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The complexity of p73 isoforms in human neoplasia

Review Article

Emanuela Giombini[1], Giovanni Blandino[1], Ada Sacchi[1], Francesco Lo-Coco[2] and Maria Giulia Rizzo[1,*]

[1]Department of Experimental Oncology, Regina Elena Cancer Institute, 00158-Rome, Italy

[2]Department of Biopathology, University "Tor Vergata", Rome, Italy

***Correspondence**: Maria Giulia Rizzo PhD, Dept. of Experimental Oncology; Regina Elena Cancer Institute, Via delle Messi d'Oro, 156, 00158-Rome, Italy; Phone: +39-06-5266-2563; Fax: +39-06-4180-526; e-mail: rizzo@ ifo.it

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Abbreviations: acute lymphoblastic leukemias, (ALLs); acute myeloid leukemia, (AML); arsenic trioxide, (ATO); B-cell chronic lymphocytic leukemia, (B-CLL); blast crisis, (BC); chronic lymphocytic leukemias, (CLL); CML-Acceleration Phase/Blast Crisis, (CML/AP/BC); CML-Bone Marrow Transplantation patients, (CML-BMT); CML-Chronic Phase patients, (CML/CP); DNA binding domain, (DBD); dominant-negative proteins, (ΔN-p); multiple myeloma, (MM); oligomerization domain, (OD); peripheral blood leukocyte, (PBL); RAR elements, (RARE); retinoic acid, (RA); sterile α motif, (SAM); transactivation domain, (TAD)

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Summary

p73, the homologue of p53, is a nuclear protein whose ectopic expression, in p53+/+ and p53 -/- cells, recapitulates the most well-characterized p53 effects, such as growth arrest, apoptosis and differentiation. Altered expression of the p73 gene has been reported in neuroblastoma, lung cancer, prostate cancer and renal cell carcinoma as well as in breast cancer, ovarian tumor, melanoma and hematopoietic neoplasia. p73 has a complex genomic organization that largely results from an alternative internal promoter in intron 3 generating NH2-terminally deleted dominant-negative proteins (ΔN-p73) and differential splicing of the COOH-terminal exons (α, β, γ, δ, ε, ζ, Ë, Ë$_1$ isoforms), of which the two major forms are p73α and p73β. These different splicing variants at the COOH-terminus were shown to have variable homo- and heterotypic interactions between themselves and with p53 whereas the ΔN-p73, that lacks the transactivation domain, exerts a dominant negative function towards p53 and p73 activity. Therefore, it is likely that the various products of this gene participate, in different ways, in a complex network that regulates cell growth, death, and differentiation giving rise to a family of proteins that adds a new level of complexity to the understanding p73 signalling in cancer cells. Indeed, several studies demonstrated that expression of p73 is markedly enhanced during differentiation of myeloid leukemic cells. We and others have shown that leukemic blasts from acute myeloid leukemia (AML) patients exhibit an increased expression of shorter p73 isoforms (γ, δ, ε). In addition, we described a distinct expression pattern of the ΔN-p73 isoform in the peculiar subset acute promyelocytic leukemia (APL) as compared to other AMLs. Here, we provide an overview of the potential role of p73 isoforms in acute myeloid leukemias (AMLs). We speculate that a complex p73 isoform profile with alterate expression pattern of a particular p73 variant (ΔN-p73 in APL) might represent non-mutational mechanisms of leukemogenesis whose study can shed light on the pathogenesis of AMLs.

I. Introduction

Leukemias can be regarded as diseases in which differentiation has been blocked at one of the steps along a specific hematopoietic lineage. The proper differentiation of hematopoietic cells might also depend from transcriptional factors whose deregulation can take part to the development of neoplasia.

p53 is a sequence-specific transcription factor that regulates the expression of genes involved in cell cycle arrest and apoptosis in response to genotoxic damage or cellular stress. P53 is the most frequently mutated gene in human cancer, being mutated or lost in 50% of most tumors (D'Amico et al, 1992). One exception is the acute myeloid leukemia (AML) where p53 mutations are found in only 5% in *de novo* cases (Fenaux et al, 1991; Stirewalt et al, 1999). This finding leads to the possibility that other oncosuppressor genes may be involved in the pathogenesis of this type of leukemia. 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 to understand the basis for their similarities and differences in terms of physical and genetic interactions with one other, their

regulation and their mechanisms of activation and /or inactivation identifying others attractive candidate to investigate in AMLs.

p73, one of the two p53 homologues, is a nuclear protein whose ectopic expression, in p53+/+ and p53-/- cells, recapitulates the most well-characterized p53 effects. Unlike p53, p73 is very rarely mutated in human cancer (Melino et al, 2002). Therefore, several studies have explored alternative mechanisms for its inactivation. Hypermethylation seems to be the predominant mechanism of p73 inactivation in lymphoid leukemogenesis (Kawano et al, 1999; Corn et al, 1999) whereas no evidence for methylation has been obtained for other hematological malignancies such as AML, non–Hodgking lymphoma and CLL. The absence of p73 methylation in *de novo* AML as well as the fact that this hematological tumor is also characterized by low frequency of p53 mutations, suggest that p73 may be altered in different ways depending on the tumor type and that, p73 may be involved in an unusual manner in the pathogenesis of AML.

p73 presents a complex genomic organization that largely results from an alternative internal promoter in intron 3 generating NH_2-terminally deleted dominant-negative proteins (ΔN-p73) and differential splicing of the COOH-terminal exons (α, β, γ, δ, ε, ζ, Ë, $Ë_1$ isoforms), (De Laurenzi et al, 1998, 1999; Scaruffi et al, 2000). It has originally been reported that short p73 isoforms are less efficient than p73α in transactivating gene target promoters and promoting growth suppression and apoptosis (De Laurenzi et al, 1998; Ueda et al, 1999; Ueda et al, 2001). However, the molecular mechanisms underlying the reduced transcriptional activity of p73γ, p73δ, and p73ε are still under investigation.

ΔN-p73 is a truncated protein that takes origin from a cryptic promoter located in the third intron of the p73 gene and lacks the transcriptional activation domain. Recent studies have shown that ΔN-p73 protein impairs both p53 and p73 transcriptional activity and p53/p73-mediated apoptosis in response to agents inducing DNA damage. Furthermore, ΔN-p73 is induced by TAp73 and p53, generating a dominant-negative feedback loop that regulates p53 and p73 functions (Grob et al, 2001; Kartasheva et al, 2002; Zaika et al, 2002). Therefore, it is likely that the several products of p73 participate, in different ways in a complex network that regulates cell growth, death and differentiation.

In myeloid leukemic cells expression of p73 has been shown to be markedly enhanced during differentiation (Fontemaggi et al, 2001; Morena et al, 2002) and we and others showed that leukemic blasts from patients present an increased expression of the shorter p73 isoforms (γ, δ, ε,) (Tschan et al, 2000; Rizzo et al, 2004). In contrast, less information on ΔN-p73 expression in leukaemia is actually available. We have recently identified the lack of ΔN-p73 as a frequent feature of acute promyelocytic leukemia (APL). Thus, a complex p73 isoform profile and the possible aberrant expression pattern of a particular p73 variant (ΔN-p73 in APL) might constitute a non-mutational mechanism with a potential role in the pathogenesis of AMLs. We will focus on recent progress that has been made in our understanding of the potential involvement of p73 isoforms in haematological malignancies.

II. Genomic organization and structure of p73

The human *p73* gene localizes on chromosome 1p36. The over 60 kb length of p73 is organized in 14 exons which originate an array of multiple protein isoforms due to differential mRNA splicing (Kaghad et al, 1997; De Laurenzi et al, 1998; Ueda et al, 1999; Scaruffi et al, 2000; Fillippovich et al, 2001) and to alternative promoter P1 and P2 usage (Kaghad et al, 1997; Yang et al, 2000).

Historically, the C-terminal complexity was described before the N-terminal complexity of the 2 gene loci. Most of alternative splicing occurs at the 3' end and involves more specifically exons 10 to 13, hence yielding transcripts that encode protein isoforms with various C-terminal structures. The two promoters, P1 in the 5'UTR upstream of a non-coding exon 1, and the P2 located within intron 3 and over 30-kb downstream, produce two diametrically opposed classes of proteins: those containing the transcriptional activator domain (TAp73) and those lacking it (ΔN-p73).

Recently, two crucial studies have indicated that: 1) TAp73 directly activates the transcription of endogenous ΔN-p73 by binding to the two p73-specific target elements located on P2 (Nakagawa et al, 2002); and 2) p53 induces ΔN-p73 both at the mRNA and protein levels, as a result of a p53 direct activation of the P2 promoter (Kartasheva et al, 2002; Vossio et al, 2002).

Structurally, p73 exhibits three typical domains of a transcription factor across various species: namely an acidic, amino-terminal transactivation domain (TAD); a central core DNA binding domain (DBD); and a carboxy-terminal oligomerization domain (OD) (Kaghad et al., 1997). **Figure 1** depicts the genomic organization of the p73 gene.

A. Amino- TAD

The TAD is known to interact with various transcriptional coactivators, thereby allowing the enhanced expression of some genes. As a consequence of the two major promoters, p73 protein can be expressed with or without the aminoterminal TA domain. Additional variation at the aminotermini occurs as a result of exon skipping (Stiewe et al, 2002b; Fillipovich et al, 2001; Ishimoto et al, 2002).

B. DBD

DBD is a core region required for the association with its cognate DNA element to act as a transcriptional factor. All the known variants of p73 invariably contain a central DBD that specifically interacts with an overlapping, if not identical set of promoter sequences. A subtle but possibly important difference consists in the strength of this interaction. p73's DBD is directly involved in the physical interaction with human tumor derived p53 mutants (Marin et al, 2000; Strano et al, 2000, 2002; Gaiddon et al, 2001;). This interaction has been shown to

play a key role in the gain of function of mutant p53 (Lang et al, 2004; Olive et al, 2004).

C. OD

The OD is immediately at the carboxyterminal side of the DNA binding domain and allows the formation of tetramers (dimers of dimers). Several investigations have also implied that the C-terminus of p73 affects its oligomerization capacity and transcriptional activity (De Laurenzi et al, 1998; Ueda et al, 1999). Therefore, the C-terminal region may have a role as a regulatory moiety for the function of p73 in response to various cellular stimuli.

The most obvious feature of the carboxyterminal portions of p73 is the fact that the α isoforms of p73 contain a sterile α motif (SAM). The precise function of the SAM domain is currently unknown, although in general, SAM domains can mediate specific interactions with a variety of cytoplasmic signalling proteins (Chi et al, 1999; Thanos and Bowie, 1999), or with RNA (Kim and Bowie, 2003) and has been found in multiple signalling proteins and transcription factors, many of which are important in developmental regulation. The crystal and solution structures of the p73 SAM domain both agree with the five-helix bundle fold characteristic of all SAM domains (Chi et al, 1999; Wang et al, 2001). The ETS transcription factor TEL, that is involved in a number of chromosome translocations in leukemia, possess a pointed domain (a SAM-like domain) that self-associate (Jousset et al, 1997), and was found fuse to other signalling and regulatory proteins in many leukemias, resulting in constitutive activation of these fusion proteins via dimerization (Golub et al, 1994; Golub, 1997; Kim et al, 2001). Other SAM-containing proteins are the members of the polycomb group of homeotic transcription factors and the ephrin receptors (Peterson et al, 1997; Kyba and Brock, 1998; Smalla et al, 1999; Stapleton et al, 1999; Thanos et al, 1999).

However, the SAM domain comprises only a minor proportion of the residues found at the carboxyterminal side of the oligomerization domain, and other portions of this region have been implicated in transcriptional activation (Ghioni et al, 2002) such an additional transactivation domain may contribute to promoter activation by the β isoform of p73 (Takada et al, 1999). Identification of the cellular proteins that bind to the p73 SAM domains and elucidation of their functions will likely and significantly improve our knowledge of the functions of these newer family members. Additional important sub domains exist within these COOH-terminal "tails." In fact, p73 contain several PXXP and PPXY (Y 5 tyrosine) motifs. The SH3 domain of the oncoprotein c-abl has been shown to bind p73 via a PXXP sequence located in the proline-rich region between the p73 DNA binding domain and the predicted oligomerization domain (Agami et al, 1999; Gong et al, 1999; Yuan et al, 1999). Similar to SH3 domains, WW domains (small 38–40 aminoacid sequences characterized by two conserved tryptophan residues 20 amino acids apart) also bind proline-rich ligands (Sudol et al, 1995). Recently, Strano et al, (2001) reported that the WW domain adaptor phosphoprotein YAP (yes-associated protein) also interacts with the PPPPY sequence of p73 (residues 482–488) but not p53. This interaction appears to increase p73 transactivation function and imparts selectivity to p73-mediated apoptosis in response to DNA damage (Chi et al, 1999; Strano et al, 2005) (**Figure 1**).

However, differences in the SAM and proline-rich domains of the different family members may reflect significant divergence in signalling and function.

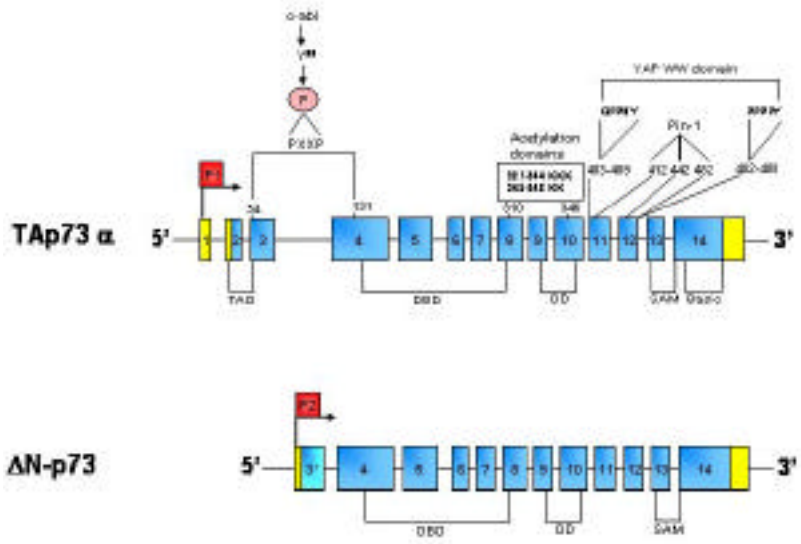

Figure 1. Genomic organization of p73 locus. TAD, transactivation domain; DBD, DNA-binding domain; OD, oligomerization domain; SAM, sterile α motif-like. Transcriptional start sites are indicated by arrows. The P1 promoter in the 5' untranslated region (yellow) upstream of a non-coding exon 1 produces TA proteins that are transcriptionally active. The P2 promoter located within intron 3 produces ΔTA-p73 proteins lacking the transcriptional activator domain. The PXXP region (between aa 54 and aa 131) indicate the motifs of direct interaction with the Src homology 3 domain of c-abl. Phosforylation (P) of tyrosine (Y) Tyr^{99} phosphorylation of p73 by c-abl. 321-344 KKK; 345-346 KK: acetylation domain. PPPP: polyproline rich domains: the C-terminal PP domain, contain Pin-1

consensus sites at residues 412, 442, and 482. PPPPY and PPSY indicate the canonical motif of the two ligand binding consensus for YAP: yes-associated protein.

III. NH_2 isoforms

The absence of the N-terminal transactivation domain characterizes a second class of p73 variants. These truncated isoforms are encoded by transcripts derived from the alternative promoter P2 in intron 3 (ΔN-p73), or by spliced transcripts, derived from P1 promoter (ΔN'-p73, p73Δex2 and p73Δex2/3). The various N-terminally truncated isoform products (ΔN-p73, p73Δex2, p73Δex2/3, ΔN'-p73) are collectively referred to as "ΔTA-p73" (**Figure 2a)**.

The human variant ΔN-p73, encoded by the first three exons of TP73, starts with 276 bases of the additional exon 3' over 30-Kb downstream, containing different ATG and unique 13 amino acids (exon 3'). This region is highly conserved between human and mouse and is in frame with exon 4. Whereas the length of the p73 intron 3' (between exon 3' and exon 4) is approximately 15 kb.

p73Δex2 and p73Δex2/3 are p73 isoforms that lack either exon 2, or exon 2 and 3, and in contrast to ΔN-p73 are generated from the same promoter (P1) as TAp73. The other TA promoter-derived transcript, ΔN'-p73, is created by splicing out exon 2 but includes the 3'-portion of the alternative exon 3' (Casciano et al, 1999; Yang et al, 2000; Fillippovich et al, 2001; Ishimoto et al, 2002; Stiewe et al, 2002b) (**Figure 2a**). Because the translation start of the full-length transcript is located in exon 2, both alternatively spliced transcripts encode ΔTA-p73 proteins starting with amino acids 49 and 72, respectively (**Figure 2b**). Whereas the third TA promoter-derived transcript, ΔN'-p73, aberrantly includes the 3'-portion (198 bp) of the alternative exon 3' and encodes for the same protein as the ΔN-p73 transcript (**Figure 2b**).

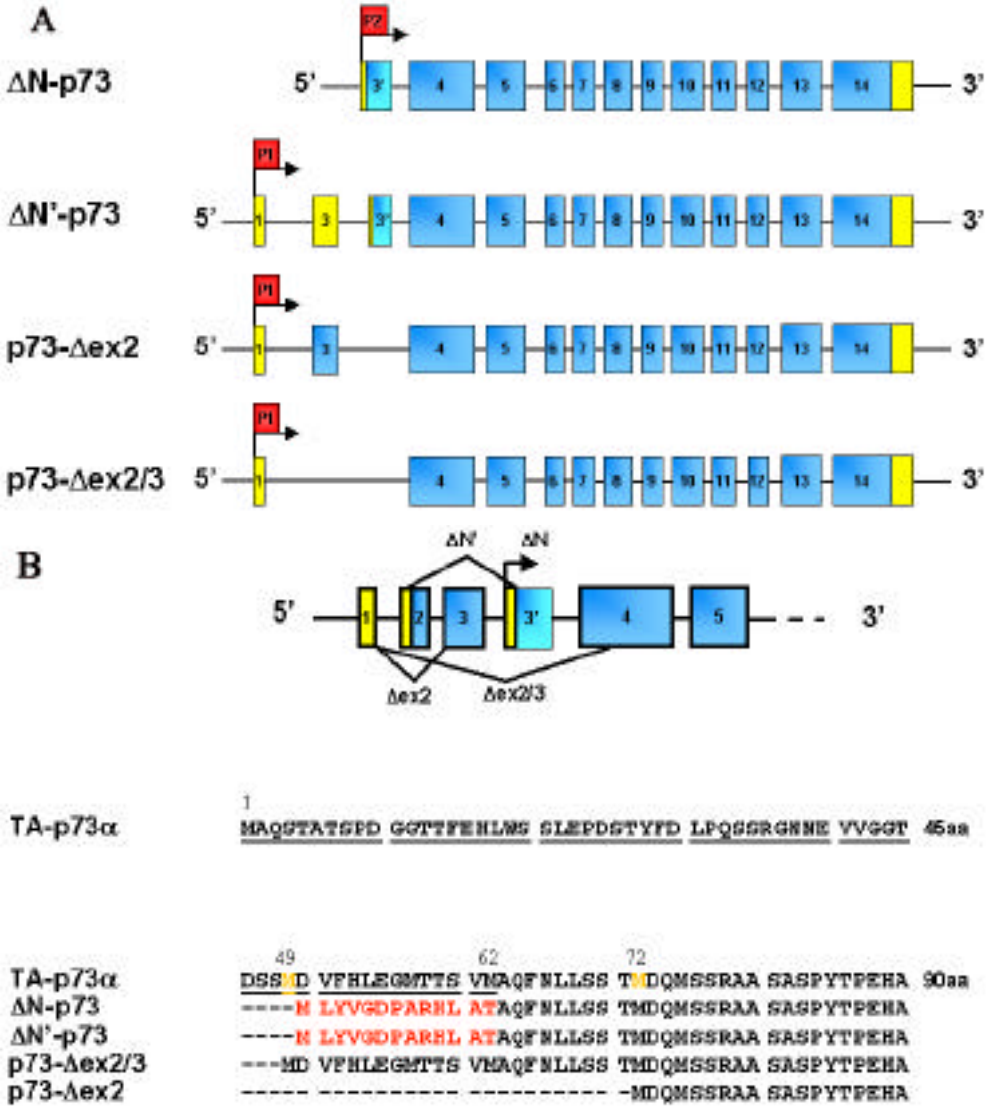

Figure 2. A. ΔTA-p73 isoforms. Trascriptional start sites are indicated by arrows. The ΔN-p73 is generated from a cryptic promoter (P2) within intron 3. p73Δex2 and p73Δex2/3 aberrantly lack either exon 2, or exon 2 and 3 and are generated from the same promoter (P1) as TAp73. ΔN'-p73 is created by splicing out exon 2 but includes the 3'portion of the alternative exon3'. B. Aminoacid alignment of p73 NH_2-terminal isoforms and full-length TA-p73. The underlined 62 aa of TA-p73 are replaced in ΔN-p73 and ΔN'-p73 isoforms, by the

unique 13 aa (in red) encoded by the alternative exon 3' located in intron 3. The yellow indicates the translation start with aminoacids 49 and 72 for p73Δex2 and p73Δex2/3 respectively.

The ΔTA isoform was initially identified in mouse, then in human (Pozniak et al, 2000; Yang et al, 2000; Grob et al, 2001; Ishimoto et al, 2002; Melino et al, 2002; Stiewe et al, 2002b). Mice functionally deficient for all p73 isoforms exhibit profound defects, including hippocampal dysgenesis, hydrocephalus, chronic infections and inflammation, as well as abnormalities in pheromone sensory pathways. ΔN-p73 is the predominant form in the developing mouse brain and might act as dominant-negative inhibitors of themselves and of other family members in vivo in the mouse and in transfected human cells (Pozniak et al, 2000; Yang et al, 2000). In situ hybridization reveals strong ΔN-p73 expression in E12.5 fetal mouse brain in the preplate layer, bed nucleus of stria terminalis, choroid plexus, vomeronasal area, and preoptic area (Yang et al, 2000). Moreover, ΔN-p73 is the only form of p73 found in mouse brain and the sympathetic superior cervical ganglia in P10 neonatal mice. Functional studies and knockout mice showed that ΔN-p73 plays an essential antiapoptotic role in vivo. ΔN-p73 is required to counteract p53-mediated neuronal death during the normal ''sculpting'' of the developing mouse neuronal system (Pozniak et al, 2000). Withdrawal of nerve growth factor, an obligate survival factor for mouse sympathetic neurons, leads to p53 induction and p53-dependent cell death. Conversely, nerve growth factor withdrawal leads to a decrease of ΔN-p73. Importantly, sympathetic neurons are rescued from cell death after nerve growth factor withdrawal when ΔN-p73 levels are maintained by viral delivery. Likewise, sympathetic neurons are rescued from Adp53-mediated neuronal death by coinfected AdΔN-p73. In pull-down assays, mixed protein complexes of p53/ΔN-p73 were demonstrated, suggesting one biochemical basis for transdominance in addition to possible promoter competition. Altogether, these data firmly put ΔN-p73 into nerve growth factor survival pathway and also explains why p73-/- mice, missing all forms of p73 including protective ΔN-p73, undergo accelerated neuronal death in postnatal superior cervical ganglia (Pozniak et al, 2000).

Using classical in vitro and in vivo transformation assays, Petrenko et al, 2003 showed that ΔN-p73 exerts oncogenic functions in primary cells facilitating immortalization of MEFs, by rescue of Ras-induced senescence, and cooperating with cMyc and E1A in driving their proliferation. Most importantly, ΔN-p73 cooperates with all the three isoforms of oncogenic Ras in inducing MEF-derived malignant fibrosarcomas in vivo. On the other hand, ΔN-p73 has no transforming capacity of its own, at least in primary fibroblasts and in the NIH 3T3 strains. Together, these data indicate that ΔN-p73 can be classified as an immortalizing protein that co-operates with a classical oncogene from the signal transduction category in completely transforming primary fibroblasts (Petrenko et al, 2003). ΔN-p73 forms have a very important regulatory role, as they exert a dominant-negative effect on p53 and TAp73 by blocking their transactivation activity, and hence their ability to induce apoptosis (Grob et al, 2001; Ishimoto et al, 2002). At least two, non-mutually exclusive, mechanisms can be evoked for the dominant-negative action of the ΔN forms. The first is the competition of these proteins, which lack an NH2-terminal transactivation domain, for binding to canonical p53 DNA-binding sites and thus preventing the binding of transcriptional activation-competent p53 family members. The second is the inhibitory function exerted at the oligomerization level (for TAp73). In fact, while unbound to DNA, they oligomerize with, and hence sequester, transcriptional activation-competent p53 family members.

Current data suggest that both of these mechanisms can occur in vivo for ΔN-p73, and play an important role in p73- and p53-mediated cell death in both malignant cells and developing neurons (Ishimoto et al, 2002; Stiewe et al, 2002a; Zaika et al, 2002).

p53 and TAp73 can induce expression of the ΔN-p73 isoform, which creates a dominant-negative feedback loop that regulates the function of both p53 and TAp73, and can fine-tune the function of p53 in a manner that is similar to the MDM2 loop (Grob et al, 2001).

The ability of p53 and TAp73 to control the expression of the ΔN-p73 isoform through this p53-responsive element has also been confirmed by numerous reports (Kartasheva et al, 2002; Melino et al, 2002; Vossio et al, 2002). The findings of Vossio et al, 2002 also are consistent with a model in which ΔN-p73 proteins may be up-regulated at the transcriptional level by stimuli that activate the p53 pathway and may act to repress p53-dependent transcription by competing for its binding site on different promoters. Both MDM2 and ΔN-p73 are direct transcriptional targets of p53, and they are able to inhibit p53 function by inducing its degradation (in the case of MDM2) or competing for its target DNA-binding sites (in the case of ΔN-p73). Perturbations of these regulatory loops in cancer cells (Stiewe et al, 2002a) or in virally infected cells (Roth et al, 1998; Allart et al, 2002), result in excess or persistent expression of MDM2 or the ΔN-p73 isoform and might contribute to the inability to activate p53 or TAp73.

Ishimoto et al, 2002), first described the identification of human ΔN-p73 by an efficient RACE method and revealed, by comparison of the ΔN-p73 cDNA to its genomic sequence, the existence of exon 3' in intron 3 of p73 gene.

This group underlined, also, the interesting feature of the two-way usage of this exon 3': one usage is the exon 3' transcribed by an alternative promoter. The other usage is that the exon 3' is being transcribed along with exon 3 by an alternative splicing event. This latter way was determined by PCR screening of human cDNA libraries using various primers. The identified transcript, ΔN'-p73 mRNA, was shown to retain all of the other exons 1 through 14 with 198 base-exon 3' insertion (**Figure 2a**). Of note, the ΔN-p73 and ΔN-'p73 transcripts encode the same protein due to the use of a second translational start site because of an upstream premature stop in ΔN'-p73 (Ishimoto et al, 2002). Furthermore, the existence of different regulations among different species has been hypothesized. In contrast with predominant expression of

ΔN-p73 in the murine brain, human ΔN-p73 expression has been detected only in the corpus callosum among eight subregions of the brain. In addition, neither TA nor ΔN isoform of human p73 was expressed in hippocampus, whereas p73-deficient mice were demonstrated to suffer from hippocampal dysgenesis (Yang et al, 2000).

To clone the human homologues of the mice ΔN-p73 isoforms Grob et al, 2001 performed a BLAST search in the genebank database using a sequence from mice exon 3' (y19235). This search allowed them to identify a genomic clone (AL136528) containing the entire human p73 gene and highly homologous to the mouse ΔN-p73 isoforms. Interestingly, the sequence of exon 3' contains two different in frame ATGs and translation can start with either one. The existence of two different translation start sites was confirmed by in vitro translation of a ΔN-p73 construct and by Western blot analysis of over-expressed and endogenous p73 (Grob et al, 2001). Stiewe et al, 2002b, detected the human alternative transcript ΔN-p73 derived from the cryptic promoter in intron 3, homologous to murine ΔN-p73 using RT-PCR analysis. This alternative transcript encodes a NH_2-terminally truncated p73 protein in which the 62 NH2-terminal amino acids are replaced by 13 aminoacids encoded by the alternative exon 3'(**Figure 2b**). In addition, they detected the three alternatively spliced transcripts (p73Δex2, p73Δex2/3, and ΔN'-p73) derived from the TA promoter.

IV. COOH isoforms

p73 undergoes multiple COOH-terminal splicing of exons 10 to 14, skipping one or several exons generating additional complexity. So far, several transcripts were found for p73: α, β, γ, δ, ε, ζ, η, and η_1 (Kaghad et al, 1997; De Laurenzi et al, 1998, 1999; Ueda et al, 1999; Zaika et al, 1999; Scaruffi et al, 2000; Ishimoto et al, 2002). Splicing of different ''tails'' further modulates the p53-like function of TA proteins, although they do not appear to vary much in their role in tumorigenesis (Ueda et al, 2001). Importantly, despite the exclusive differences at their carboxyl-terminal portions, all of these isoforms have three functional domains. **Figure 3** gives an overview of the genomic organization of p73 C-terminus isoforms.

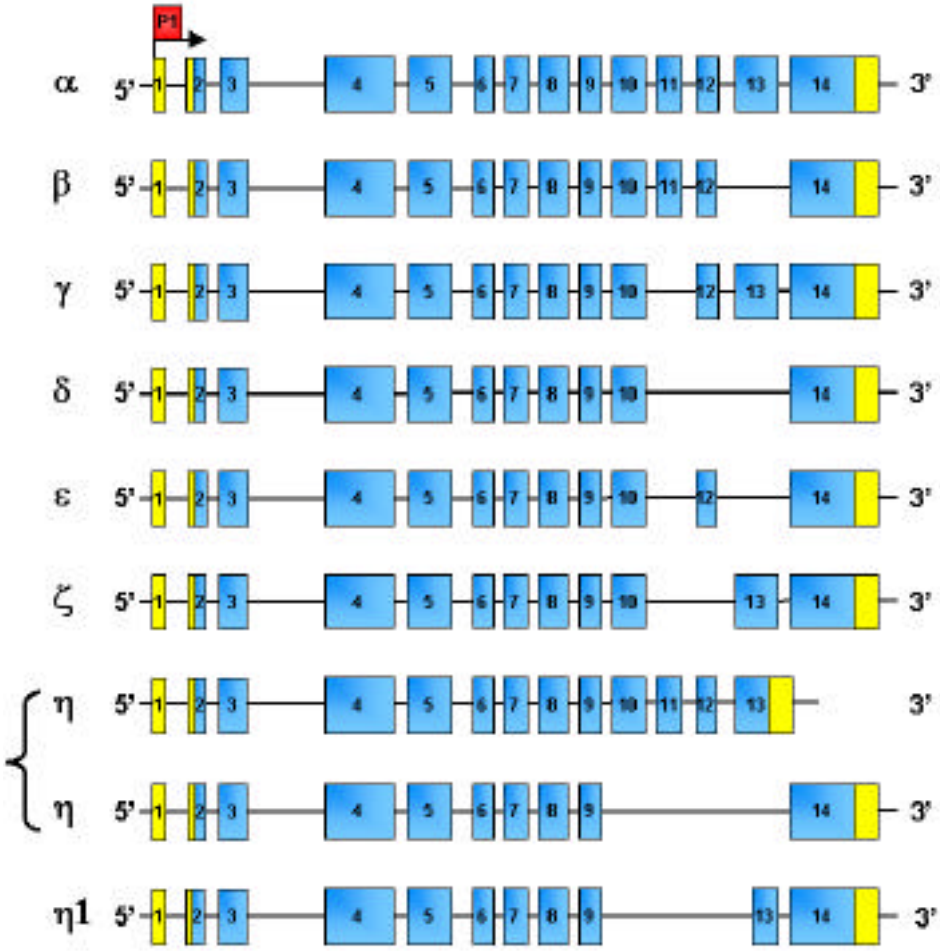

Figure 3. Genomic organization of p73 C-terminus isoforms. α: full length, 636 aa (Kaghad et al, 1997); β: exon spliced out 13, 489 aa (Kaghad et al, 1997); γ: exon spliced out 11, 475 aa (De Laurenzi et al, 1998; Ueda et al, 1999); δ: exon spliced out 11, 12, 13, 403 aa (De Laurenzi et al, 1998); ε: exon spliced out 11, 13, 555 aa (Ueda et al, 1999; De Laurenzi et al, 1999); ζ: exon spliced out 11, 12, 540aa (De Laurenzi et al, 1999); η exon spliced out 10, 11, 12 and 13, 468 aa (Scaruffi et al, 2000); η_1 exon spliced out 10, 11 and 12, 362 aa (Scaruffi et al, 2000); η lacking exon 14 and a long exon 13 (853 bp) with stop codon, 571 aa (Ishimoto et al, 2002). yellow: untraslated region; blue: translated region.

The p73 α and β were the first p73 forms identified. The cDNA encoding p73 was fortuitously discovered by Kaghad et al, 1997 in a hybridization screen of a COS cell cDNA library using degenerative oligonucleotide primers for the IRS-1-binding domains but the coding sequence of *p73* was found to lack any homology to IRS-1 binding domains. Subsequently, libraries of normal human colon tissue cDNAs were screened by hybridization to yield cDNAs encoding p73α and p73β, (Kaghad et al, 1997).

Structurally, the p73α is the larger form of p73 and is the only one containing at the C-terminus a region, with a strike structural similarity to the SAM domain (Bork and Koonin, 1998; Chi et al, 1999). This domain is absent in the β, γ or δ isoforms and only partially present in ε and ζ isoforms of p73.

The study of homotypic and heterotypic interactions between p73 isoforms and p53 were performed by employing the yeast two-hybrid system. The α and β forms of p73 interact weakly with p53, whereas the γ, δ, and ε forms establish only homotypic interactions. The different forms of p73 interact each other with variable intensity. p73 γ forms strong heterotypic interactions with α, β, and δ and with β being the strongest, whereas p73 δ forms strong interactions with α and γ, but binds to β much weaker than p73γ (Kaghad et al, 1997; De Laurenzi et al, 1998). Moreover, p73α has a very low tendency to form homotypic interactions, whereas p73β homodimerizes tightly and binds weakly to p73α. These results indicate that all homotypic and several heterotypic interactions are possible between the p73 variants and suggest that in vivo this interaction might fine-tune the system.

At the functional level, p73γ is very weak compared with p53 and p73 β in suppressing colony formation in SaoS-2 cells and in transactivating the Waf-1 gene promoter (De Laurenzi et al, 1998). Interestingly, even though p73 γ by itself activates poorly transcription, it has no inhibitory effect on coexpressed p73 β that is consistently the strongest transactivator of p53 targets in ectopic expression (De Laurenzi et al, 1998; Roth et al, 1998; Zhu et al, 1998). p73δ, like p73α, shows intermediate strength in transactivation and suppression. (De Laurenzi et al, 1998). The short isoforms are mainly located, as p73α and p73β, in the nucleus indicating that the varied transcriptional activities are not due to differences in subcellular localization (Jost et al, 1997; Ueda et al, 2001). Hence, the various isoforms seem to have very different biological effects from weak to strong or neutral.

Further work is necessary to understand the complex regulatory network generated by: (*a*) multiple p73 COOH-terminal isoforms and their idiosyncratic expression profiles that vary among tissues and individuals; (*b*) homo- and heterotypic interactions among themselves; and (*c*) the resulting differentials in their activities. Unfortunately, the studies of these variants at the C-terminus are hampered by the fact that commercially available antibodies only react with p73-α and p73-β, and these two isoforms of p73 have been detected in both normal and cancer cells. The translations of the other spliced variants have to be verified through the preparation of isoform-specific antibodies.

A. p73 α 636 aa (Kaghad et al, 1997), p73β 499 aa (Kaghad et al, 1997)

p73 α is a full-length 636 aa protein that has distinct developmental roles. TP73 expression is required for neurogenesis of specific neural structures, for pheromonal signalling, and for normal dynamics of cerebrospinal fluid (Yang et al, 2000). The hippocampus that continues to develop throughout adulthood is central for learning and memory. p73-null animals exhibit hippocampal dysgenesis due to the selective loss of large bipolar neurons called Cajal-Retzius in the marginal zone of the cortex and the molecular layers of the hippocampus. These Cajal-Retzius neurons that are responsible for cortex organization coexpress ΔN-p73 and the secretory glycoprotein reelin. In addition, p73-null mice have severe malformations of the limbic telencephalon. They also suffer from hydrocephalus (20%) probably due to hypersecretion of cerebrospinal fluid by the choroid plexus and from a hyperinflammatory response (purulent but sterile exudates) of the respiratory mucosa likely due to mucus hypersecretion. Moreover, the animals are runted and show abnormal reproductive and social behaviour due to defects in pheromone detection. The latter abnormality is due to a dysfunction of the vomeronasal organ, which normally expresses high levels of p73.

The 499 aa p73β protein is encoded by transcripts lacking the 96 nucleotides corresponding to exon 13. This deletion interrupts the open reading frame, yielding a polypeptide of 499 amino acids (**Figure 4**). Both p73α and β transcripts were detected by PCR in several human tissues including brain, kidney, placenta, colon, heart, liver, spleen, and skeletal muscle, indicating a wide-spread, albeit low level, expression of these proteins.

B. p73 γ 475 aa (Ueda et al, 1999; De Laurenzi et al, 1998)

The γ isoform was contemporary identified by De Laurenzi et al, in 1998 and Ueda et al, in 1999.

Ueda et al, 1999) cloned the new splicing variant γ by reverse transcriptase-polymerase chain reaction (RT-PCR) using purified polyA-RNA of MCF-7 cells as a template. The tissue-specific expression of this variant were than analysed by RT-PCR of RNA samples extracted from normal human tissues including the brain, heart, lung, liver, fetal liver, spleen, pancreas, kidney, small intestine, skeletal muscle, testis and thymus. In the testis, p73γ was expressed at much higher levels than in other tissues. Instead, De Laurenzi et al, 1998 identified p73γ by amplification of the 3' end of the p73 mRNA from the SH-Sy5y neuroblastoma cell line. The expression pattern was examined using a radioactive PCR, that amplifies exons 10–14 in the p73 mRNA (bp 1198–1735 of the full-length mRNA), in normal human lymphocytes, primary keratinocytes, and several tumor cell lines (neuroblastoma, glioblastoma, melanoma, hepatoma, and leukaemia) including MCF-7.

The γ isoform contains a long alternative reading frame that leads to the formation of a different, 76-residue C terminus and it is the p73 form that most closely resemble p53 itself, harbouring just a small COOH-terminal extension beyond the last 30 aa stretch (**Figure 4**) of p53 but, surprisingly, TAp73γ is rather weak in transactivation and apoptosis assay (De Laurenzi et al, 1998; Ueda et al, 1999).

C. p73 δ 403aa (De Laurenzi et al, 1998)

The splicing of exon 11, 12, and 13 in p73 δ result in a shorter truncated form of p73 of 403 aa. As γ also δ p73 variant is expressed in human peripheral blood lymphocytes, primary keratinocytes, and different tumor cell lines, including neuroblastoma, glioblastoma, melanoma, hepatoma, and leukemia.

D. p73 ε 555aa (De Laurenzi et al, 1999; Ueda et al,1999)

De Laurenzi et al, 1999 identified a new p73 isoform called p73ε by amplification from normal peripheral blood leukocyte (PBL). Whereas, Ueda et al, 1999 identified p73ε by the analysis of different types of cDNA obtained when full-length p73 cDNA was synthesized by RT-PCR using purified polyA-RNA of human breast cancer cell line MCF-7 as a template. Cloning and sequencing p73ε showed that it lacks exons 11 and 13. In particular loss of exon 11 deletes 50 aa with a frame shift to the reading frame of the γ isoform; while splicing of exon 13 deletes an additional 31 aa and reverts the reading frame to the α variant (**Figure 4**). Thus, p73ε results composed of parts of the γ and α reading frames. Moreover, because the ε isoform lacks the first three and a half helices the SAM domain it is unlikely be folded and may not be functional.

Of note, Zaika et al, 1999) also reported the identification of a p73 variant that appeared as novel amplification products of 273 bp, together with p73 α-δ, in a single PCR reaction, that was named φ.

This p73 φ splices exon 11 and exon 13 thus resulting identical to p73 ε described by others authors (De Laurenzi etal, 1999; Ueda et al, 1999). As already described for p73 ε this variant is different from all other isoforms between residues 400 and 445, and is followed by a novel stretch of 110 aa and a premature stop at residue 555 (**Figures 3 and 4**).

So we clarify this issue saying that De Laurenzi ε= Ueda ε= Zaika φ.

To confirm the existence of p73ε, a panel of normal and tumour tissues and cell lines (brain, heart, lung, liver, fetal liver, spleen, pancreas, kidney, small intestine, skeletal muscle, testis, thymus and human hepatoma line HepG2) were screened, showing that the expression vary among the different tissues (Ueda et al, 1999).

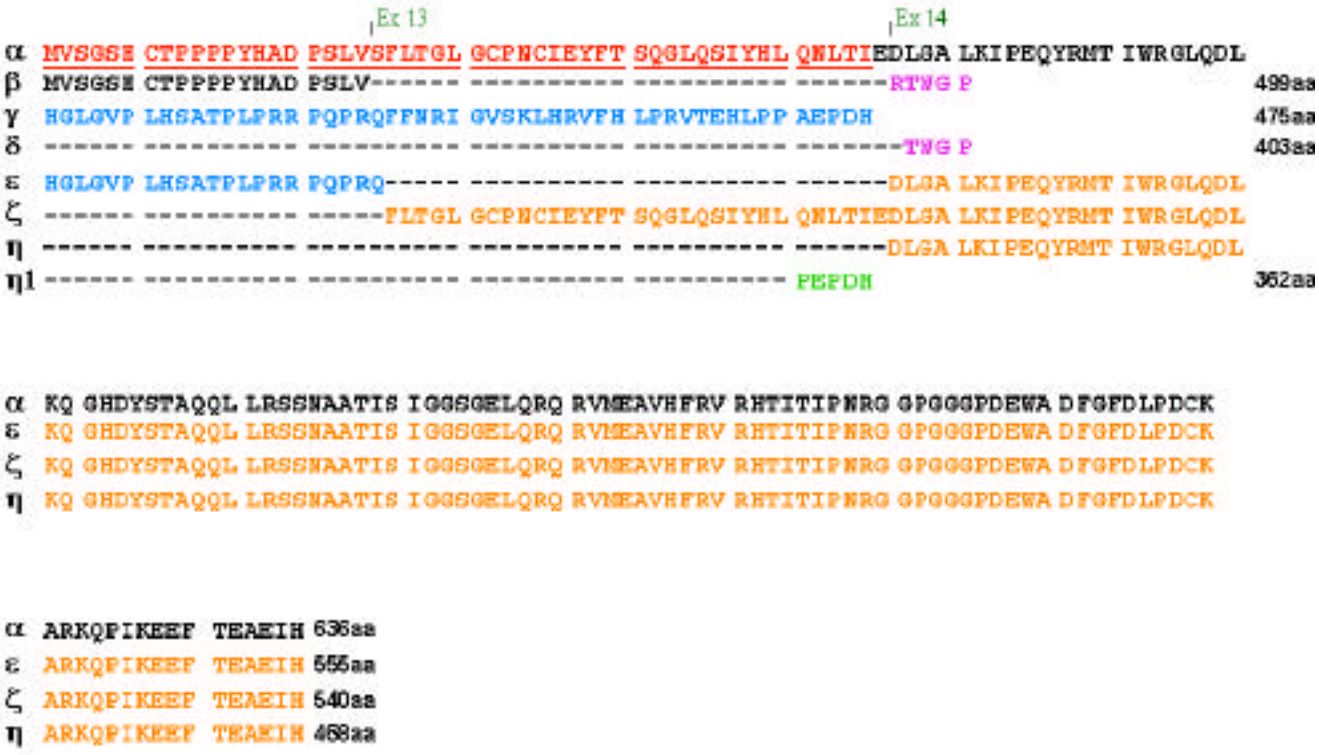

Figure 4 Aminoacid alignment of the C-terminal region of the different p73 splicing variants. The corresponding exons are indicated in green. Underlined red sequence indicates a reading frame of p73α which are modify in the splicing variant γ, ε.Blue sequence indicates the reading frame which is different from that of the α isoform (76-residue C-terminus for γ; 46-residue for ε). Green in the η sequence indicates the translation of 5 aa followed by premature stop codons. The orange of ε, η, and ζ isoforms indicate the reverts of the reading frame to the α variant. The violette in β and δ isoforms indicate premature stop codon.

E. p73 ζ 540 aa (De Laurenzi et al, 1999)

p73 ζ is a further splice variant which lacks exons 11 and 12, and results in the loss of 96 aa by internal deletion, (residues 400-496 of the p73α), the sequence continuing with the C-terminus of the α form (**Figure 4**).

The ζ isoform contains most of the SAM domain sequence but lacks a crucial hydrophobic residue (L493 in p73α) that contributes to the folding and stability of this domain. Thus, it is not clear whether this isoform contains a folded, functional SAM domain (Arrowsmith, 1999). The ζ isoform was identified in the MCF-7 human breast cancer cell line and in a human skin biopsy.

As discussed for ε isoform we remark that the nomenclature of the isoforms ε and ζ is confusing. Indeed, the so called ε isoform described by Zaika et al, 1999 is generated by splicing exons 11 and 12, resulting in a frame-shift starting at residue 400, followed by 141 novel aa and a premature stop codon at residue 540 as the so called ζ isoform described by De Laurenzi et al, 1999. As consequence, De Laurenzi ζ=Zaika ε.

F. p73η 468aa p73η 571aa and p73η_1 362 aa (Scaruffi et al, 2000; Ishimoto et al, 2002)

The η isoform was first described as a 468 aa protein by Scaruffi et al, 2000) and then as a 571 aa protein by Ishimoto et al, 2002).

1. p73η and η_1 (Scaruffi et al, 2000)

p73η and η_1 were identified by Nested RT-PCR amplification of p73 mRNA from leukemic cells with a primer set encompassing exons 9 to 14 (Scaruffi et al, 1999; Romani et al, 1999). p73η lacks exons 10, 11, 12 and 13. The splicing of these exons generates an in–frame deletion of 168 aa followed by a –COOH tail of 110 residues identical to that of p73α (**Figures 3 and 4**).

In contrast to others C–terminal isoforms, the η isoform has, so far, only been detected in neoplastic but not in normal cells. Indeed, p73η was detected in primary neuroblastoma and breast carcinoma cell line MCF–7 but not in the B–lymphocytes derived from seven healthy donors. On the contrary p73η_1 is also present in non-tumor lymphocytes.

p73η_1 results from the complete splicing of exons 10, 11 and 12; two nucleotides at the 3'-end of exon 9 are deleted, whereas the last 18 nucleotides of exon 13 are retained. The splicing generating p73η_1 result in frame-shifts leading to the translation of 5 aa followed by premature stop codons (**Figures 3 and 4**). The predicted –COOH terminus of p73η_1 is almost identical to that of the γ isoform.

The result of the splicing of exon 10 in these variants is the loss of tetramerization domain (the region of homology with the p53 oligomerization domain) and the second transactivation domains at the COOH-terminus which are unique to p73α and β (Takada et al, 1999). However, these variants have not been cloned.

2. p73η (Ishimoto et al, 2002)

In contrast with the other isoforms, this η form was identified by rapid amplification of cDNA ends (3'-RACE) and is generated by alternative termination.

p73 η mRNA has a long exon 13 (853 bp) containing a stop codon, encoding a 571 aa protein (**Figure 4**). The p73η transcript was only detected in lymphnode. Although not as potent, TAp73η showed significant transactivation potential comparable with that of TAp73α and TAp73γ against mdm2-P2, BAX, p21waf1, 14-3-3 δ.

V. p73 in normal and tumor tissues

The chromosomal location of the p73 gene, initially led to speculation that it was a tumor suppressor gene. p73 is located at chromosome 1p36, a region that is frequently deleted in a variety of human tumors including neuroblastoma, melanoma, breast, and colon cancer (Irwin and Kaelin, 2001). The mouse *p73* gene is also located in a chromosome region frequently involved in murine cancer. Specifically, mouse *p73* maps to the distal part of chromosome 4, a region lost in γ-radiation-induced murine T-cell lymphomas (Herranz et al, 1999). Despite these initial reports suggesting tumor-associated deletion of p73, several lines of evidence argue against p73 being a classical tumor suppressor. TP73-deficient mice lack a spontaneous tumor phenotype (Yang et al, 2000), and inactivating mutations in human tumors are extremely rare [more that 900 tumors covering a broad tissue spectrum have been analyzed to date (Kaghad et al, 1997; Nomoto et al, 1998; Yokomizo et al, 1999; Yokozaki et al, 1999; Zaika et al, 1999; Moll et al, 2001; Stiewe and Pulzer, 2001)]. The scenario is even more complicated by the fact that various tumors express higher levels of p73 than tissues from which they originated. These tumors included neuroblastoma (Kovalev et al, 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), prostate cancer (Takahashi et al, 1998), hepatocellular carcinoma (Tannapfel et al, 1999a), liver cholangiocarcinoma (Tannapfel et al, 1999b), and B-cell chronic lymphocytic leukemia (Novak et al, 2001). These results were difficult to reconcile with the current understanding of p73 function and raised the question about additional activities of p73 in cancer. Therefore, attempts have been made to correlate p73 status with disease prognosis: studies of large-sized patient groups with hepatocellular carcinomas (Tannapfel et al, 1999a), colorectal carcinoma (Sun, 2002), and breast cancer (Dominguez et al, 2001) indicated a trend for poor prognosis in tumors with high p73 expression levels.

Thus far, the TP73 story seems to be more complex than that of classical tumour-suppressor genes. The intricacies of p73 regulation are only now beginning to be understood. One of the more obvious complications in assessing the role of p73 in tumour development is the fact that the TP73 locus encodes both a tumour suppressor (TAp73) and a putative oncogene (ΔN-p73) and several TA variants with unclear roles.

The p73 isoforms that arise from alternative splicings are found in different proportions, depending on the cell

species analyzed. Most notably, many tumor cell lines express enhanced levels of TP73-derived mRNA species that occur as a result of exon skipping (De Laurenzi et al, 1998; Casciano et al, 1999; De Laurenzi et al, 1999; Zaika et al, 1999; Zwahlen et al, 2000; Tschan et al, 2000; Rizzo et al, 2004). Considering the complexity of p73-isoforms, their different transactivation potential and apoptotic activity, a detailed expression analysis of the various p73-isoforms in tumour tissues was needed. So far, the most complex p73 isoform pattern associated with higher levels of total p73 mRNA and protein has been documented in ovarian and breast cancers as well as in acute myeloid leukemias (Zaika et al, 1999; Tschan et al, 2000; Zwahlen et al, 2000; Rizzo et al, 2004). Zaika et al, 1999, reported overexpression of wild-type p73 in breast cancer cell lines. Tumors and cell lines with p73 overexpression tended to exhibit a "complex" profile of C-terminal p73 variants, whereas normal tissues (breast, kidney, thyroid, ovary, uterus, placenta, neutrophils, and lymphocytes) and transformed tissues with low level of p73 mRNA predominantly expressed p73α.

The survey on p73 gene expression in benign and malignant ovarian neoplasms has yielded comparable features. A detailed analysis of p73 isoforms indicated that, as a general feature, ovarian carcinomas and cancer cell lines exhibited multiple complex isoform patterns, which were not seen to this extent in the adenomas (Zwahlen et al, 2000). We and others analyzed the RNA expression pattern of TA-p73α and related isoforms such as p73γ, p73δ and p73ε in primary blasts from patients with AML. In agreement with previously reported findings, in breast and ovarian tumors, also the AMLs tend to overexpress a complex profile of shorter C-terminal splice variants as compared to the absence in normal human leukocytes, granulocytes, monocytes, CD34+progenitors and spleen (Tschan et al, 2000; Rizzo et al, 2004). These data on p73 isoform expression in tumors indicate that instability in the splicing of p73 exons occurs in cancer cells and tumors exhibit a "complex" pattern of isoforms in contrast to the "simple" profile (simple profile is termed the minor expressions of shorter isoforms) detectable in various normal tissues. However, apart from a shift toward expression of the shorter C-terminal isoforms in tumor cells, the in vivo function of p73 isoforms (dominant negative molecules?) and their interference with the transcriptional machinery are still unclear, and little evidence exists to support their role in tumorigenesis (Zaika et al, 1999; Tschan et al, 2000; Novak et al, 2001).

The recent identification, as an additional group of p73 proteins, of N-terminally truncated and transactivation-deficient p73 isoforms, may contribute in the future to better understanding the role of p73 in tumorigenesis (Yang et al, 2000; Pozniak et al, 2000; Fillippovich et al, 2001; Grob et al, 2001; Sayan et al, 2001; Ishimoto et al, 2002; Stiewe et al, 2002b).

Indeed, the interpretation of the initial data that identify p73 overexpression in primary human tumors was hampered by the lack of discrimination between expression of the TAp73 and ΔN-p73 isoforms. The emerging evidence that in some cancers, the dominant-negative ΔTAp73 forms, rather than TAp73, are the physiologically relevant components of tumor-associated p73 overexpression and are functionally overriding the frequently concomitant increase in TAp73 calls for *isoform-specific* assessment of p73 overexpression, thus investigators have started to use isoform-specific RT-PCR.

ΔN-p73 is the predominant form in the developing mouse brain and it is the only form in the neonatal brain and sympathetic ganglia (Pozniak et al, 2000; Yang et al, 2000). Mouse ΔN-p73 plays an essential antiapoptotic role during developmental associated, p53-driven neuronal death in vivo by acting as a dominant-negative inhibitor of p53 (Pozniak, 2000). Given the existence of this powerful transdominant p53 inhibitor in the mouse, the possibility arose that this isoform might in part be responsible for the overexpression seen in human tumors. The human counterpart of ΔN-p73, was identified and its level specifically analyzed in a large spectrum of human tumor to determine its potential role in cancer. It is was found that a variety of human cancers, including breast, ovary, endometrium, cervix, vagina, neuroblastoma, liver cancer and melanoma but not their relative normal tissues, frequently overexpress ΔN-p73 (Ng et al, 2000; Filippovich et al, 2001; O'Nions et al, 2001; Casciano et al, 2002; Douc-Rasy et al, 2002; Stiewe et al, 2002b; Zaika et al, 2002; Concin et al, 2004; Tuve et al, 2004). In addition, in some ovarian and vulvar cancers up-regulation of the alternatively spliced transcript p73Δex2, either alone or in combination with ΔN-p73, has been described. A large and comprehensive analysis of all NH2-terminal p73 isoforms in ovarian carcinoma of all histological types showed high prevalence of up-regulation of ΔN'-p73 in the vast majority of this tumor type (Concin et al, 2004). A potential function of ΔTA-p73 splice isoforms in melanoma progression were also suggested by Tuve et al, 2004 that showed a significant correlation between TA-p73 mRNA expression and high levels of oncogenic p73Δex2 and p73Δex2/3 splice isoforms in melanoma metastases. So far, however, there is only one study on the prognostic value of ΔN-p73-isoform expression in neuroblastoma patients (Casciano et al, 2002). A study of 52 patients affected by neuroblastoma showed that expression of ΔN-p73 isoform is a strong adverse prognostic marker, independent of stage and *MYCN* amplification. ΔN-p73-negative patients had overall and progression-free survival rates significantly higher than ΔN-p73-positive patients. In another study on neuroblastoma, a p73 variant was detected which lacked exon 2. If translated, such a variant would lack the transactivation domain of the protein. Moreover, different patterns of splicing variants of p73 were found in different subareas of the same neuroblastoma tumor indicating that the splicing pattern of p73 may reflect the biological heterogeneity of the tumor and also that instability in the splicing of p73 exons occurs in cancer cells (Casciano et al, 1999). Nevertheless the basis for this instability and its role is still unclear.

These data support an oncogenic function of ΔTA-p73 and provide a possible explanation for the observed overexpression of p73 in human cancers. Taken together, the reported data indicate that different scenarios can be

envisioned, suggesting that the TP73 gene embodies the "two genes in one" idea with products that play opposing or, at least, different roles within the family circuitry.

VI. p73 alterations in haematological malignancies

Several studies suggest that mutations or structural alterations of the p73 locus occur very unfrequently in hematological malignancies (Corn et al, 1999; Kawano et al, 1999; Schltheis et al, 1999; Stirewalt et al, 1999; Leupin et al, 2004; Stoffel et al, 2004; Sahu et al, 2005). This does not however rule out the potential involvement of p73 through alternative mechanisms such as, for example, gene hypermethylation.

There is increasing evidence that many genes are hypermethylated in human leukemias (Issa et al, 1997; Baylin et al, 1998; Melki et al, 1999; Singal and Ginder, 1999; Chim et al, 2001a, b, 2002) and because mutations or structural alteration of p73 appear rare, the methylation status of the p73 promoter CpG island has been analysed in both normal hematopoietic tissue and leukemia samples. Methylation of p73 promoter was observed in specific types of hematological malignancies, including acute lymphoblastic leukemias (ALLs), Burkitt's lymphomas, B-non-Hodgkin's lymphomas (B-NHLs). In the ALLs, p73 promoter methylation was more frequent in T-cell than B-cell ALL (Corn et al, 1999; Kawano et al, 1999). Most recently, tumor cells in cutaneous T-cell lymphomas were also found to display widespread promoter hypermethylation associated with p73 inactivation, among other tumor suppressor genes (Van Doorn et al, 2005). In contrast, no evidence for p73 promoter methylation was found in any other hematopoietic malignancies including AML, non-Hodgkins lymphomas and chronic lymphocytic leukemias (CLL) (Corn et al, 1999; Kawano et al, 1999; Liu et al, 2000). The absence of promoter hypermethylation in AMLs was also confirmed by gene promoter methylation studies in the peculiar AML subtype known as acute promyelocytic leukemia (APL, Chim et al, 2003). These data implicate that p73 promoter hypermethylation is an important and predominant mechanism in regulating p73 expression in ALLs. Hypermethylation of the p73 gene has also been investigated in multiple myeloma (MM). Distinct from the study by Chim et al, 2004) who reported no p73 methylation, recent investigations by other groups (Galm et al, 2004; Seidl et al, 2004) described hypermethylation of p73 promoter in MM.

The absence of promoter p73 methylation in *de novo* AMLs as well as the fact that these hematological tumors have also a relatively low incidence of p53 mutations suggest that p73 alterations may be different depending on the tumor type and that, in AMLs, p73 may be altered in a distinct manner.

We and others have recently reported that primary blasts from leukemia patients show an increased expression of shorter p73 isoforms (γ, δ, ε) (Tschan et al, 2000; Rizzo et al, 2004). In particular, we investigated the potential involvement of p73 in AMLs analyzing p73 expression pattern in samples from AML patients assigned to different FAB (French–American–British) subtypes. We detected p73 expression of shorter isoforms in AML irrespective of FAB subtypes, but of note, the analysis of ΔN-p73 expression, which has been reported to inactivate both p53 and p73 antitumor effects, revealed a rather peculiar pattern. In fact, ΔN-p73 transcript and protein were detectable in 27/28 (96.4%) cases of M0, M1, M2, M4, M5 and M6 AML and in 13/41 (31.7%) cases of PML-RARα positive M3 acute promyelocytic leukemia (APL, Rizzo et al, 2004).

APL is a distinct subtype of AML with specific biologic and clinical features. These include a unique t (15;17) chromosome translocation resulting in the fusion gene PML/RARα, and a striking differentiative response to retinoids and arsenic trioxide (ATO). Our recent finding that ΔN-p73 is expressed less frequently in APL as compared to other AMLs further contributes to distinguish this leukemia subset (Rizzo et al, 2004). We speculate that the lack of the expression of a dominant-negative ΔN-p73 protein may contribute to the well known responsiveness of APL to currently adopted treatments, which include anthracycline-based chemotherapy in addition to retinoic acid (RA) (Tallman et al, 2002). In line with this hypothesis, it is remarkable that missense mutations of the *TP53* gene have been shown to occur very rarely in APL (Longo et al, 1993; Trecca et al, 1994). Recently, it has been shown that the PML gene contains p53-binding sites, which confer responsiveness to p53. Therefore, PML has been proposed as a direct target modulating p53 biological activity (Gostissa et al, 2003; De Stanchina et al, 2004). The p53/PML cross-talk is likely to be impaired in APL carrying the PML/RARα fusion. It is conceivable to hypothesize that such impairment could be balanced by the lack of ΔN-p73 inhibitory effects on p53 activity. Interestingly, it has been shown that PML/RARα induces hypermethylation of the $RAR\beta_2$ promoter with subsequent silencing of RARβ2 expression (Di Croce et al, 2002). These findings introduce another potential molecular mechanism underlying the infrequent ΔN-p73 expression in APL. In fact, computer analysis indicate that the ΔN-p73 promoter contains RAR elements (RARE) (unpublished observation) suggesting a possible modification by PML/RARα that, as it occurs for the $RAR\beta_2$ promoter, might bind to ΔN promoter and induce its transcriptional silencing. In contrast, no RAR elements are present in the TA-p73 promoter.

Chronic myelocytic leukemia (CML) is a frequent neoplasm of hematopoietic pluripotent stem cells. The molecular genetic changes of CML occurring in the chronic phase of the disease have been well characterized. In contrast, although the evolution from chronic phase to blast crisis (BC) occurs in virtually 100% of CML patients, very little is known on the mechanisms responsible for transformation. Abnormalities of tumor suppressor genes have been reported to occur during the progression from chronic phase to BC in some CML patients (Ahuja et al, 1991; Melo, 1996). Because the loss of chromosomal locus 1p36 where p73 resides has been associated with progression from chronic phase to BC in a subset of CML patients, p73 has been indicated as one of the putative tumor suppressor genes potentially involved in CML progression (Mori et al, 1998).

Peters et al (1999) identified a distinct p73 mRNA expression in CML patient samples with the highest p73 mRNA expression in samples from CML-Acceleration Phase/Blast Crisis (CML/AP/BC), intermediate levels in CML-Chronic Phase patients (CML/CP) and low levels in CML-Bone Marrow Transplantation patients (CML-BMT). In addition it was found that such p73 high expression in CML was associated with the expression of multiple p73 isoforms (Tschan et al, 2000).

In contrast, the analysis of p73 C-terminal splice variants in B-cell chronic lymphocytic leukemia (B-CLL) patients showed that the shorter p73 variants γ, and η prevail in the normal B-lymphocyte population studied, while the expression of the p73α protein is significantly more frequent in B-CLL patient samples than in normal CD19+ -B-lymphocytes (Novak et al, 2001). However, expression of the p73 gene may differ between various other subpopulations of B cells including CD5+-B-cells, which have been indicated as the normal counterparts of B-CLL cells (Lydyard et al, 1999). A comprehensive survey of p73 expression in all normal B-cell subtype, as well as, other B-cells malignancies expressing the CD5 marker such as mantle cell lymphomas would be of interest and warrant further investigation

Thus, we speculate that a complex p73 isoform profile and/or the possible aberrant expression pattern of a particular p73 variant (ΔN-p73 in APL) can represent a non-mutational mechanism with a potential role in the pathogenesis of some hematological tumor (AML, CML, B-CLL).

Overall, it is conceivable that deregulated expression of the p73 gene rather than loss of its function (due to mutations or deletions) contributes to the phenotype observed in some hematological tumor such as AML and B-CLL. The p73 gene, by the use of alternative exon splicing and/or alternative promoter, could generate an impressive modular complexity by combining a specific ”head” with a particular “tail”. This imply that our understanding of its biological role will greatly depend on determining which forms get expressed under what circumstances. Again, these data suggest that the inactivation and consequently the effects of p73 expression may vary according to the specific tissue and tumor type (**Table 1**).

VII. Conclusion

The complexity of p73 resulting from alternative gene promoter usage and differential splicings produces a number of alternative proteins with variable homotypic and heterotypic interactions with key regulatory molecules such as p53. This scenario suggests that various gene products participate in a complex network regulating cell growth, death and differentiation. Studies on human neoplasia indicate that non-mutational mechanisms such as promoter hypermethylation or aberrant expression of specific isoforms (¢Np73), rather than structural alterations, may contribute to the pathogenesis of certain hematopoietic tumors including APL and CLL. It is likely that both p73 gene normal function and potential involvement in human tumors will further attract either basic or translational investigators in the years ahead.

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Table 1. Predominant alterations of the p73 locus in haematological malignancies

Hematopoietic Disease	p73 Alteration	References
ALL	Hypermethylation	Corn et al, 1999; Kawano et al, 1999; Liu et al, 2000
Burkitt’s lymphomas	Hypermethylation	Corn et al, 1999; Kawano et al, 1999
B-NHLs	Hypermethylation	Corn et al, 1999; Kawano et al, 1999
T-cell limphomas	Hypermethylation	Van Doorn et al, 2005
MM	Hypermethylation	Galm et al, 2004; Seidl et al, 2004
AML (M_0, M_1, M_2, M_4, M_5, M_6)	Increased expression of shorter isoforms and expression of ΔN-p73	Tschan et al, 2000; Rizzo et al, 2004
APL(M_3)	Lack of ΔN-p73	Rizzo et al, 2004
B-CLL	Prevalent expression of p73α but high levels of ΔN-p73	Novak et al, 2001; Leupin et al, 2004
CML-BC	Increased expression of shorter isoforms	Tschan et al, 2000

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