Review

Biological functions of p53 isoforms through evolution: lessons from animal and cellular models

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The *TP53* tumour-suppressor gene is expressed as several protein isoforms generated by different mechanisms, including use of alternative promoters, splicing sites and translational initiation sites, that are conserved through evolution and within the *TP53* homologues, *TP63* and *TP73*. Although first described in the eighties, the importance of p53 isoforms in regulating the suppressive functions of p53 has only become evident in the last 10 years, by analogy with observations that p63 and p73 isoforms appeared indispensable to fully understand the biological functions of *TP63* and *TP73*. This review summarizes recent advances in the field of 'p53 isoforms', including new data on p63 and p73 isoforms. Details of the alternative mechanisms that produce p53 isoforms and *cis*- and *trans*-regulators identified are provided. The main focus is on their biological functions (apoptosis, cell cycle, aging and so on) in cellular and animal models, including mouse, zebrafish and *Drosophila*. Finally, the deregulation of p53 isoform expression in human cancers is reviewed. Based on these latest results, several developments are expected in the future: the identification of drugs modulating p53 isoform expression; the generation of animal models and the evaluation of the use of p53 isoform as biomarkers in human cancers.

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Thirty years of research have shown that the p53 tumour-suppressor protein, encoded by *TP53* gene (OMIM 191170), integrates endogenous and exogenous signals to modulate cell fate to stress and cellular environments.¹ In addition, it has emerged that p53 is more than just a 'stress response' factor as it regulates embryo implementation.² The ability of p53 to integrate signals implies the existence of multiple and subtle levels of regulation. Over the years, transcriptional, translational and post-translational regulatory mechanisms have been uncovered.^{3,4} This biochemical diversity echoes the genetic diversity of the *TP53* locus, which contains multiple genetic polymorphisms defining over 100 distinct *TP53* haplotypes.⁵ Recently, an additional layer of regulatory mechanism has emerged through identification of p53 isoforms, which are physiological proteins expressed in normal cells from the *TP53* gene owing to the use of alternative promoters, splicing sites and/or translational initiation sites.^{6,7}

The p53 isoforms were first identified in early studies investigating p53 expression patterns. In 1984, Matlashewski *et al.* cloned an N-terminal variant of the human p53 mRNA, whereas in 1985, Rotter and co-workers detected an alternatively spliced C-terminal variant of mouse p53, latter isolated in human cells.^{8–10} However, the 'p53 isoform' field

has only really emerged in the past 10 years, when it became clear that *TP53* retained the elaborate patterns of isoform expression that characterizes its homologues, *TP63* and *TP73*. The rapid accumulation of descriptive, functional and clinical data on p53 isoforms has led to the emergence of a research community, which held its First International Meeting at the International Agency for Research on Cancer in Lyon, France, in September 2010.¹¹ This review provides a brief survey of the 'p53 isoforms' field at a time when it is emerging at the forefront of p53 research.

TP63 and *TP73*: the Isoform Paradigm

The two p53-related proteins, p63 and p73, share strong structural, biochemical and biological homologies.^{12,13} In particular, they bind specifically to DNA onto conserved p53 response elements (p53REs) by using their DNA-binding domain. In the late nineties, the cloning of *TP63* and *TP73* revealed an elaborate pattern of mRNA expression resulting in several protein isoforms.^{14,15} Several N-terminal forms, produced by the use of alternative promoters and/or alternative splicing (i.e., TA forms, which contains the transactivation domain (TAD), *versus* Δ N forms, produced from an

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internal promoter resulting in the presence of a different TAD), were found combined with several C-terminal forms generated by alternative splicing (five for p63: α to ϵ ; seven for p73: α to η).¹³ Far from being of minor component, the Δ Np63 and Δ Np73 forms are the major forms expressed in certain cell types.^{14,16} In mice, knockout of the entire *TP63* or *TP73* loci (targeting all isoforms) revealed the roles of p63 and p73 in epithelial differentiation and neuronal development, respectively,^{15,17} whereas no impact was observed in stem cell commitment.¹⁸ However, with the generation of isoform-specific knockout mice, a subtle interplay between the N-terminal isoforms has recently emerged, with the dynamic expression of N-terminal p63 or p73 isoforms appearing critical for maintaining the normal sequence of cell development (from stem to committed progenitors and then differentiated cells).^{19,20} Building on this idea, Aberdam and co-workers analysed the impact of Δ Np63 and TAp63 isoforms in cellular commitment. In murine embryonic stem cells, Δ Np63, but not TAp63, is highly expressed during epidermal commitment and is critical for the expression of the cytokeratins K14 and K5, two markers of keratinocyte proliferation, indicating that only Δ Np63 is required for the commitment of ectodermal into epidermal cells.^{21,22} Mills *et al.* observed that Δ Np63 α overexpression in mouse embryonic fibroblasts (MEFs) resulted in the bypass of Ras-mediated senescence and enhanced carcinoma development in mice, suggesting that Δ Np63 α inhibits senescence and therefore acts as an oncogene.²³ By contrast, overexpression of TAp63 forms in p53^{-/-} MEFs increased senescence and reduced tumour development *in vivo*, consistent with a p53-independent effect.²⁴ Studying mice deficient for specific p73 protein isoforms, Mak, Melino and co-workers revealed that, like p63, the different p73 isoforms had dual cellular roles. In particular, TAp73^{-/-} mice are spontaneously tumour-prone whereas Δ Np73^{-/-} cells show impaired tumour formation in nude mice.^{25,26} In addition to these cancer-related effects, knockout of either TAp73 or Δ Np73 isoforms resulted in isoform-specific defects in neurogenesis or neurodegeneration, respectively. Overall, these studies show that each N-terminal p63/p73 form has specific roles in regulating distinct cell differentiation pathways. Moreover, they demonstrate that the Δ N forms, by preventing senescence and maintaining progenitor cell status, may act as oncogenes, whereas TA forms, through their capacity to switch cells into a post-mitotic state, may act as tumour suppressors.

Generation of Human p53 Isoforms by Diverse Regulatory Mechanisms

Like *TP63* and *TP73*, the human *TP53* gene encodes several p53 protein isoforms through conserved mechanisms.^{6,7} The main and most abundant p53 isoform is the canonical p53 protein, also termed TAp53 α , as it contains an entire TAD and the longest C-terminal domain (Figure 1). In addition to the TA forms, three Δ N forms have been identified that differ by their translation initiation site. This is used to designate them as Δ 40p53, Δ 133p53 and Δ 160p53. The four N-terminal p53 forms can be combined with three different C-terminal domains (α , β , γ). Recently, some *cis*- and *trans*-regulators

have been identified as specific modulators of p53 isoform expression (Figure 1).

Δ 40p53 expression: one form, several mechanisms.

Compared with p53, the human N-terminal Δ 40p53 forms lack the first 39 amino acids corresponding to the main TAD (Figure 1c). Matlashewski *et al.*²⁷ identified a p53 mRNA species retaining the entire intron-2 (p53I2), indicating that an alternative splicing event leading to the retention of the *TP53* intron-2 can occur (Figure 1b). They later observed that stop codons in intron-2 of the p53I2 mRNA prevent p53 expression from the first AUG.²⁸ However, p53I2-transfected cells were found to express a 45-kDa protein, undetectable using antibodies recognizing the p53-TAD epitopes (DO1 or DO7), that corresponds to Δ 40p53 initiated at a second AUG at codon-40, encompassed within a strong Kozak consensus.²⁹ Alternative splicing of intron-2 can be regulated through *G-quadruplex* structures located in intron-3 of the p53 pre-mRNA.³⁰ Using reporter assays and RNA-*G-quadruplex* ligands, it appears that *G-quadruplex* structures promote the correct splice-out of intron-2, leading to the fully spliced p53 (FSp53) mRNA encoding the full-length p53 protein; *G-quadruplex* disruption however favours the retention of intron-2 and thus p53I2 mRNA expression. This observation is the first clue that the *TP53* sequence itself can modulate its own isoforms' expression through regulation of alternative splicing.

In addition to alternative splicing, Δ 40p53 forms can be encoded from the FSp53 mRNA through an internal initiation of translation at codon-40.²⁹ Two internal ribosomal entry sequences (IRES) have been identified that regulate the translation of either p53 or Δ 40p53 (Figure 1b).^{31,32} However, the relative contribution, *in vivo*, of each of these mechanisms, which are producing Δ 40p53, remains to be fully established.

Production of Δ 133p53 and Δ 160p53 forms from the internal P2 promoter. As for *TP63* and *TP73*, *TP53* contains an internal promoter that controls the expression of two N-terminal forms (Figure 1a).³³ In addition to the proximal P1 promoter regulating p53 and Δ 40p53 expression, an internal P2 promoter located between intron-1 and exon-5 regulates the transcription of p53 mRNAs initiated in intron-4 (p53I4) (Figure 1b).³³ This internal P2 promoter is different from the P* promoter in *TP53* intron-1 identified by Reisman *et al.*^{34,35} that regulates the expression of an unrelated p53 transcript encoded by *TP53* intron-1. Site-directed mutagenesis and siRNA methods revealed that translation of p53I4 mRNAs can be initiated at two distinct codons, AUG133 and AUG160, leading to the expression of the Δ 133p53 and Δ 160p53 proteins, respectively, that lack the TAD and part of the DNA-binding domain (Figure 1c).³⁶ Surprisingly, Δ 160p53 forms are expressed in K562 cells, which are considered as 'p53-null' cells. The *TP53* mutation in K562 cells results in a premature stop codon between AUG133 and AUG160, thus preventing the expression of the TA, Δ 40 and Δ 133 forms without compromising the Δ 160p53 reading frame. Thus, it should be kept in mind that some cells or tumours considered as 'p53-null' because of the presence of frameshift or nonsense mutations, may retain the capacity to express one or several p53 isoforms.^{29,36}

Figure 1 A schematic representation of human p53 isoforms. (a) The human *TP53* gene structure. The *TP53* gene, which consists of 11 exons (coloured boxes, coding exons; grey boxes, non-coding exons), expresses several p53 isoforms owing to usage of alternative promoters (↑), splicing sites (Δ) or translational initiation sites (I). (b) Human p53 mRNA variants. The proximal promoter P1, located upstream from exon-1, regulates the transcription of two transcripts: the fully spliced p53 mRNA (FSp53), which encodes both p53 (from ATG1) and Δ40p53 forms (from ATG40), and the p53l2 mRNA, retaining the entire intron-2 by alternative splicing, which generates Δ40p53 forms from ATG40, owing to the presence of stop codons (*) in the reading frame starting from ATG1. The internal P2 promoter, described as encompassing the region from intron-1 to exon-5, produces p53l4 mRNA, initiated in intron-4 and encoding the N-terminal Δ133p53 (from ATG133) and Δ160p53 forms (from ATG160). Three different C-terminal p53 forms have been described owing to alternative splicing of intron-9: the α-forms resulting from the excision of the entire intron-9, and the β- and γ-forms produced by retention of two small parts of intron-9. Some cis- and trans-regulators driving p53 isoform expression have been described (purple boxes). Endogenous expression of most of the p53 mRNA variants in human cells has been reported (references shown in parentheses). Grey box, non-coding sequence; NR, not yet reported. (c) Human p53 protein isoforms. The canonical p53 protein contains a TAD (blue), a proline-rich domain (PXXP, purple), a DNA-binding domain (DBD, orange) and an OD (green) that encompasses a nuclear localization domain (NLS, green) and five regions conserved through evolution (I–V in grey boxes). Compared with p53, the Δ40p53 forms lack the first TAD, whereas the Δ133p53 and Δ160p53 isoforms lack the entire TAD and parts of the DBD. At the C-terminal, the α-peptide corresponds to the OD that is replaced by new residues, the β- and γ-peptides (brown). On the right is indicated the theoretical molecular weight, the detection at endogenous levels (reference in brackets) as well as the different names of the isoforms used in the literature. The color reproduction of this figure is available at the *Cell Death and Differentiation* journal online

Experimental studies showed a p53-dependent regulation of Δ133p53 expression.^{37,38} The internal P2 promoter contains p53REs located at the junction of exon-4/intron-4. Promoter deletion, site-directed mutagenesis and chromatin

IP experiments demonstrate that the direct binding of p53 onto the p53REs results in an increased expression of both Δ133p53 mRNAs and the Δ133p53α protein. Some experimental evidence suggests that protein expression of

$\Delta 133p53\alpha$ can be induced through p53-independent mechanisms. In particular, the accumulation of the $\Delta 133p53\alpha$ protein was observed in response to the knockdown of p68, a DEAD Box helicase involved in multiple transcriptional regulatory processes, and more recently in response to the expression of some p63 or p73 isoforms.³⁹

The C-terminal spliced p53 forms, β and γ . The alternative splicing of intron-9 of human *TP53* produces three different C-terminal p53 forms (α , β and γ) (Figure 1a).^{8,33} Complete excision of intron-9 results in the expression of the α -forms corresponding to the classical p53 C-terminal domain (oligomerization domain, OD) (Figures 1b and c). On the other hand, partial retention of intron-9 generates the β - or γ -forms, in which the OD is replaced by 10 or 15 new amino acids, respectively. However, the mechanisms that control the alternative splicing of intron-9 are unknown.

The classical p53 C-terminal domain contains the main post-translational modification sites regulating p53 stability, such as the lysines residues ubiquitinated by Mdm2, an E3-ubiquitin ligase regulating p53 stability and activity.³ Their absence in β - and γ -forms led to the investigation as to whether the stability of the p53 β and p53 γ proteins is regulated by the Mdm2–ubiquitin–proteasome pathway. To date, results remain controversial. For instance, whereas constitutively overexpressed FLAG-tagged p53 β or p53 γ do not appear to interact with Mdm2 or to be degraded in a proteasome-dependent manner,⁴⁰ Bourdon and co-workers observed that the endogenous p53 β protein can be degraded by the proteasome in an Mdm2-dependent manner.

Biological Functions of Human p53 Isoforms

Based on early studies detailed below, the N-terminal isoforms lacking the TAD (i.e., $\Delta 40p53$, $\Delta 133p53$ and $\Delta 160p53$) were expected to only act as dominant-negative regulators of p53 activity. For instance, ectopic $\Delta 40p53\alpha$ expression downregulated the p53-induced transactivation on reporter genes and counteracted p53-dependent growth suppression in colony formation assays.^{28,29} In addition, in human diploid fibroblast WI38 cells, expression of endogenous $\Delta 40p53\alpha$ increased during the G₁/S transition, in parallel with decreased expression of p21.²⁹ Furthermore, as $\Delta 40p53\alpha$ lacks the Mdm2-binding site, it escapes Mdm2-mediated degradation and does not accumulate in response to DNA damage, its expression persisting at low but stable amounts in many cell types.^{29,41} These data support the notion that $\Delta 40p53\alpha$ inhibits basal p53 activities during cell-cycle progression. However, whether $\Delta 40p53\alpha$ can exert p53-independent effects is still unknown. Hainaut and co-workers observed that $\Delta 40p53\alpha$ contains an intact DNA-binding domain able to bind to p53REs *in vitro*. Thus, $\Delta 40p53\alpha$ may also exert intrinsic regulatory effects by competing with p53 for p53RE binding and thereby modulating their accessibility for other transcription factors, regulating the cell fate outcome depending upon cell type and cell context. In addition, $\Delta 40p53\alpha$ lacks the first TAD, which has been shown to be dispensable for p53 transcriptional activity, and retains the second TAD, which can regulate gene expression.^{42,43} Therefore, with our current knowledge, one should be

cautious in considering $\Delta 40p53$ as a simple dominant-negative inhibitor of p53.

Compared with $\Delta 40p53\alpha$, the available data have clearly revealed that $\Delta 133p53$ controls p53 activity. In reporter assays, $\Delta 133p53\alpha$ can also behave as a dominant-negative inhibitor of p53.^{13,33} $\Delta 133p53\alpha$ does not bind to consensus p53REs *in vitro*, consistent with its partial lack of the DNA-binding domain, and thus can also behave as a dominant mutant p53 protein.³⁸ Experimental studies suggested that, instead of being a strict dominant inhibitor, $\Delta 133p53\alpha$ is instead a fine modulator of p53's suppressive activity as its expression determines cell fate in response to stress. The knockdown of $\Delta 133p53\alpha$ expression promotes p53-mediated apoptosis and G₁ cell-cycle arrest in response to doxorubicin treatment, without altering the p53-dependent G₂ cell-cycle arrest.³⁷ These effects may be due to the ability of $\Delta 133p53\alpha$ to modulate gene expression in a promoter-dependent manner, as observed for *p21^{WAF1}*, *Mdm2* and *Bcl-2*.³⁷ Interestingly, $\Delta 133p53$ silencing has also been associated with replicative, but not oncogene-induced, senescence in normal human fibroblasts through transcriptional regulation of p53-target genes, including *p21^{WAF1}* and *mir-34a*.⁴⁴ Overall, these results are consistent with an oncogenic capacity of $\Delta 133p53$.

The biological functions of the C-terminal p53 isoforms (i.e., p53 β and p53 γ) remain poorly described and controversial. Bourdon *et al.*³³ showed that, in the absence of stress, endogenous p53 β bound to the *Bax* and *p21^{WAF1}* promoters, but only weakly to that of *Mdm2*. Moreover, in luciferase reporter assays in the absence of stress, the co-expression of p53 β and p53 enhanced the p53 transcriptional activity on the *p21^{WAF1}* promoter but not on the *Bax* promoter, suggesting a promoter-dependent effect. These observations are consistent with the demonstration that p53 β cooperates with p53 to accelerate senescence in human fibroblasts.⁴⁴ By contrast, experimental studies failed to observe binding of FLAG-tagged p53 β or p53 γ onto p53RE consensus and to show a role of FLAG-tagged p53 β or p53 γ in p53-dependent apoptosis or senescence in cells constitutively overexpressing FLAG-tagged p53 β or p53 γ , and selected to grow in presence of neomycin.⁴⁰ Thus, there is still debate on whether p53 β or p53 γ exert their activities in an autonomous manner or through an interaction with p53. Furthermore, there is no evidence of distinct biological activities for p53 β or p53 γ .

Overall, current experimental data on the biological roles of p53 isoforms are fragmented. Given that p53 isoforms differ from each other in the three functional domains (TAD, DNA-binding and OD), their potential to modulate p53-dependent responses is expected to be diverse and cell type-dependent. Further insight into which of these functions are of physiological or pathological relevance may come from animal model studies.

Animal Models: clues to the Physiological Significance of Isoforms

The simplest animal model to study p53 isoforms, *Drosophila melanogaster*. The diversification of the p53 gene family into three members occurred in vertebrates.

Thus, invertebrates such as *Drosophila* contain a single p53-related gene, which encodes three protein isoforms (Figure 2): Dp53, corresponding to the human full-length

p53; Δ Np53, a general counterpart of the human N-terminal p53 forms as it is encoded by an mRNA transcribed from an internal promoter (i.e., human Δ 133) and contains a

Figure 2 p53 isoforms in animal models. (a) Structural organization of p53 isoforms through evolution. Like humans, mouse, *Drosophila* and zebrafish express a full-length p53 protein, which conserves a TAD (blue), a DNA-binding domain (DBD, orange) and an OD (green). Only the mouse Mp53 protein presents a proline-rich domain (PXXP, purple) and a nuclear localization signal (NLS, green). In addition, all these animals express some p53 isoforms that have the same structural organization as the human p53 isoforms owing to the use of alternative promoters and splicing sites. M, mouse protein; D, *Drosophila* protein; Z, zebrafish protein; N-terminal p53 isoform identification, Δ forms; N-terminal p53 isoform denomination, codon number, when initiated ATG occurs in the coding sequence, or N, when initiation occurs in a non-coding sequence; C-terminal p53 isoform identification, AS (alternative splicing, green boxes); grey box, different residues compared with the full-length p53 protein. (b) Localization of translation initiation sites in animal p53 sequences. Red, ATG1 generating the full-length p53 protein; green, the methionine used to produce the homologues to the human Δ 40p53 forms; blue, the methionine used to produce the homologues to the human Δ 133p53 forms; orange, the methionine used to produce the homologues to the human Δ 160p53 forms. The color reproduction of this figure is available at the *Cell Death and Differentiation* journal online

Table 1 Available p53 animal models

Species	Name	Modulation of p53 isoforms	Ref.
<i>Drosophila</i>		Overexpression of DΔNp53 in cells	45
		Overexpression of transgenic DΔNp53 under the control of tissue-specific promoters	47,49–53
		Overexpression of transgenic dominant-negative mutant DΔNp53	53,54
		Inactivation of <i>Dp53</i> locus	49,53
Zebrafish	Tg(Δ113p53:gfp)	Overexpression of transgenic ZΔ113p53-GFP morpholino (Δ113p53-MO)	61
		Overexpression of ectopic ZΔNp53	59
Mouse	MΔ41p53/MΔ41p53 wt/MΔ41p53	Overexpression of transgenic MΔ41p53	65
	MΔ122p53/MΔ122p53 wt/MΔ122p53 m/m wt/m	Overexpression of transgenic MΔ122p53	68
		'm' = deletion of exon 1–6	69

truncated TAD followed by a complete DBD and OD (i.e., human Δ40); and Dp53ΔC, encoded by a short transcript leading to a putative isoform bearing only the TAD.^{33,45–47} The *Drosophila p53* gene is activated by irradiation and exerts broad suppressive effects recapitulating those of the p53-family members, including regulation of apoptosis, aging, autophagy, differentiation and growth.⁴⁸ Historically, DΔNp53 was the first form identified and previously termed Dp53; hence most of the functional studies to date have focused on the role of this particular isoform (Table 1).

Studies on the morphogenesis of imaginal discs have highlighted the role of DΔNp53 isoforms in the control of cell death.^{45,47,49,50} In this system, DΔNp53 controls apoptosis through the Reaper–Hid–Grim (RHG) cascade. Indeed, irradiated imaginal discs from flies with a mutant *Dp53* gene show reduced apoptosis but normal cell-cycle arrest, suggesting a specific regulatory role of the *Dp53* gene product in apoptosis.^{45,49} So far, the main isoform implicated in apoptosis appears to be DΔNp53, which directly regulates *reaper* (*rpr*) expression.⁴⁵ However, DΔNp53 also exerts effects through other pathways. In particular, DΔNp53 may activate either apoptotic or non-apoptotic responses in photo-receptor cells depending on the cell differentiation status.^{50–52} Moreover, DΔNp53 appears to inhibit cellular differentiation in the retina independently of its apoptotic function.⁵⁰

The *Dp53* gene product also exerts important roles in controlling lifespan in a sex- and stage-dependent manner. When overexpressed in adult flies (Table 1), DΔNp53 limited lifespan in females and extended it in males. By contrast, when overexpressed during development, DΔNp53 exerted a similar dose-dependent effect on longevity in both sexes.⁵³ Conversely, inactivation of the *Dp53* locus increased lifespan in females but had only minor effects in males. Similar phenotypes were observed in *Drosophila* expressing dominant-negative Dp53 mutant transgenes or overexpressing DΔNp53, suggesting that DΔNp53 interferes with Dp53 activity.⁵⁴ The role of *Drosophila p53* has also been investigated in detail in other cellular functions such as DNA repair or compensatory proliferation.^{48,55,56} However, the specific roles of each *Drosophila p53* isoform, Dp53 and DΔNp53, have not been studied in sufficient detail to understand the exact contributions of each isoform to development, stress responses or longevity.

Danio rerio, the historical model to study p53 isoforms. The zebrafish p53 protein, Zp53, recapitulates

the suppressive and pro-apoptotic functions of human p53 upon genotoxic stress.⁵⁷ So far, only N-terminal Zp53 isoforms have been identified (Figure 2): Zp53, corresponding to the human p53 protein;⁵⁸ ZΔNp53, produced through an alternative splicing of intron-2 and thus similar to the human Δ40p53 forms;⁵⁹ and ZΔ113p53, produced by an internal promoter located within the zebrafish *Zp53* gene that is regulated by Zp53 itself, and thus equivalent to the human Δ133p53 isoform.^{37,38,60} In contrast to the human Δ40p53, AUG1 of ZΔNp53 is located within the partial intronic sequence retained by alternative splicing.⁵⁹ Thus in ZΔNp53, the 38 N-terminal residues containing the TAD are replaced by 33 residues derived from the intron-2 sequence.

There is evidence that the ZΔNp53 transcript accumulates in response to γ-ray irradiation.⁵⁹ In addition, ectopic expression of ZΔNp53 resulted in a strong developmental phenotype with hypoplasia and malformation of the head, eyes and somites (Table 1). This phenotype is dependent upon the presence of Zp53, the two isoforms forming a protein complex through their ODs. Overexpression of an OD-mutant ZΔNp53 or wild-type ZΔNp53 in a mutant Zp53 background is phenotypically ineffective. These observations suggest that ZΔNp53 exerts its effects by modulating the activity of Zp53 during zebrafish development.

ZΔ113p53 was discovered in a different context (Table 1). Cheng and co-workers found that in zebrafish embryos, loss of the *Def* gene (Digestive organ Expansion Factor) led to defects in the morphogenesis of digestive organs. In a genome-wide screen, they identified a shorter form of Zp53 whose expression was upregulated in *def*^{−/−} embryos.⁶⁰ Upregulation of ZΔ113p53 correlated with increased expression of p53-target genes involved in cell-cycle progression such as *cyclin-G1* and *p21*^{WAF1}, whereas pro-apoptotic genes such as *Bax* and *Reprimo* were not activated. Furthermore, ZΔ113p53 was found to selectively upregulate the *Bcl-2L* anti-apoptotic gene.⁶¹ This pattern of effects is consistent with the notion that ZΔ113p53, like Δ133p53, operates as a modulator of p53 in selectively activating defined target genes.

Mouse, the next generation of animal models. Six p53 isoforms have been described in mice, resulting from combination of three N-terminal p53 isoforms with two different C-terminal isoforms (Figure 2). In addition to the full-length mouse Mp53, Rotter and co-workers identified an alternative p53 mRNA retaining part of intron-10 that encodes a shorter isoform with new residues in place of

the usual OD (Mp53AS), homologous to the human p53 β .^{10,62} Later, Mowat *et al.*⁶³ isolated M Δ 41p53, the mouse counterpart of human Δ 40p53 forms. In addition, Khoury *et al.* isolated a shorter N-terminal form produced by an internal promoter within the mouse p53 gene, M Δ 157p53, equivalent to the human Δ 160p53 form. Khoury *et al.* also showed that M Δ 41p53 and M Δ 157p53 can be expressed as a C-terminal AS variant. As for the human p53 isoforms, Rotter and co-workers reported that Mp53AS modulates Mp53-mediated apoptosis and Mp53 transcriptional activity in luciferase reporter assays.⁶⁴

In 2004, Maier *et al.*⁶⁵ described a transgenic mouse overexpressing M Δ 41p53 (Table 1). When expressed in a p53-null background, this isoform did not induce any particular phenotype. However, when expressed in a p53-competent background, an increased dosage of M Δ 41p53 led to reduced size, accelerated aging and a shorter lifespan associated with hypo-insulinemia and glucose insufficiency.^{65–67} These effects were attributed to the hyper-activation of the insulin-like growth factor (IGF)-signalling axis by M Δ 41p53, setting in motion a cascade that clamps unimpeded growth through p21.⁶⁵ Furthermore, these M Δ 41p53-overexpressing mice show cognitive decline and synaptic impairment early in life, also attributable to the hyper-activation of the IGF-1-signalling pathway.⁶⁷ These observations are consistent with studies performed *in vitro* and in the zebrafish model, indicating that Δ 40p53 isoforms may regulate growth suppression through modulation of p53 activity.

A premature aging phenotype was also reported in a non-physiological knock-in p53 mouse model expressing a 'M Δ 122p53' form, truncated for the first 122 residues (Table 1).⁶⁸ Although M Δ 122p53 has no physiological equivalent in mouse, it can be considered as an 'intermediate' between the M Δ 41p53 and M Δ 157p53 isoforms, as it lacks the TAD and part of the DNA-binding domain. During adulthood, transgenic p53 ^{Δ 122p53/ Δ 122p53} mice showed premature aging symptoms, such as balding and arthritis, similar to that observed in the p53^{+/-m} mice (deletion exon 1–6).^{68,69} In addition, earlier tumour onset and shortened lifespan were observed in p53^{+/- Δ 122p53} mice as compared with p53^{+/-} mice. Overall, these results support the hypothesis that mouse N-terminal p53 forms may operate as dominant oncogenes to promote cell proliferation and inflammation.

The studies summarized above highlight common themes in the 'p53 isoform' field. First, overall patterns of isoform

expression are well-conserved throughout evolution. Second, N-terminal isoforms have a major role as regulators of physiological processes related to development, aging, life-span and, possibly, carcinogenesis. In this respect, two key mechanisms are emerging. The Δ 40p53 form exerts regulatory effects on signalling cascades controlled by p53, perhaps through direct interaction between the two isoforms. Conversely, isoforms corresponding to Δ 133p53 α modulate cell response by regulating gene expression in a p53-dependent and -independent manner. It should, however, be remembered that our current view of isoform activities remains fragmentary and that further studies are needed to better understand their roles and underlying mechanisms.

p53 Isoforms and Human Cancers

Genetic polymorphisms: effects on p53 isoform expression. The *TP53* gene is highly polymorphic, with over 80% of known single-nucleotide polymorphisms (SNPs) located within introns or non-coding 5' and 3' sequences.⁷⁰ Hainaut and co-workers showed that *G-quadruplex* structures, formed in intron-3 and regulating intron-2 splicing (and thus Δ 40 form expression), overlap a common polymorphism, *TP53* PIN3, which consists of a 16-bp duplication (A1, non-duplicated allele; A2, duplicated allele).^{30,71} This polymorphism may therefore modulate the structure and/or the stability of the *G-quadruplexes*, and affect Δ 40p53 expression (Table 2). Consistent with a functional effect, *TP53* PIN3 has recently been identified as a strong genetic modifier of germline *TP53* mutations. Indeed, carriers bearing A1A1 genotypes developed their first cancer on average 20 years earlier than carriers with an A1A2 genotype.⁷² This effect was detected in a Brazilian cohort, in which the p.R337H mutation of partial penetrance is very common owing to a widespread founder effect.⁵ It remains to be demonstrated whether a similar effect is observed in carriers of other *TP53* mutation types.

Landi and co-workers have identified 11 different haplotypes defined by eight SNPs in a region from exon-3 to intron-4, overlapping part of the internal P2 promoter regulating Δ 133p53 and Δ 160p53 expression.⁷³ Using these different haplotypes as promoters to drive *luciferase* expression, they found significant differences in basal promoter activity that were confirmed by analysing endogenous

Table 2 Role of p53 isoforms in human cancers

Cancer	Isoform	Observation	Ref.
Breast cancer	Δ 133p53 α	Overexpression	33
	p53 β	Loss of expression	33,78
	p53 γ	Loss of expression	33,79
		Prognosis of mutp53/p53 γ as good as wtp53	
Acute myeloid leukaemia	p53 β / γ	Increased expression in response to chemotherapy	78
Ovarian cancer	p53 β	Serous and poorly differentiated tumours/worse recurrence-free and overall survival	77
	Δ 40p53	Improved recurrence-free survival	74
Li–Fraumeni syndrome	Δ 40p53	<i>G-quadruplex</i> structure overlapping <i>TP53</i> PIN3/early age at first cancer diagnosis in patients carrying A1A1 compared with patients carrying A1A2	72

$\Delta 133p53$ expression in samples of normal colonic mucosa. Furthermore, *in silico* and *in vitro* DNA binding analyses suggested that an SNP in intron-4 (rs179287; C > T) affects proteins binding within the P2 promoter.⁷³ These results suggest that genetic polymorphisms may modulate basal $\Delta 133p53$ expression. Whether these effects influence cancer risk remains to be demonstrated.

Mutations affecting the production of isoforms. Given the role of N-terminal p53 forms as inhibitors of p53 transactivation, it can be expected that overexpression of certain isoforms may represent an alternative to a mutation in *TP53* for inactivating p53 in cancers, as observed in some small clinical studies.^{33,74,75} Analysis of the IARC *TP53* database identified 1019 somatic cancer mutations that are predicted to disrupt the p53 coding sequence in the N-terminal region while leaving intact the sequence encoding at least $\Delta 160p53$. This represents 3.65% of all somatic mutations reported to date (version R15; www-p53.iarc.fr).⁷⁰ Therefore, rare and/or silent mutations in *TP53* may differentially affect the expression of p53 isoforms, as observed recently for mutations present in the IRES regulating the relative expression of p53 and $\Delta 40p53$ forms encoded by the full-length FSp53 mRNA.⁷⁶ With respect to C-terminal isoforms, Hofstetter *et al.*⁷⁷ have used an yeast-base functional assay and RT-PCR to show that mutations at splice sites of *TP53* intron-9 can lead to aberrant expression of p53 β mRNA in primary cultures of ovarian cancer cells. By contrast, somatic mutations at splice sites of introns 6 and 9 were found to generate spliced mutant p53 mRNAs (p53 ζ , p53 δ and p53 ϵ), distinct from the physiological isoforms. However, the role of such mutant isoforms in carcinogenesis is not known.

Isoform expression and cancer outcomes. There is emerging evidence that p53 isoform expression is deregulated in human cancers (Table 2). In a study of 245 primary ovarian cancers, Hofstetter *et al.*⁷⁷ observed that

expression of p53 β was associated with serous and poorly differentiated cancers, and, when expressed together with the functional p53 protein, it was correlated with poor recurrence-free and overall survival. By contrast, tumours expressing functional p53 and $\Delta 40p53$ showed improved recurrence-free survival of patients compared with tumours expressing no $\Delta 40p53$. Studies of patients with acute myeloid leukemia have shown that elevated expression of p53 β and/or p53 γ in blood cells was correlated with improved responses to chemotherapy,⁷⁸ which may predict decreased chemoresistance and improved overall survival. An effect of isoform expression has also been observed on breast cancer prognosis. In a cohort of 127 breast cancer patients, Thompson and co-workers reported that patients whose tumours expressed both mutant p53 and p53 γ mRNAs had a prognosis as good as patients whose tumours expressed a wild-type p53, suggesting that the expression of p53 γ may abrogate the poor prognosis commonly associated with *TP53* mutations.⁷⁹

Conclusion

The field of 'p53 isoforms' is still in its infancy, but the increasing number of genetic, biochemical and clinical studies have clearly established that p53 isoforms are fundamental and important components of the p53 pathway. Data obtained from animal and cellular models indicate that p53 isoforms regulate the cell fate in response to developmental defects and cell damages by differentially regulating gene expression, both in a p53-dependent and -independent manner. Furthermore, the current data suggest that p53 isoforms have roles in all biological activities regulated by p53 (Figure 3). Therefore, one can reasonably expect that the characterization of the biochemical and biological activities of p53 isoforms will impact on the fields of cancer, embryo development and aging.

Future experiments will be needed to gain further insight into how p53 isoforms modulate the different biological

Figure 3 p53 isoforms in the p53 network. p53 integrates the different stress signals to adapt cell fate to the intensity and the nature of stress by regulating several biological functions to maintain genomic and cellular integrity. In addition, p53 controls physiological functions under basal conditions. Recent data suggest that p53 isoforms modulate p53-mediated cell fate outcome and may thus be key components of the p53-mediated decision not only in response to stress but also under basal conditions. It has also been reported that p53 isoforms have p53-independent activities and directly regulate cell-cycle arrest and apoptosis. In addition, genetic alterations, such as *TP53* SNPs and *TP53* mutations, affect the expression of p53 isoforms, which may result in tumorigenesis

activities. However, based on our current understanding of the p63, p73 and p53 isoforms, we have come to realize that the p53 pathway should no longer be considered as regulated by only the p53 protein, but by a set of p63, p73 and p53 isoforms that interplay with each other in regulating physiological functions. Further progress on this front will require the development of robust and standardized tools for the identification and quantification of p53, p63 and p73 protein isoform expression in experimental systems as well as in human tissues.

Conflict of Interest

The authors declare no conflict of interest.

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Facts

- At least nine different mRNAs are expressed from the *TP53* gene encoding 12 distinct protein isoforms through the use of alternative promoters, splicing sites and internal initiation sites of translation.
- p53 isoforms regulate cell fate in response to developmental defects and cell damage by differentially regulating gene expression, both in a p53-dependent and -independent manner.
- The N-terminal p53 isoforms, $\Delta 40$ and $\Delta 133$, are not strict dominant-negative inhibitors of p53's suppressive function as they have p53-independent activities.
- The expression patterns and biological activities of p53 isoform are conserved through evolution (human, mouse, zebrafish and *Drosophila*).
- *TP53* SNPs and mutations alter the pattern of p53 isoform expression.
- p53 isoform expression is deregulated in human cancers and is associated with clinical prognosis.

Open Questions

- Development of standardized and robust tools is needed to systematically identify and quantify p53 isoforms in human and animal models.
- Characterization of the biochemical activities of p53 isoforms as well as their molecular targets.
- Is each p53 isoform specialized in a particular biological response? Have they redundant activities?
- Evaluation of the interplay between p53-, p63- and p73-family isoforms.
- Study the potential use of p53 isoform expression in human cancers as biomarkers and as therapeutic targets.

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