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# The p53 gene family

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**p73 and p63 are two recently discovered p53 homologs. Like p53, these proteins can recognize canonical p53 DNA-binding sites and, when overproduced, can activate p53-responsive target genes and induce apoptosis. Unlike p53, these genes undergo complex alternative splicing which, at least in the case of p63, yields proteins with widely divergent biological properties. In addition p73 and p63 are, in contrast to p53, rarely mutated in human cancer. Furthermore, p73 inactivation is not required for viral transformation. Thus, there is currently no firm evidence that p63 and p73 should be considered tumor suppressors. The early suggestion that monoallelic expression of p73 contributed to carcinogenesis needs to be interpreted cautiously in light of data showing interindividual and intraindividual variation with respect to monoallelic expression of p73 and the finding that p73 mRNA levels are generally increased, rather than decreased, in a host of tumors relative to normal cells.**

**Keywords:** p53; p73; p63; KET; p51; p40

## Introduction

p53 is the most intensively studied gene in molecular oncology. The considerable interest in this gene stems from the fact that most human tumors have subverted the function of the p53 protein. In most cases, this is due to mutations of the p53 gene itself. In other cases, the p53 protein is degraded at the hands of molecules such as HDM2 or the human papillomavirus E6 protein or loses the ability to respond to certain oncogenic stimuli due to loss of p14ARF. Finally, in some cases p53 is sequestered in the cytoplasm through unknown mechanisms. The importance of p53 is further underscored by the observation that germ line mutations of p53 give rise to tumors in both mice and men. Conversely, restoring p53 function in human cancer cells can induce either a cell-cycle block, apoptosis, or chemosensitization depending upon the model system under study. Any of these outcomes would be expected to translate into therapeutic benefit if achieved in human tumors *in vivo*.

The ability of p53 to act as a tumor suppressor is due, at least in part, to its ability to form homotetramers that bind to specific DNA sequences and consequently activate the transcription of target genes implicated in cell-cycle control and apoptosis. Examples of such genes include p21, 14-3-3$\sigma$, GADD45, BAX, and a family of genes involved in oxidative stress (Adams and Kaelin, 1998). A number of signals, notably DNA damage and activation of oncogenes, activate p53 (Adams and Kaelin, 1998). The latter involves the induction of p14ARF which prevents HDM2 from inhibiting p53. The former appears to involve changes in p53 phosphorylation mediated by kinases such as ATM and DNA-PK. These phosphorylation events affect the stability and/or DNA-binding capability of p53. Recent insights into the biochemical and biological properties of p53 are discussed in greater detail elsewhere in this issue.

Most genes are, in fact, members of gene families. Until recently, p53 proved a striking exception to this rule. In 1997, however, Caput and coworkers serendiptiously identified a human homolog of p53 which they called p73 (Kaghad *et al*., 1997). Shortly thereafter, several groups identified a third member of the family variably called Ket, p40, p51, p73L and p63 (Osada *et al*., 1998; Schmale and Bamberger, 1997; Senoo *et al*., 1998; Trink *et al*., 1998; Yang *et al*., 1998). This review will outline the similarities and differences between p53 and these two proteins.

## Structures of p73 and p63

Both p73 and p63 are most highly similar to p53 in the regions corresponding to the p53 N-terminal transactivation, central DNA-binding, and C-terminal oligomerization domains. In addition, the residues that directly contact DNA in the crystal structure of p53 bound to DNA are highly conserved among these proteins. In keeping with this high degree of similarity, both p73 and p63 can bind to canonical DNA binding sites, can activate transcription from p53-responsive promoters, and can induce apoptosis when overproduced in cells (De Laurenzi *et al*., 1998; Di Como *et al*., 1999; Jost *et al*., 1997; Kaghad *et al*., 1997; Osada *et al*., 1998; Yang *et al*., 1998; Zhu *et al*., 1998).

p73 and p63 are most closely related to one another than either is to p53. Remarkably, all three genes share a very similar exon/intron organization (Kaghad *et al*., 1997; Yang *et al*., 1998). Unlike p53, however, both p73 and p63 undergo complex alternative splicing (De Laurenzi *et al*., 1998; Kaghad *et al*., 1997; Kong *et al*., 1999; Osada *et al*., 1998; Yang *et al*., 1998) (see Figures 1 and 2). In addition, p63 utilizes an alternative promoter to generate proteins lacking its N-terminal transactivation domain (Yang *et al*., 1998). Intriguingly, Yang and coworkers showed that these proteins were produced in cells and could act in a dominant-negative manner with respect to p53 (Yang *et al*., 1998).

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## Expression pattern on p73 and p63

p73 mRNA can be detected at low levels in a variety of normal tissues using sensitive RT–PCR assays (De Laurenzi *et al*., 1998; Kaghad *et al*., 1997; Kovalev *et al*., 1998). p73$\alpha$ and p73$\beta$ protein has been detected in a variety of tumor cell lines by immunoblot analysis using monoclonal antibodies that are specific to p73 (Jost *et al*., 1997).

p63 mRNA has been detected in skin, cervix, urothelium, prostate, thymus, placenta, testis, and skeletal muscle (Osada *et al*., 1998; Schmale and Bamberger, 1997; Senoo *et al*., 1998; Trink *et al*., 1998; Yang *et al*., 1998). Some disparities among these various reports may relate to differences in the nature of the probes and assays chosen. This is an important consideration given the existence of multiple p63 isoforms and the potential for crosshybridization of probes with various p53 family members. Using a monoclonal anti-p63 antibody, McKeon and coworkers confirmed high levels of p63 in the proliferating basal cells of various epithelial structures (Yang *et al*., 1998).

## Alteration of p73 and p63 in human cancers

p73 attracted immediate attention by virtue of its localization to chromosome 1p36, a region that is frequently deleted in a variety of tumors including neuroblastoma (Ichimiya *et al*., 1999; Kaghad *et al*., 1997; Lo Cunsolo *et al*., 1998). If p73 were behaving as a classical tumor suppressor gene, one would anticipate that the remaining p73 allele would be mutated in such cases. This is rarely, if ever, true. Look and coworkers found no p73 mutations among

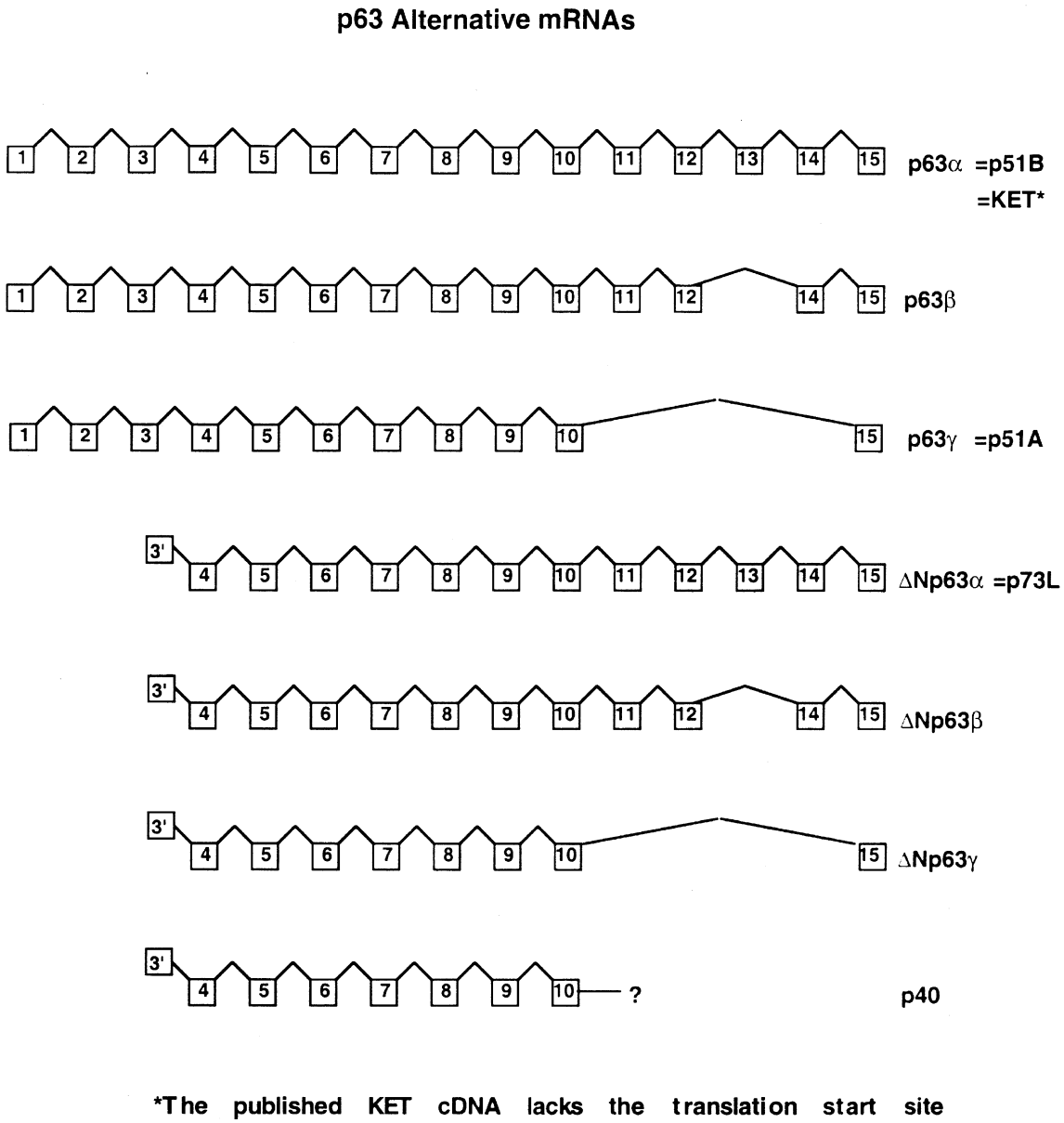


**Figure 1** Alternative p63 mRNA isoforms. Exons are indicated by numbered boxes. For simplicity, the sizes of introns and exons are not drawn to scale. p63 can be transcribed from a cryptic promoter/exon, designated 3′, present in intron 3. The 3′ end of the p40 cDNA has not been mapped to a known p63 exon. Nomenclature is according to (Osada *et al*., 1998; Schmale and Bamberger, 1997; Senoo *et al*., 1998; Trink *et al*., 1998; Yang *et al*., 1998)

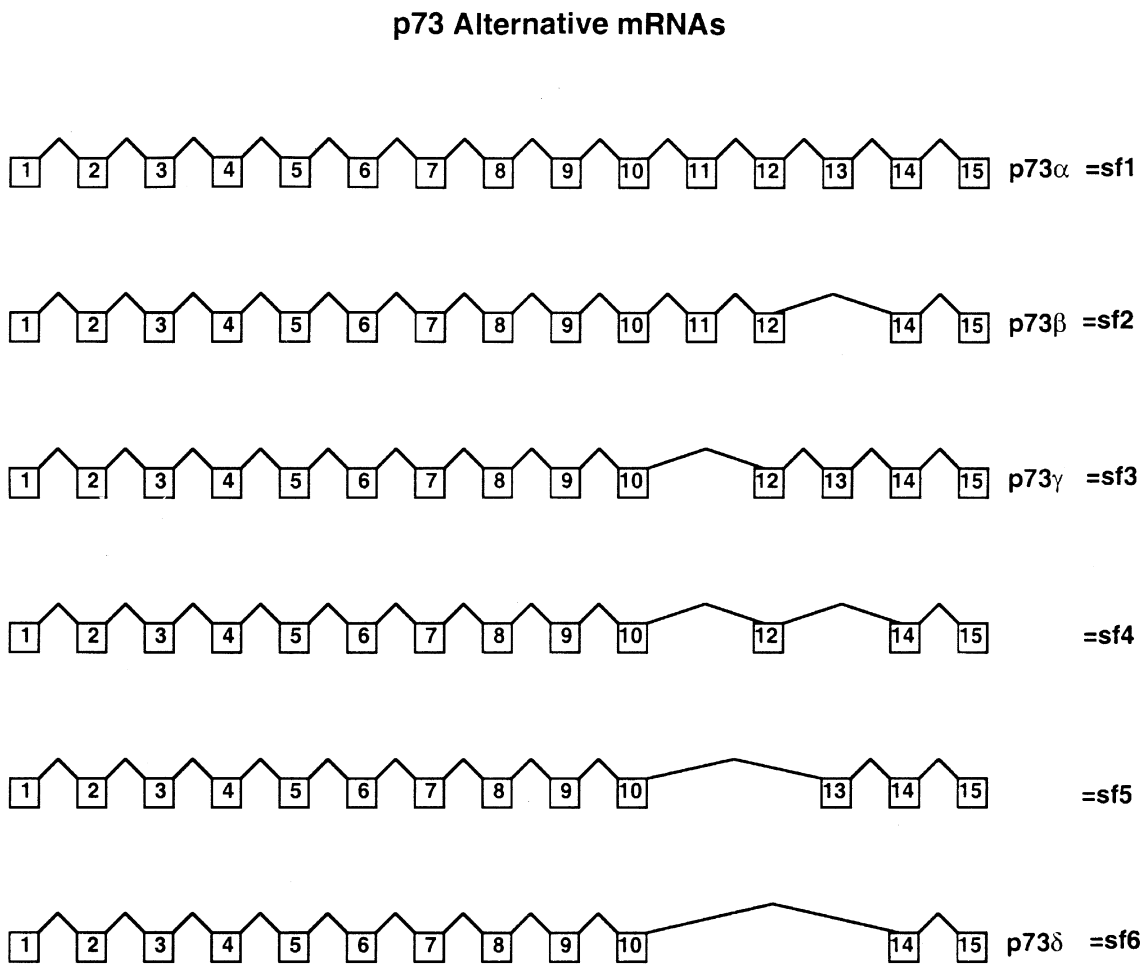


**Figure 2** Alternative p73 mRNA isoforms. Exons are indicated by numbered boxes. For simplicity, the sizes of introns and exons are not drawn to scale. Nomenclature is according to (De Laurenzi *et al*., 1998; Kaghad *et al*., 1997; Kong *et al*., 1999)

18 neuroblastoma cell lines and 32 primary neuroblastomas (Kong *et al*., 1999). Similarly, Caput and coworkers identified no p73 mutations among eight neuroblastomas examined, and Moll and coworkers found no p73 mutations among 16 neuroblastomas examined (Kaghad *et al*., 1997; Kovalev *et al*., 1998). In the largest study to date, Ichimiya found p73 mutations in two out of 140 neuroblastomas (Ichimiya *et al*., 1999). Both of these mutations, one of which was detected in the germ line, mapped to the p73 C-terminus and reportedly affect p73 transactivation function. Furthermore, p73 falls outside of the smallest region of overlap among 1p deletions in neuroblastomas but may be affected by the large 1p deletions seen in neuroblastomas associated with N-myc amplification (Ichimiya *et al*., 1999; Lo Cunsolo *et al*., 1998). No p73 mutations have been identified in the other cancers examined to date including lung (Mai *et al*., 1998; Nomoto *et al*., 1998), prostate (Takahashi *et al*., 1998), bladder (Yokomizo *et al*., 1999), esophageal (Nimura *et al*., 1998), and renal carcinomas (Mai *et al*., 1988), as well as melanoma (Kroiss *et al*., 1998; Tsao *et al*., 1999).

One potential explanation for the absence of p73 mutations in tumors with 1p36 deletion was offered by Caput and coworkers, and related to the observation that p73 is monoallelically expressed (Kaghad *et al*., 1997). In this scenario, loss of the transcribed allele would be sufficient to promote tumorigenesis. This model does not account, however, for the absence of p73 mutations in tumors where both copies of the p73 gene are retained. In addition, others have found evidence for biallelic p73 expression although it remains possible that monoallelic expression is restricted to certain tissues or varies between different individuals (Kovalev *et al*., 1998; Mai *et al*., 1988; Nomoto *et al*., 1998; Tsao *et al*., 1999). More importantly, however, multiple groups have shown that p73 mRNA levels are generally increased, rather than decreased, in the tumors examined to date relative to normal tissue (Kovalev *et al*., 1998; Mai *et al*., 1998; Takahashi *et al*., 1998). In summary, there is presently no genetic evidence that p73 acts as a classical tumor suppressor.

p63 maps to chromosome 3p27-28, a region that is altered in several cancers including lung, cervical, and ovarian carcinomas (Senoo *et al*., 1998; Trink *et al*., 1998; Yang *et al*., 1998). Ikawa and coworkers found three examples of p63 mutations among 101 evaluable tumors and tumor cell lines (Osada *et al*., 1998). Thus p63, like p73, appears to be rarely mutated in human cancers, in striking contrast to p53.

## Differential binding of p53 family members to viral oncoproteins

Viral oncoproteins such as SV40 large T antigen, the adenoviral E1B 55 kD protein, and the human papillomavirus E6 protein inactivate p53 during the course of viral infection, presumably to prevent cellular apoptosis prior to viral replication. T antigen binds to, and disables, the core p53 DNA binding domain. Despite the high degree of similarity between p53 and p73 in this region, T antigen does not interact with p73 (Dobbelstein and Roth, 1998; Marin *et al*., 1998). E6 interacts with the p53 core and C-terminus and, in conjunction with E6AP, targets p53 for ubiquitin-dependent proteolysis. E6 does not bind to p73 however and does not target it for destruction (Marin *et al*., 1998; Prabhu *et al*., 1998). Indeed, El-Deiry and coworkers confirmed that p73 could induce apoptosis in cells overproducing E6 (Prabhu *et al*., 1998). Finally, E1B 55 kD binds to, and silences, the p53 transactivation domain. E1B 55 kD does not, however, bind to p73 (Marin *et al*., 1998; Roth *et al*., 1998). Dobbelstein and coworkers showed that this was related to a 5 amino acid colinear sequence in p53 that was not conserved in p73 (Roth *et al*., 1998).

The adenovirus E4orf6 protein also binds to, and antagonizes, p53. Together with E1B 55 kD, E4orf6 can promote the degradation of p53. Dobbelstein and coworkers reported that E4orf6 did not affect p73 stability or transcriptional activation function (Roth *et al*., 1998). In contrast, Shenk and coworkers reported that E4orf6 could bind to p73 and inhibit its function in transcription-based reporter assays and colony suppression assays (Higashino *et al*., 1998). Further studies are required to clarify the differences between these two reports.

No reports have been published related to the interaction of viral oncoproteins and p63.

## Interaction of HDM2 and p53 family members

MDM2 (HDM2 in humans) binds to the N-terminal transactivation domain of p53. This results in impaired p53-dependent transactivation function and, more importantly, targets p53 for ubiquitin-dependent proteolysis. This activity of HDM2 is antagonized by p14ARF. The region of p53 that binds to HDM2 is highly conserved in p73 and, indeed, overproduced HDM2 can bind to p73 and inhibit its ability to serve as a transcriptional activator (Zeng *et al*., 1999). Interestingly, however, HDM2 does not target p73 for degradation (Zeng *et al*., 1999). Livingston and coworkers have suggested that efficient degradation of p53 requires the formation of a trimeric complex containing p53, p300, and HDM2 (Grossman *et al*., 1998). In contrast to p53, the binding of p300 and HDM2 to p73 is mutually exclusive, perhaps accounting for the failure of p73 to be ubiquitinated by HDM2 (Zeng *et al*., 1999). Whether HDM2 interacts with p73 under physiological conditions is not known. Similarly, it is not known whether ARF modulates the interaction of HDM2 with p73 and whether HDM2 binds to p63.

## Homotypic and heterotypic interactions among p53 family members

p53 binds to DNA as a homotetramer. p73 and p63 possess seemingly homologous C-terminal oligomerization domains and thus are suspected of likewise binding to DNA as homoteteramers, although this has not been formally proven. In yeast two-hybrid assays, p53 preferentially interacts with itself and binds only weakly to p73$\alpha$ and p73$\beta$ (De Laurenzi *et al*., 1998; Kaghad *et al*., 1997). Furthermore, the isolated p53 and p73 oligomerization domains form homodimers but not heterodimers. The various p73 isoforms $\alpha$, $\beta$, $\gamma$, $\delta$ bind variably to themselves as well as to one another in yeast two-hybrid assays (De Laurenzi *et al*., 1998). Marin and coworkers showed that p73$\alpha$ and p73$\beta$ formed complexes *in vitro* as measured by reciprocal coimmunoprecipitation assays (Marin *et al*., 1998).

Interestingly, Di Como *et al*., (1999) reported that two p53 mutants could bind to, and inactivate, p73 when overproduced. This is intriguing given earlier studies that suggested that certain p53 mutants exhibit a gain of function that can be measured in cells lacking p53 (Dittmer *et al*., 1993; Li *et al*., 1998; Shaulsky *et al*., 1991). Whether hetero-oligomerization of mutant p53 with p73 occurs under physiological conditions and is a general property of gain-of-function p53 mutants is not known. Nor has p63 been studied in this regard.

## Are p73 and p63 tumor suppressors?

As described above, there are currently no genetic data to support the contention that p73 and p63 are classical tumor suppressor genes. Furthermore, the fact that p73 is not targeted for inactivation by SV40 T, adenovirus E1B, and E6 argues that p53 and p73 are not wholly redundant. Indeed, that viral oncoproteins discriminate between p53 and p73, despite the high degree of similarity between these two proteins, raises the heretical possibility that p73 may augment, rather than inhibit, viral and cellular transformation. This latter hypothesis is intriguing in light of the data cited above that shows that p73 mRNA levels are generally increased, rather than decreased, in tumor cells relative to normal cells.

One reconciliation of these findings would be that certain critical afferent and efferent functions performed by p53 are not shared with p73 and p63. In this regard, preliminary data suggest that p73, unlike p53, is not induced by DNA damage, although these observations need to be replicated in different systems (Kaghad *et al*., 1997). Secondly, it is unknown whether p73 is induced by oncogenes via the p14ARF/HDM2 pathway. As described above, however, the functional consequences of HDM2 binding appear to differ between p53 and p73. In terms of 'downstream' targets, preliminary data suggest that p53 and p73 preferentially activate subsets of 'p53-responsive' promoters in transfection experiments (Di Como *et al*., 1999; Zhu *et al*., 1998). It will be important to complement these types of experiments with biochemical experiments which ask whether p53, p73, and p63 interact with the same DNA sequences in unbiased binding site selection assays.

## Therapeutic opportunities

As described above, it is unclear whether p73 and p63 are tumor suppressor proteins under physiological conditions. Nonetheless, the overproduction studies done to date establish the proof of concept that activation of wild-type p73 (or p63) might lead to cell death in cancer cells. This hypothesis is intriguing given the fact that p73 and p63 are rarely mutated in cancer. In principle, this activation might be achieved in one of several ways. For example, it might be possible to activate p73 with a small molecule that blocked its interaction with HDM2 (provided HDM2 is a physiologically relevant inhibitor of p73). In theory, molecules already in development that block the interaction of HDM2 with p53 might also function in this setting. Secondly, it might be possible to design or discover drugs that block the interaction of mutant p53 with p73 (provided this finding is shown to be of general significance). Finally, a more detailed understanding of the upstream signals that impinge upon p73 and p63 might allow for the design of drugs which, for example, would activate the transcription of these genes or stabilize their protein products.

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