlieb, T. M. and Oren, M. (1998). p53 and apoptosis. *Semin. Cancer Biol.* **8**, 359-368.

Han, S. Semba, S., Abe, T., Makino, N., Furukawa, T., Fukushige, S., Takahashi, H., Sakurada, A., Sato, M., Shiiba, K., Matsuno, S., Nimura, Y. and Nakagawara, A. (1999). Infrequent somatic mutations of the p73 gene in various human cancers. *Eur. J. Surg. Oncol.* **25**, 194-198.

Higashino, F., Pipas, J. M. and Shenk, T. (1998). Adenovirus E4orf6 oncoprotein modulates the function of the p53-related protein, p73. *Proc. Nat. Acad. Sci. USA* **95**, 15683-15687.

Hollstein, M., Sidransky, D. and Vogelstein, C. C. (1991). p53 mutations in human cancers. *Science* **253**, 49-53.

Hollstein, M., Shomer, B., Greenblatt, M., Soussi, T., Hovig, E., Montesano, R. and Harris, C. C. (1996). Somatic point mutations in the p53 gene of human tumors and cell lines: updated compilation. *Nucl. Acids Res.* **24**, 141-146.

Ichimiya, S., Nimura, Y., Kageyama, H., Takada, N., Sunahara, M., Shishikura, T., Nakamura, Y., Sakiyama, S., Seki, N., Ohira, M., Kaneko, Y., McKeon, F., Caput, D,. and Nakagawara, A. (1999). p73 at chromosome 1p36. 3 is lost in advanced stage neuroblastoma but its mutation is infrequent. *Oncogene* **18**, 1061-1066.

Ikawa, S., Nakagawara, A. and Ikawa, Y. (1999). p53 family genes: structural comparison, expression and mutation. *Cell Death Differ.* **6**, 1154-1161.

Jost, C. A., Marin, M. C. and Kaelin, W. G. Jr. (1997). p73 is a human p53 related protein that can induce apoptosis. *Nature* **389**, 191-194.

Kaghad, M., Bonnet, H., Yang, A., Creacier, L., Biscan, J. C., Valent, A., Minty, A., Chalon, P., Lellas, J. M., Dumont, X., Ferrara, P., McKeon, F. and Caput, D. (1997). Monoallelically expressed gene related to p53 at 1p36, a region frequentely deleted in neuroblastoma and other human cancers. *Cell* **90**, 809-819.

Ko, L. J. and Prives, C. (1996). p53: puzzle and paradigm. *Genes Dev.* **10**, 1054-1072.

Kovalev, S., Marchenko, N., Swendeman, S., LaQuaglia, M., Moll, U. M. (1998). Expression level, allelic origin, and mutation analysis of the p73 gene in neuroblastoma tumors and cell lines. *Cell Growth Differ.* **9**, 897-903.

Kroiss, M. M., Bosserhoff, A. K., Vogt, T., Buettner, R., Bogenrieder, T., Landthaler, M. and Stolz, W. (1998). Loss of expression or mutations in the p73 tumour suppressor gene are not involved in the pathogenesis of malignant melanomas. *Melanoma Res.* **8**, 504-509.

Lane, D. P. (1992). Cancer. p53, guardian of the genome. *Nature* **358**, 15-16.

Lee, C. W. and La Thangue, N. B. (1999). Promoter specificity and stability control of the p53-related protein p73. *Oncogene* **18**, 4171-4181.

Levine, A. J. (1997). p53, the cellular gatekeeper for growth and division. *Cell* **88**, 323-331.

Levrero, M., De Laurenzi, V., Costanzo, A., Gong, J., Melino, G. and Wang, J. Y. J. (1999). Structure, function and regulation of p63 and p73. *Cell Death Differ.* **6**, 1146-1153.

Liu, Z. G., Baskaran, R., Lea-Chou, E. T., Wood, L. D., Chen, Y., Karin, M. and Wang, J. Y. (1996). Three distinct signalling responses by murine fibroblasts to genotoxic stress. *Nature* **384**, 273-276.

Lohrum, M. A. E. and Vousden, K. H. (1999). Regulation and activation of p53 and its family members. *Cell Death Differ.* **6**, 1162-1168.

Mai, M., Qian, C., Yokomizo, A., Tindall, D. J., Bostwick, D., Polychronakos, C., Smith, D. I. and Liu, W. (1998). Loss of imprinting and allele switching of p73 in renal cell carcinoma. *Oncogene* **17**, 1739-1741.

Marin, M. C., Jost, C. A., Irwin, M. S., DeCaprio, J. A., Caput, D. and Kaelin, W. G. Jr (1998). Viral oncoproteins discriminate between p53 and the p53 homolog p73. *Mol. Cell. Biol.* **18**, 6316-6324.

Mihara, M., Nimura, Y., Ichimiya, S., Sakiyama, S., Kajikawa, S., Adachi W., Amano, J. and Nakagawara A. (1999). Absence of mutation of the p73 gene localized at chromosome 1p36. 3 in hepatocellular carcinoma. *Br. J. Cancer* **79**, 164-167.

Mills, A. A., Zheng, B., Wang, X. J., Vogel, H., Roop, D. R. and Bradley, A. (1999). p63 is a p53 homologue required for limb and epidermal morphogenesis. *Nature* **398**, 708-713.

Nimura, Y., Mihara, M., Ichimiya, S., Sakiyama, S., Seki, N., Ohira, M., Nomura, N., Fujimori, M., Adachi, W., Amano, J., He, M., Ping, Y. M. and Nakagawara, A. (1998). p73, a gene related to p53, is not mutated in esophageal carcinomas. *Int. J. Cancer* **7**, 437-440.

Nomoto, S., Haruki, N., Kondo, M., Konishi, H., Takahashi, T., Takahashi, T. and Takahashi, T. (1998). Search for mutations and examination of allelic expression imbalance of the p73 gene at 1p36. 33 in human lung cancers. *Cancer Res.* **58**, 1380-1383.

Ongkeko, W. M., Wang, X. Q., Siu, W. Y., Lau, A. W., Yamashita, K., Harris, A. L., Cox, L. S. and Poon, R. Y. (1999). MDM2 and MDMX bind and stabilize the p53-related protein p73. *Curr. Biol.* **15**, 829-833.

Oren, M. (1999). Regulation of the p53 tumor suppressor protein. *J. Biol. Chem.* **274**, 36031-36034.

Osada, M., Ohba, M., Kawahara, C., Ishioka, C., Kanamaru, R., Katoh, I., Ikawa. Y,, Nimura, Y., Nakagawara, A., Obinata, M. and Ikawa, S. (1998). Cloning and functional analysis of human p51, which structurally and functionally resembles p53. *Nature Med.* **4**, 839-843.

Prabhu, N. S., Somasundaram, K., Satyamoorthy, K., Herlyn, M. and el-Deiry, W. S. (1998). p73beta, unlike p53, suppresses growth and induces apoptosis of human papillomavirus E6-expressing cancer cells. *Int. J. Oncol.* **13**, 5-9.

Prives, C. and Hall, P. A. (1999). The p53 pathway. *J. Pathol.* **187**, 112-126.

Roth, J., Konig, C., Wienzek, S., Weigel, S., Ristea, S. and Dobbelstein, M. (1998). Inactivation of p53 but not p73 by adenovirus type 5 E1B 55-kilodalton and E4 34-kilodalton oncoproteins. *J. Virol.* **72**, 8510-8516.

Shaul, Y. (2000). c-Abl: activation and nuclear targets. *Cell Death Differ.* **7**, 10-16.

Shishikura, T., Ichimiya, S., Ozaki, T., Nimura, Y., Kageyama, H., Nakamura, Y., Sakiyama, S., Miyauchi, M., Yamamoto, N., Suzuki, M., Nakajima, N. and Nakagawara A. (1999). Mutational analysis of the p73 gene in human breast cancers. *Int. J. Cancer* **84**, 321-325.

Steegenga, W. T., Shvarts, A., Riteco, N., Bos, J. L. and Jochemsen, A. G. (1999). Distinct regulation of p53 and p73 activity by adenovirus E1A, E1B, and E4orf6 proteins. *Mol. Cell Biol*. **9**, 3885-3894.

Sunahara, M., Ichimiya, S., Nimura, Y., Takada, N., Sakiyama, S., Sato, Y., Todo, S., Adachi, W., Amano, J. and Nakagawara, A. (1998). Mutational analysis of the p73 gene localized at chromosome 1p36.3 in colorectal carcinomas. *Int. J. Oncol.* **13**, 319-323.

Takada, N., Ozaki, T., Ichimiya, S., Todo, S. and Nakagawara, A. (1999). Identification of a transactivation activity in the COOH-terminal region of p73 which is impaired in the naturally occurring mutants found in human neuroblastomas. *Cancer Res.* **59**, 2810-2814.

Takahashi, H., Ichimiya, S., Nimura, Y., Watanabe, M., furusato, M., Wakui, S., Yatani, R., Aizawa, S. and Nakagawara, A. (1998). Mutation, allelotyping, and transcription analyses of the p73 gene in prostatic carcinoma. *Cancer Res.* **58**, 2076-2077.

Tannapfel, A., Wasner, M., Krause, K., Geissler, F., Katalinic, A., Hauss, G., Mossner, J., Engeland, K. and Wittekind, C. (1999a). Expression of p73 and its relation to histopathology and prognosis in hepatocellular carcinoma. *J. Nat. Cancer Inst.* **91**, 1154-1158.

Tannapfel, A., Engeland, K., Weinans, L., Katalinic, A., Hauss, J., Mossner, J. and Wittekind C. (1999b). Expression of p73, a novel protein related to the p53 tumour suppressor p53 and apoptosis in cholangiocellular carcinoma of the liver. *Br. J. Cancer* **80**, 1069-1074.

Tao, W. and Levine, A. J. (1999). P19(ARF) stabilizes p53 by blocking nucleo-cytoplasmic shuttling of Mdm2. *Proc. Nat. Acad. Sci. USA* **96**, 6937-6941.

Tsao, H., Zhang, X., Majewski, P. and Haluska, F. (1999). Mutational and expression analysis of the p73 gene in melanoma cell lines. *Cancer Res.* **59**, 172-174.

Ueda, Y., Hijikata, M., Takagi, S., Chiba, T. and Shimotohno, K. (1999). New p73 variants with altered C-terminal structures have varied transcriptional activities. *Oncogene* **18**, 4993-4998.

Yang, A., Kaghad, M., Wang, Y., Gillett, E., Fleming, M. D., Dotsch, V., Andrews, N. C., Caput, D. and McKeon, F. (1998). p63, a p53 homolog at 3q27-29, encodes multiple products with transactivating, death-inducing, and dominant-negative activities. *Mol. Cell.* **2**, 305-316.

Yang, A., Kaghad, M., Wang, Y., Gillett, E., Fleming, M. D., Doetsch, V., Andrews, N. C., Caput, D. and McKeon, F. (1999). p63 is essential for regenerative proliferation in limb, craniofacial and epithelial development. *Nature* **398**, 714-718.

Yang, A., Walker, N., Bronson, R., Kaghad, M., Oosterwegel, M., Bonnin, J., Vagner, C., Bonnet, H., Dikkes, P., Sharpe, A., McKeon, F. and Caput, D. (2000). p73-deficient mice have neurological, pheromonal and inflammatory defects but lack spontaneous tumours. *Nature* **404**, 99-103.

Yokomizo, A., Mai, M., Tindall, D. J., Cheng, L., Bostwick, D. G., Naito, S., Smith, D. I. and Liu, W. (1999). Overexpression of the wild type p73 gene in human bladder cancer. *Oncogene* **18**, 1629-1633.

Yokozaki, H., Shitara, Y., Fujimoto, J., Hiyama, T., Yasui, W. and Tahara, E. (1999). Alterations of p73 preferentially occur in gastric adenocarcinomas with foveolar epithelial phenotype. *Int. J. Cancer* **83**, 192-196.

Yu, J., Zhang. L., Hwang, P. M., Rago, C., Kinlzer, K. W. and Vogelstein, B. (1999). Identification and classification of p53-regulated genes. *Proc. Nat. Acad. Sci. USA* **96**, 14517-14522.

Yuan, Z. M., Shioya, H., Ishiko, T., Sun, X., Gu, J., Huang, Y. Y., Lu, H., Kharbanda, S., Weichselbaum, R. and Kufe, D. (1999). p73 is regulated by tyrosine kinase c-Abl in the apoptotic response to DNA damage. *Nature* **399**, 814-817.

Zaika, A. I., Kovalev, S., Marchenko, N. D. and Moll, U. M. (1999). Overexpression of the wild type p73 gene in breast cancer tissues and cell lines. *Cancer Res.* **59**, 3257-3263.

Zeng, X., Chen, L., Jost, C. A., Maya, R., Keller, D., Wang, X., Kaelin, W. G. Jr, Oren, M., Chen, J. and Lu, H. (1999). MDM2 suppresses p73 function without promoting p73 degradation. *Mol. Cell Biol.* **5**, 3257-3266.

Zeng, X., Li, X., Miller, A., Yuan, Z., Yuan, W., Kwok, R. P., Goodman, R. and Lu, H. (2000). The N-terminal domain of p73 interacts with the CH1 domain of p300/CREB binding protein and mediates transcriptional activation and apoptosis. *Mol. Cell Biol.* **20**, 1299-1310.

Zhang, Y. and Xiong, Y. (1999). Mutations in human ARF exon 2 disrupt its nucleolar localization and impair its ability to block nuclear export of MDM2 and p53. *Mol. Cell* **5**, 579-591.

Zhu, J., Jiang, J., Zhou, W. and Chen, X. (1988). The potential tumor suppressor p73 differentially regulates cellular p53 target genes. *Cancer Res.* **58**, 5061-5065.