

Uncovering the role of p53 splice variants in human malignancy: a clinical perspective

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Abstract: Thirty-five years of research on p53 gave rise to more than 68,000 articles and reviews, but did not allow the uncovering of all the mysteries that this major tumor suppressor holds. How p53 handles the different signals to decide the appropriate cell fate in response to a stress and its implication in tumorigenesis and cancer progression remains unclear. Nevertheless, the uncovering of p53 isoforms has opened new perspectives in the cancer research field. Indeed, the human *TP53* gene encodes not only one but at least twelve p53 protein isoforms, which are produced in normal tissues through alternative initiation of translation, usage of alternative promoters, and alternative splicing. In recent years, it became obvious that the different p53 isoforms play an important role in regulating cell fate in response to different stresses in normal cells by differentially regulating gene expression. In cancer cells, abnormal expression of p53 isoforms contributes actively to cancer formation and progression, regardless of *TP53* mutation status. They can also be associated with response to treatment, depending on the cell context. The determination of p53 isoform expression and p53 mutation status helps to define different subtypes within a particular cancer type, which would have different responses to treatment. Thus, the understanding of the regulation of p53 isoform expression and their biological activities in relation to the cellular context would constitute an important step toward the improvement of the diagnostic, prognostic, and predictive values of p53 in cancer treatment. This review aims to summarize the involvement of p53 isoforms in cancer and to highlight novel potential therapeutic targets.

Keywords: p53, isoforms, p63, p73, alternative splicing, cancer

Introduction

The tumor suppressor p53 has been dubbed the “guardian of the genome”, given its crucial role in maintaining genetic stability and the prevention of cancer formation. To assume this role, once activated after cell injury, p53 induces a number of cellular responses, such as cell repair and survival or programmed cell death.

In the late nineties, two p53-related proteins, p63 and p73, which share strong structural, biochemical, and biological homologies with p53, were identified. Six and at least 14 protein isoforms are expressed from the *p63* and *p73* genes, respectively, with $\Delta Np63$ and $\Delta Np73$ being the predominant isoforms expressed in several human cancers.^{1,2} In vivo studies carried out with different p53, p63, and/or p73 mouse models highlight the synergistic effects of the p53 family in tumor suppression.³

Canonical p53 protein (also named p53, FLp53, p53 α or TAp53 α) was the first p53 isoform to be identified. After being thought for 25 years to be the only isoform encoded by the human *TP53* gene, we and others have described that at least twelve p53

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protein isoforms are encoded by the *TP53* gene (p53, p53 β , p53 γ , Δ 40p53 α , Δ 40p53 β , Δ 40p53 γ , Δ 133p53 α , Δ 133p53 β , Δ 133p53 γ , Δ 160p53 α , Δ 160p53 β , and Δ 160p53 γ). p53 isoforms are obtained through alternative initiation of translation, usage of alternative promoters, and alternative splicing. p53 protein isoforms all share a common part of the deoxyribonucleic acid (DNA)-binding domain (of canonical p53 protein), and contain distinct transactivation and C-terminal regulatory domains, enabling them differentially to regulate gene expression.⁴

p53 isoforms are differentially expressed in several human cancer types and were shown to exhibit several biological functions, modulating p53 transcriptional activity and tumor-suppressor functions. The biological activities of p53 isoforms, as well as their clinical implication in cancer, will be the subject of this review.

Physiological roles of p53

p53 family members: p63 and p73

The p53-related proteins p63 and p73 share significant structural and functional homologies with p53, particularly in the DNA-binding domain, including conservation of all essential DNA contact residues.⁵⁻⁷ p63 and p73 contain the three typical domains of a transcription factor: the amino-terminal transactivation domain (TAD), the DNA-binding domain (DBD) and the carboxyl-terminal oligomerization domain (OD). These two p53 homologues are involved in cellular responses to stress and development.⁸ They possess several functional properties and work together with p53 to regulate tumorigenesis. In response to stress signals, they can bind p53-target genes and induce their transcription. In the absence of cellular stress, p63 and p73 have important roles in the regulation of cellular differentiation and development. While p63 is important in the development of squamous epithelia, p73 has been shown to be involved in neuronal differentiation as well as nervous and olfactory system development. p53 family members are important in the development of congenital abnormalities in humans.⁹ Importantly, not only the individual roles of each p53 family member but also their interaction with one another are important for tumor suppression.³

p53, a tightly regulated major tumor suppressor

p53 is a 53 kD protein that is activated in response to alteration of normal cell homeostasis, including DNA damage, nutrient starvation, heat shock, virus infection, pH change, hypoxia, and oncogene activation.¹⁰ p53 maintains genetic stability by

regulating different processes, such as cell-cycle arrest, DNA synthesis and repair, programmed cell death, and energy metabolism. In the absence of stress signals, p53 protein is present at low levels, due to a dynamic and finely tuned balance between its transcription and its degradation. This balance is of great importance, as too much p53 can be lethal to cells, whereas too little can allow cancers to develop. p53 protein is tightly regulated in response to various cellular stresses at the transcriptional and translational level and by different posttranslational modifications, such as phosphorylation, acetylation, ubiquitination, neddylation, sumoylation, and methylation.¹¹ In particular, p53 protein level is regulated by ubiquitin ligases, such as HDM2 (also known as MDM2, for mouse double minute 2) and Pirh2.^{12,13}

All those modifications control the activation of p53 protein, as well as its subcellular localization, degradation, the choice of its protein partners, and therefore the outcome of the cellular response after stress: life or death. Indeed, after stress, p53 is activated by numerous mediators upstream in the pathway (ATM, CHK, ARF, among others), inducing an accumulation of p53 protein as a result of the inhibition of its repressors, such as MDM2 (Figure 1).

p53 is a transcription factor that binds directly and specifically as a tetramer to p53-responsive elements on DNA to induce or repress gene expression.^{14,15} It is estimated that more than 3,600 target genes are directly regulated by p53.¹⁶ Physiologically, p53 prevents damaged cells from proliferating. This function is of great benefit, as damaged cells are more likely to contain mutations and therefore exhibit abnormal cell growth, which can lead to the development of cancer.^{17,18}

Depending on the type of stress, activated p53 triggers either cell-cycle arrest and DNA repair or cell death, but the mechanism determining the choice between these fates has not yet been elucidated.

p53 biological activities as the “guardian of the genome”

Once activated, p53 can induce cell-cycle arrest in either the G₁ or G₂ phase of the cell cycle. Indeed, following DNA damage, p53 induces *p21*, a cyclin-dependent kinase inhibitor that mediates cell-cycle arrest at the G₁ and S phases of the cell cycle, allowing DNA repair. On the other hand, p53 can activate GADD45 (growth arrest and DNA damage), which regulates cell-cycle arrest in the G₂/M phases.¹⁹ Other p53-target genes also involved in cell-cycle control include miR-34a and the 14-3-3 proteins. Altogether, the presence of a functional p53 is important for the different checkpoints of the cell cycle, thereby giving cells time to repair DNA damage.²⁰

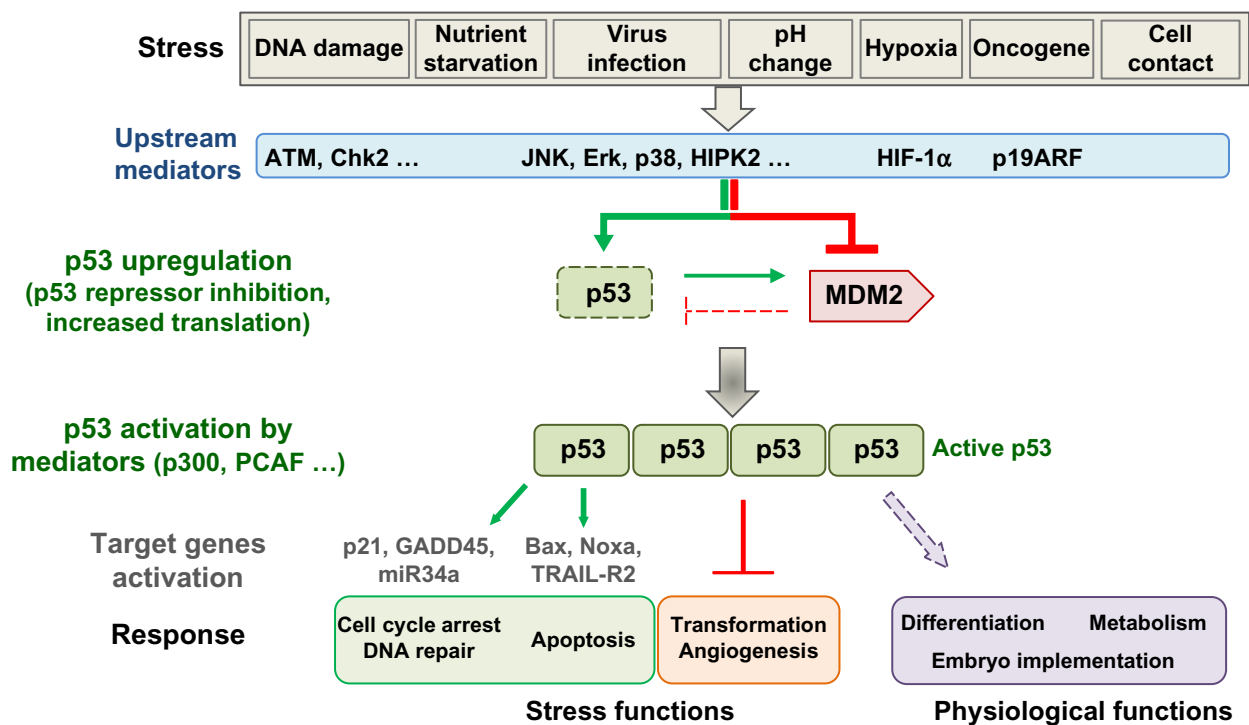


Figure 1 The p53 pathway. Different cellular stresses induce the activation of the upstream mediators (eg, ATM, Chk2, p19ARF), which upregulate p53 by inhibiting p53 interaction with MDM2, its main ubiquitin ligase. Then, p53 activated by mediators activates or represses its target genes according to the final outcome expected. In the absence of stress, p53 regulates many other physiological functions.

Abbreviations: DNA, deoxyribonucleic acid; ATM, ataxia telangiectasia mutated; JNK, c-Jun N-terminal kinase; HIPK2, homeodomaininteracting protein kinase 2; HIF-1 α , hypoxia-inducible factor-1 α ; MDM2, mouse double minute 2; PCAF, p300/CBP-associated factor; GADD45, growth arrest and DNA damage; TRAIL-R2, TNF-related apoptosis-inducing ligand-receptor2.

When damage is beyond repair, p53 triggers programmed cell death, and it is thought that p53-mediated apoptosis is the primary cause of tumor suppression. Many genes involved in apoptosis are p53-target genes, especially in the intrinsic pathway of apoptosis. Indeed, some of the proapoptotic members of the Bcl-2 family (Bax, Bid, Noxa, and Puma), as well as the mitochondrial proteins apoptotic protease activating factor 1 and second mitochondria-derived activator of caspases/DIABLO can be upregulated by p53.²¹ Conversely, the expression of antiapoptotic proteins, such as Bcl-2, Bcl-xL, or survivin can be repressed by p53. Regarding the extrinsic pathway of apoptosis, it has been shown that expression of the death receptors tumor necrosis factor-related apoptosis-inducing ligand R2 and Fas is controlled by p53.^{22–24} Moreover, it was highlighted that p53 can promote apoptosis in a transcriptional-independent manner.^{25,26} Indeed, after a stress signal, a part of the cytoplasmic pool of p53 is rapidly translocated to mitochondria to increase mitochondrial depolarization and thus cell death.^{27,28} More recently, it has emerged that p53 is involved in other types of cell death, such as autophagy and necroptosis.²⁹

More than 30 years of research on p53 have revealed that this tumor suppressor controls many biological activities, including energy metabolism, cell differentiation,

angiogenesis, cell migration, and embryo implantation.^{30–33} In brief, the literature accumulated over the last 34 years indicates that p53-mediated cell responses to damage are diverse because they are adapted (proportionately) to the nature, the extent, and the intensity of the damage, as well as to the cell type and the activated oncogenes (eg, Ras, epidermal growth-factor receptor, estrogen receptor 1). Although p53 is expressed in every cell of our body (except red blood cells), p53 does not impose, regardless of the tissue origin, a universal cell response to a given damage. Cells are of different tissue origin, and thus are composed of very different molecular components (eg, proteins, lipids, chromatin structure), which interact differentially with p53 and thus constrain p53 activity. It is essential to take account of the biological constraints of a cell in order to understand why a p53-mediated biological activity is triggered rather than another one.

p53 as a tumor suppressor in cancer

The p53 protein was first identified in 1979 as a cellular protein overexpressed in simian virus 40-transformed mouse cells and in cancer cells.^{34,35} Given that earlier studies had shown that this protein could promote cell proliferation and transformation, it was initially thought to be an oncogene.^{36,37}

However, in the late 1980s, different studies have not only revealed that the studied *TP53* genes bore missense mutations^{38,39} but also wild-type (WT) p53 protein could inhibit cell transformation induced by an oncogene.^{40–43} *TP53* mutations were then highlighted in germ-line cells of patients with the cancer-prone Li–Fraumeni syndrome^{44,45} and in numerous types of cancer cells. Moreover, Donehower et al¹⁷ have shown that mice deficient in *TP53* are susceptible to spontaneous tumorigenesis. As a consequence, *TP53* has been characterized as a tumor-suppressor gene, and is now commonly referred to as the “guardian of the genome”.

All events that are likely to promote cancer cell formation, such as an abnormal proliferation increase, oncogene activation, DNA damage, or an aberrant cell cycle, lead to an activation of p53. This tumor suppressor stops damaged cells from proliferating, triggers cell death, and thus prevents cancer formation. Because of its nodal role in integrating extracellular and intracellular signals to maintain cell homeostasis, p53 is inactivated in more than half of all human cancer.^{46,47} It can also be inactivated at the protein level through interaction with viral/cellular proteins, or epigenetic events (hypermethylation) of *TP53*.⁴⁸ Many studies have shown that the p53 pathway can also be inactivated indirectly via an increase of p53 degradation (MDM2 overexpression, *PTEN* or *INK4A/ARF* mutation, and deregulation of the Akt pathway, among others), an increased nuclear exclusion, or abnormalities in pathways upstream of p53 (ATM and Chk2 mutation) (Figure 2).

Mutations of *TP53* are mostly located in the DNA-binding domain, reducing or completely abolishing the function of the protein. Moreover, in recent years, p53 mutants have shown new properties, named “gain of function”, which are

different from those of p53 and enhance tumorigenicity.^{49,50} Frequently, *TP53* mutation or deletion is associated with chemotherapeutic failure, tumor progression, metastasis, and a shortened overall survival. However, some types of cancer have a low frequency of *TP53* mutation or deletion (breast cancer, acute myeloid leukemia [AML], and cervical cancer), and these abnormalities are not always associated with a poor prognosis. Therefore, in those types of cancer, the p53 pathway seems to be disrupted in other ways.

Introduction to the p53 isoforms

The p53 protein is encoded by the *TP53* gene, which is highly conserved through evolution and is located on the human chromosome 17p13.1. This gene comprises eleven exons, of which the first is noncoding (Figure 3A), and it contains multiple genetic polymorphisms defining over 100 distinct *TP53* haplotypes, some of which have been shown to be correlated with an increased risk of cancer.^{51–53} p53 isoforms were first identified in the late 1980s in humans by Matlashewski et al and in mice by Wolf et al.^{54,55} Thereafter, an alternative splicing of the *TP53* gene has been described.^{56,57} Using more sensitive technologies, we and others have shown that the *TP53* gene encodes at least twelve different p53 protein isoforms.^{56,58,63,64} The canonical p53 protein (also named p53, FLp53, p53 α , or Tap53 α) constitutes the most abundant isoform encoded by *TP53*. This protein of 393 amino acids has seven main functional domains (Figure 3B). The N-terminal transactivation domain is composed of two parts – TAD1 and TAD2 – which were shown to be required to induce a distinct subset of p53-target genes. The p53 protein also contains a proline-rich domain (PXXP) and a DNA-binding domain. The C-terminus domain encompasses an oligomerization domain

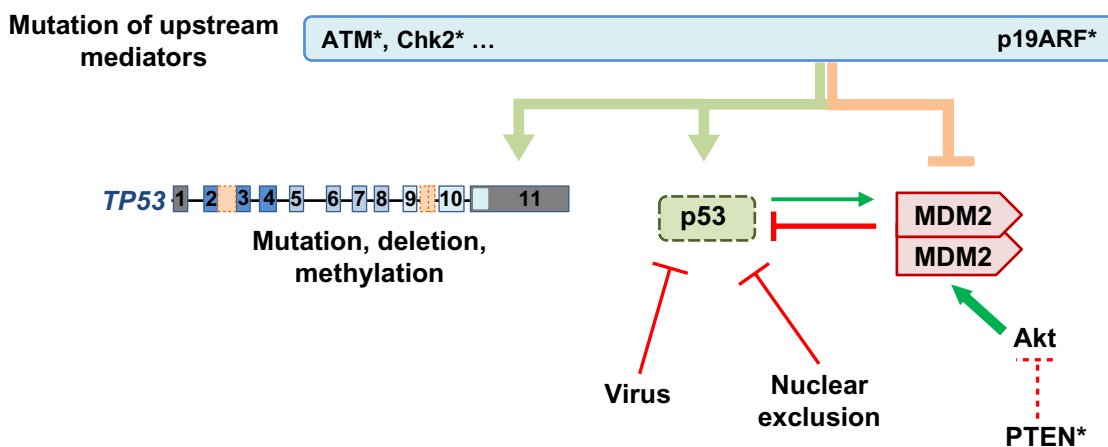


Figure 2 Many ways to inactivate the p53 pathway in cancer. The p53 pathway can be inactivated directly by mutation, deletion, or methylation of *TP53*, or indirectly by mutation (*) of mediators of the p53 pathway, proteins encoded by viruses, or a nuclear exclusion of p53.

Abbreviations: ATM, ataxia telangiectasia mutated; MDM2, mouse double minute 2; PTEN, phosphatase and tensin homolog.

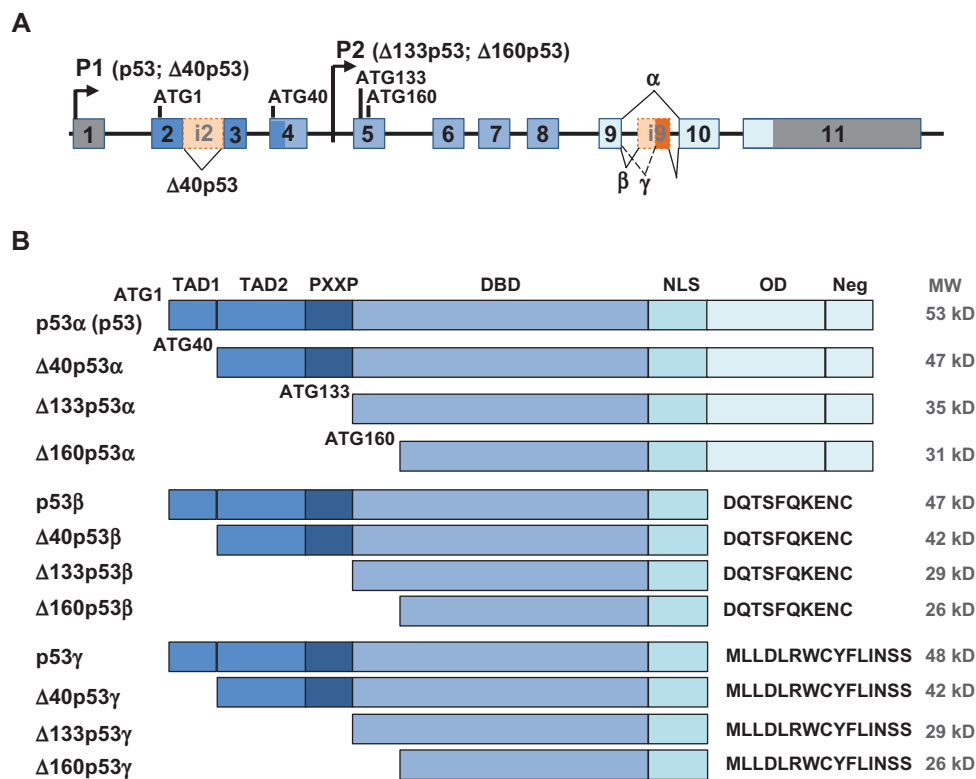


Figure 3 (A and B) The *TP53* gene encodes twelve different isoform proteins. **(A)** The human *TP53* gene structure. The *TP53* gene comprises eleven exons and encodes several p53 isoforms using alternative promoters (\leftarrow), splicing sites (\wedge), or translational initiation sites (I). **(B)** Human p53 isoforms. The canonical p53 (p53 α) has two transactivation domains (TAD1 aa 1–42 and TAD2 aa 43–63), a proline-rich domain (PXXP aa 64–92), a DNA-binding domain (DBD aa 102–306), a nuclear localization domain (NLS aa 316–325), an oligomerization domain (OD aa 307–355), and a negative-regulation domain (Neg aa 364–393).

Abbreviations: MW, molecular weight; kD, kilo Dalton.

(OD) that holds a nuclear localization signaling domain and a negative-regulation domain. The other p53 isoforms are the result of different conserved molecular mechanisms that include alternative splicing, alternative promoter usage, and alternative initiation of translation. Indeed, *TP53* contains an internal promoter (*P2*) in addition to its proximal promoter (*P1*). The proximal promoter controls the expression of p53 (TAp53 α , - β , and - γ) and Δ 40p53 (α , β , γ), while the internal promoter regulates the expression of Δ 133p53 (α , β , γ) and Δ 160p53 (α , β , γ). From these promoters, the twelve p53 isoforms are produced by the use of alternative splicing as well as alternative initiation of translation (Figure 3A).

p53 α , - β , - γ

p53 isoforms obtained from the *P1* and *P2* promoters of the *TP53* gene can be classically or alternatively spliced at their C-terminus to produce the α , β or γ isoforms. Indeed, a complete exclusion of intron 9 of *TP53* generates the α -form bearing the canonical C-terminal domain with a functional OD, whereas a partial retention of intron 9, also named exon 9b and exon 9g, gives rise to the β or the γ form, respectively. The entire OD of β and γ isoforms is replaced by

ten and 15 new amino acids, respectively, due to the presence of a stop codon in exon 9b and exon 9g (Figure 3B). The OD of p53 is very important for the regulation of the protein, as it contains the ubiquitination site for most ubiquitin ligases, such as MDM2, its best-characterized ubiquitin ligase. The molecular mechanisms that control the alternative splicing of intron 9 as well as the degradation of the β and γ isoforms remain poorly understood.

Contrary to p53, p53 β binds preferentially to the proapoptotic *Bax* promoter, whereas it binds poorly to the *MDM2* promoter. In the absence of cellular stress, p53 β can enhance p53 transcriptional activity on the *p21* and *Bax* promoters. p53 β can also induce apoptosis in a p53-independent manner, but to a lesser extent than p53.⁵⁸ Furthermore, it has been shown that cotransfection of p53 and p53 β induces senescence and enhances p53-mediated apoptosis.⁵⁹ Endogenous p53 and p53 β are able to oligomerize and form a protein complex, although it is thought that this complex requires the involvement of other proteins to occur.⁵⁸ Altogether, p53 β can regulate p53 tumor-suppressor activity by modulating its transcriptional activity, and thus the selection of target genes regulated.

p53 γ is expressed in normal human tissues. It differs from p53 in that it lacks the last 60 amino acids (α -domain), with the addition of 15 amino acids, which are referred to as the peptide γ . It localizes to both the nucleus and the cytoplasm.⁵⁸ Contrary to p53 β , it can enhance p53 transcriptional activity on the *Bax* promoter, but not on the *p21* promoter. p53 γ can also regulate gene expression independently of p53,⁶⁰ and has cytotoxic activity.⁶¹

$\Delta 40p53\alpha$, $\Delta 40p53\beta$, $\Delta 40p53\gamma$

The $\Delta 40p53$ isoforms (also named p47 or $\Delta Np53$) are generated by alternative splicing of intron 2 and/or alternative initiation of translation. Indeed, the retention of a part of intron 2 (i2) generates a stop codon between exons 2 and 3 in the p53i2 messenger ribonucleic acid (mRNA) that leads to the initiation of translation at AUG40. Moreover, two internal ribosome entry sites (IRES) have been identified in p53 mRNA, which allow its translation in a cap-independent manner in stress conditions. Consequently, these IRES sequences contribute to the alternative initiation of translation at codon AUG40 instead of AUG1 and therefore to $\Delta 40p53$ expression.^{62–64} Compared to p53, $\Delta 40p53$ lacks the first 39 amino acids and thus the main transactivation domain of p53 (TAD1), but retains the secondary one (TAD2) (Figure 3B). Little is known about $\Delta 40p53\alpha$, β and γ , but it has been shown that $\Delta 40p53\alpha$ has a dominant-negative effect toward p53, inhibits its transcriptional activities, and impairs p53-mediated growth suppression.⁶⁴ In addition, $\Delta 40p53\alpha$ influences p53 ubiquitination and subcellular localization.⁶⁵

$\Delta 133p53$ (α , β , γ), $\Delta 160p53$ (α , β , γ)

$\Delta 133p53$ and $\Delta 160p53$ isoforms are produced from the internal promoter (*P2*) of *TP53* in intron 4. Due to alternative initiation of translation, the translation of $\Delta 133p53$ is initiated at codon 133, while that of $\Delta 160p53$ starts at codon 160. Therefore, those splice variants lack the two transactivation domains (TAD1 and -2), as well as a part of the DNA-binding domain (Figure 3B).

$\Delta 133p53$ and $\Delta 160p53$ isoforms are differentially expressed in normal human tissues.⁵⁸ $\Delta 133p53\alpha$ is transactivated by p53, p63, and p73 isoforms after genotoxic stress.^{60,66} It modulates gene expression through direct interaction with p53, regulating differentially p53 transcriptional activity in a promoter-dependent manner.^{59,66,67} In addition, $\Delta 133p53\alpha$ has been shown to regulate gene expression independently of p53(α).^{60,68} Thus, $\Delta 133p53\alpha$ prevents p53-mediated replicative senescence, G_1 cell-cycle arrest (but not G_2 arrest), and apoptosis by regulating gene expression in a

p53(α)-dependent and -independent manner. $\Delta 133p53\alpha$ can also promote endothelial cell migration, blood vessel formation, and metastasis formation by regulating expression of angiogenic genes independently of p53.⁶⁸ Altogether, it suggests that $\Delta 133p53\alpha$ plays an active role in tumor formation and progression. Interestingly, $\Delta 133p53\alpha$ can promote gastric epithelial cell proliferation and tumorigenesis upon infection by *Helicobacter pylori*, by directly interacting with p73 isoforms to modulate expression of nuclear factor κB and increased survival of infected cells.^{69,70}

Little is known about $\Delta 133p53\beta$ and $\Delta 133p53\gamma$, which are expressed in normal human tissue and have different subcellular localization. Their role in cells remains unclear for now. $\Delta 160p53\alpha$, $\Delta 160p53\beta$, and $\Delta 160p53\gamma$ lack the first 159 amino acids of p53. It is thought that $\Delta 160p53\beta$ may have a role in erythroid differentiation.⁴⁹ Of note, this group of isoforms has been found to be expressed in K562 cells, which were previously thought to be “p53-null” cells.^{71,72}

Altogether, p53 isoforms can modulate the response to cellular stress either indirectly by regulating transcriptional activity of p53 family proteins or directly by binding to gene promoters implicated in apoptosis (*Bax*) or cell-cycle arrest (*p21* and *miR34a*). As a consequence, they can inhibit or enhance p53 tumor-suppressor activity. Nevertheless, it is thought that p53 isoforms have various functional and biological activities that can be p53-independent.⁷³

Role of p53 isoforms in human cancer and other pathologies

It is well established that the tumor-suppressor protein p53 has a major role with regard to the development and progression of cancer, as well as response to chemotherapy. But in numerous clinical studies, it is difficult to predict the clinical outcome and the therapeutic response based on the p53 status. Moreover, in some types of cancer, such as breast cancer or AML, mutation of *TP53* is not a frequent event. In fact, as described earlier, the p53 pathway can be inactivated in different manners in cancer. In recent years, given that p53 isoforms are differentially expressed in tumors compared with normal tissue, their role in carcinogenesis has emerged.⁷⁴

While normal breast tissues express p53 α , p53 β , and p53 γ , 60% of breast tumors have lost the expression of p53 β and p53 γ . Moreover, among these, 40% overexpress the isoform $\Delta 133p53$.⁷⁵ Regarding melanoma, it has been shown that the isoforms p53 β and $\Delta 40p53$ are expressed in tumor cells but not in melanocytes or fibroblasts.⁷⁶ In renal cell carcinoma (RCC), p53 β and $\Delta 133p53$ isoforms are overexpressed in

tumor cells compared with normal cells.⁷⁷ Other studies have shown that p53 isoforms are also abnormally expressed in AML, cholangiocarcinoma, glioblastoma, head and neck tumors, colon carcinoma, and ovarian and lung tumors,^{78–83} and it can be expected that numerous other types of cancer are concerned.

It has been described that some pathogens could modulate p53 isoform expression.⁸⁴ Indeed, it is well known that the canonical p53 protein (p53 α) can be inactivated by viral proteins.^{85,86} Moreover, it has been shown that the pathogenic bacteria *H. pylori* interacts with gastric epithelial cells and induces the expression of amino-terminally truncated $\Delta 133p53$ and $\Delta 160p53$ variants, thus increasing cell survival and possibly allowing cancer to develop.⁶⁹ On the other hand, polymorphism or mutations in the noncoding region of the *TP53* gene can affect the expression of isoforms. Indeed, a mutation in the IRES sequence, introns, or in splice sites can either abrogate the expression of certain isoforms or induce the transcription of tumor-specific p53 mRNA.^{78,87,88}

As described earlier, p53 isoforms have the ability to switch p53 activity between p53-mediated prosurvival activity and p53-mediated cell death (Figure 4). Consequently, when they are abnormally expressed, they can be a factor for cancer development and progression as well as chemotherapy sensitivity. From these results, several mouse models were developed to investigate the role of some p53 isoforms in cancer and other pathologies.

Slatter et al⁷⁰ have generated a mouse expressing a mutant of p53 deleted of the first 122 amino acids ($\Delta 122p53$) in order

to mimic human $\Delta 133p53$. However, contrary to human $\Delta 133p53$, mouse $\Delta 122p53$ mutant expression is not driven by the endogenous p53 internal promoter. Therefore, $\Delta 122p53$ is not expressed in a tissue-dependent manner, and thus does not exactly recapitulate the roles of human $\Delta 133p53$ in cancer formation. Despite this limitation, the $\Delta 122p53$ mouse model is useful, because it demonstrated that mutant $\Delta 122p53$ protein is active in promoting hyperproliferation and cancer development, despite having lost both transactivation domains and part of the DNA-binding domain. In addition, $\Delta 122p53$ mice show a profound proinflammatory phenotype having increased serum concentrations of interleukin 6 and other proinflammatory cytokines, with aggregation of lymphocytes in the lung and liver. Therefore, $\Delta 133p53$ may promote cell proliferation and inflammation, contributing to tumor development.

This is consistent with recent publications on gerbils demonstrating that endogenous $\Delta 153p53$ isoforms, homologous to human $\Delta 133p53/\Delta 160p53$, are induced upon infection by *H. pylori*, promoting gastric epithelial cell proliferation, cytokine expression, and tumorigenesis formation.^{69,70} Furthermore, Bernard et al⁶⁸ have demonstrated in a mouse xenograft model and chick chorioallantoic membrane assay that $\Delta 133p53\alpha$ could stimulate cell migration, angiogenesis, and thus cancer progression by regulating angiogenic gene expression. Altogether, this confirms that $\Delta 133p53\alpha$ is an active protein that has an important biological relevance.

By comparing $\Delta 122p53$ and $\Delta 40p53$ mouse models, it appears that $\Delta 122p53$ and $\Delta 40p53$ have different biological

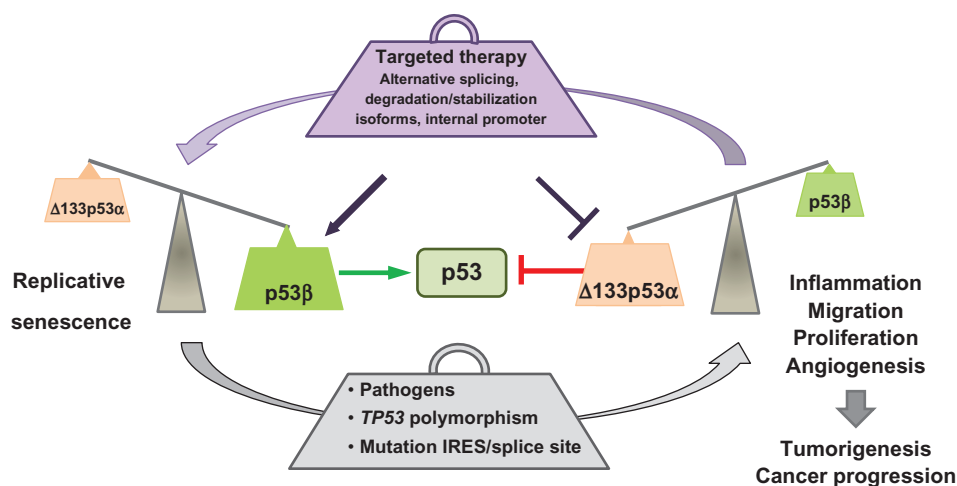


Figure 4 p53 isoforms in cancer and targeted therapy. Depending on the cell context, some p53 isoforms, such as $\Delta 133p53\alpha$, inhibit the antitumor role of the canonical p53 α , while others potentiate its activity. In cancer, the imbalance between the p53 isoforms can promote a prosurvival effect, and thus allows cancer cells to survive. The prosurvival phenotype of these cells can be changed toward the proapoptotic phenotype by altering the p53 isoform-expression profile. Using small molecules targeting the alternative splicing of p53, protein degradation, or p53 internal promoter activity, it could be possible to decrease expression of antiapoptotic isoforms, or on the contrary to increase proapoptotic isoform expression.

Abbreviations: IRES, internal ribosome entry site.

activities and are thus not equivalent. While overexpression of $\Delta 122p53$ or induction of endogenous $\Delta 153p53$ by *H. pylori* induces cancer, overexpression of transgenic $\Delta 40p53$ in mice expressing WT *TP53* alleles induces premature ageing and diabetes, but not cancer.^{89–93} This is consistent with a role of $\Delta 40p53$ in regulating cell stemness.⁹¹

This suggests that p53 isoforms have roles in pathologies other than cancer. Indeed, we recently reported that p53 isoforms are also differentially regulated in response to flu virus infection and play an active role in regulating flu virus production.^{84,94–96}

Overview of the clinical implications of p53 isoform activity in cancer

Numerous clinical studies have demonstrated that the expression profile of p53 isoforms can be linked with tumor progression, clinical response, and/or prognosis. Contrary to p53 β , which promotes replicative senescence, $\Delta 133p53\alpha$ promotes proliferation and senescence escape (Figure 4). As a consequence, Fujita et al⁹⁹ have described that an inversion of the p53 β / $\Delta 133p53\alpha$ ratio (a decrease of p53 β expression associated with an increase of $\Delta 133p53\alpha$ expression) could allow cancer progression from colorectal adenoma to carcinoma. In RCC, p53 β overexpression in tumors is related to tumor stages, and could be a good predictor of cancer progression.⁷⁷ Regarding mucinous ovarian cancer, Hofstetter et al have shown that p53 β and $\Delta 133p53$ are abnormally expressed. In addition, the expression of $\Delta 40p53\alpha$ in WT p53 ovarian cancer was associated with an improved recurrence-free survival.⁹⁷ Importantly, in serous ovarian cancer, it was reported that $\Delta 133p53\alpha$ expression was associated with higher disease-free survival and overall survival in p53 mutant advanced serous ovarian carcinoma cancer cases, while $\Delta 40p53\alpha$ was associated with higher disease-free survival and overall survival in WT p53 advanced serous ovarian carcinoma patients.⁹⁸ This suggests that p53 mutation status can affect the prognostic value of p53 isoforms. In addition, p53 β expression was associated with adverse clinicopathologic markers, such as serous and poorly differentiated cancers, and was associated with worse recurrence-free survival in patients exhibiting functionally active p53.⁷⁸ Moreover, the expression of a tumor-specific splice-site mutant p53 mRNA (p53 δ) is associated with therapeutic failure and thus a poor prognosis.⁷⁸ In breast cancer, determination of p53 γ isoform expression in p53 mutant breast cancer patients allows the identification of the ones with a particularly poor prognosis. Indeed, mutant p53 breast patients expressing p53 γ have as good a prognosis as WT p53 breast cancer patients, while mutant p53 breast

cancer patients not expressing p53 γ have a particularly poor prognosis, and would benefit from additional treatment.⁷⁵ In cholangiocarcinoma, $\Delta 133p53$ overexpression is associated with a shortened overall survival.⁸³ In acute myeloid leukemia, determination of p53 isoform expression revealed distinct p53 protein biosignatures correlating with clinical outcome.^{81,99}

As p53 isoforms modulate p53 tumor-suppressor activity and gene expression, it is expected that their overexpression or loss of expression in cancer is associated with carcinogenesis. However, in some cases, they could represent an alternative way to abrogate gain of function of mutant p53 in cancers. For example, $\Delta 133p53$ overexpression in mutant p53 ovarian cancer or p53 γ expression in mutant p53 breast cancer overcomes the adverse impact of *TP53* mutation.^{75,98}

A new therapeutic strategy currently in development consists in the reactivation of p53 in cancer cells by reactivating mutant p53 protein or by inhibiting p53 repressors, such as MDM2.^{100,101} The uncovering of p53 isoforms enlarges this research field. Moreover, based on the observation that aberrant p53 isoform expression is associated with cancer development and progression, it can be assumed that they could become tumor markers and efficient targets for cancer therapy.

It is well known that different cellular stresses, such as DNA damage, change the mRNA-splicing process.¹⁰² Indeed, several studies have already shown that expression of p53 isoforms can be modulated in vivo in response to chemotherapy.^{76,81} These changes of splicing can be correlated with an increase of sensitivity, or on the contrary generate chemoresistance. From these observations, it is expected that the identification of splicing factors or IRES-transacting factors involved in isoform expression will allow defining novel therapeutic targets and new treatments. Some regulators of p53 isoforms have already been identified, such as the IRES transacting factors polypyrimidine tract-binding protein (PTB), dyskerin, death-associated protein 5 (DAP5), annexin A2, and PTB-associated splicing factor (PSF).^{103–106} Treatments targeting these factors and thus isoform expression could reverse the adverse effect of an elevated $\Delta 133p53\alpha$ /p53 β ratio in order to stop proliferation and cancer progression (Figure 4). In addition, it is possible to manipulate p53 isoform expression by regulating their protein-degradation pathway. The entire ubiquitinase network of p53 family proteins is not known yet, but it has been shown that p53 isoforms are differently affected by the main E3 ligase of p53 α – MDM2.¹⁰⁷ The MDM2 antagonist nutlin-3a has already been shown to be effective in stabilizing p53 α to sensitize cells to chemotherapy.^{101,108} The identification of

other ubiquitin ligases involved in p53 isoform degradation could allow the design of new targeted therapies.

Conclusion

Since the discovery of p53 more than 30 years ago, the need to acquire more knowledge about this protein has constantly increased, given its prominent role in cancer. p53 is involved in many physiological processes, the most studied being its tumor-suppressor function. p53 integrates much cell signaling from damaged internal subcellular organelles, as well as cell–cell contact, extracellular matrix, hormones, cytokines, and nutrient level. Based on these signals, p53 contributes to the cell-fate decision to trigger cell survival, senescence, differentiation, cell migration, or programmed cell death. However, the underlying molecular mechanisms are still unclear. Owing to various technical advances in biochemistry and genetics, different studies have highlighted that the *TP53* gene encodes several p53 protein isoforms, which cooperate with p53 and modulate its activity toward either promoting cell survival or death. Given that p53 isoforms play a fundamental role in the regulation of the p53 pathway, their expression is often deregulated in cancer. One could therefore wonder whether WT *TP53* can act as a tumor-suppressor gene or oncogene, depending on the p53 isoform-expression profile. In some cancer types, abnormal p53 isoform expression has been correlated with clinical response, cancer recurrence, and/or overall survival. Moreover, certain isoforms seem to be potential markers for cancer therapy. However, p53 isoforms cannot be categorized into oncogenic or tumor-suppressor classes, since their biological activities and thus their prognostic value are associated with the cell context. Indeed, $\Delta 133p53\alpha$ expression is associated with cancer formation and progression in cholangiocarcinoma, as well as in colon and gastric cancers, while $\Delta 133p53\alpha$ is associated with a lower risk of cancer recurrence and death in mutant p53 serous ovarian cancer. Similarly, p53 β is associated with clinicopathological markers of good prognosis in colon cancer and AML, while p53 β is associated with worse recurrence-free survival in serous ovarian cancer patients exhibiting functionally active p53.

Further clinical studies will be necessary to develop a deeper understanding of their involvement in cancer. On the other hand, research on molecules that “reactivate” the p53 pathway in tumor cells appears to be a promising way as long as the cell context is taken into account. It is expected that some p53 isoforms will be good therapeutic targets in defined cancer subtypes, such as luminal or triple-negative breast cancer. To achieve this, future experiments will focus on defining the cell contexts that constrain WT or mutated

p53 isoforms to promote cell survival or cell death in response to a given damage or treatment. The results will allow better understanding as to how p53 isoform expression is regulated, thus enabling the design of efficient treatments.

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Disclosure

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