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Review

p53 mutation heterogeneity in cancer

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Abstract

The p53 gene is inactivated in about 50% of human cancers and the p53 protein is an essential component of the cell response induced by genotoxic stresses such as those generated by radiotherapy or chemotherapy. It is therefore highly likely that these alterations are an important component in tumor resistance to therapy. The particular characteristics of these alterations, 80% of which are missense mutations leading to functionally heterogeneous proteins, make p53 a unique gene in the class of tumor suppressor genes. A considerable number of mutant p53 proteins probably have an oncogenic activity per se and therefore actively participate in cell transformation. The fact that the apoptotic and antiproliferative functions of p53 can be dissociated in certain mutants also suggests another level of complexity in the relationships between p53 inactivation and neoplasia.

Keywords: *TP53* tumor suppressor gene mutations; Oncogene; Gain of function; Apoptosis

p53 mutations are found in approximately 50% of human cancers [1]. Apart from the fact that tumor cells must select for inactivation of the *p53* network that safeguards the cell from various types of insults, these mutations are oncogenic and have been the subject of extensive studies providing a better understanding of their origin [2,3]. The *p53* protein is a transcription factor that binds a very loose DNA recognition sequence found in several hundred genes that are differentially activated depending on the cell type, identity, and extent of damage, and various other parameters that have yet to be identified [4–6]. The unique feature of *TP53* compared to other tumor suppressor genes is its mode of inactivation. While most tumor suppressor genes are inactivated by mutations leading to absence of protein synthesis (or production of a truncated product), more than 80% of *p53* alterations are missense mutations that lead to the synthesis of a stable full-length protein [1]. This selection to maintain mutant *TP53* in tumor cells is believed to be required for both a dominant negative activity to inhibit wt *TP53* expressed by the remaining allele, and for a gain of function that transforms mutant *TP53* into a dominant oncogene [7–9]. An important feature of the *TP53* protein is the extreme flexibility and fragility of the DNA binding domain (residues 90–300) [10], as more than 200 of the 393 residues have been found to be modified and several residues have sustained multiple alterations. Most *TP53* mutations are localized in the DNA binding domain of the protein (residues 100–300) leading to a bias of *TP53* mutation analysis, as more than 80% of *TP53* mutation studies focus on exons 5–8 (residues 126–306) [1]. One of the most puzzling aspects of mutant *p53* proteins is their structural, biochemical, and biological heterogeneity, a topic that will be specifically developed in this review.

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The p53 gene: a tumor suppressor gene or an oncogene? A caretaker or a gatekeeper?

The history of p53 is a chaotic voyage from the world of oncogenes to the world of tumor suppressor genes, while retaining a certain degree of individuality [8]. Apart from artefactual problems related to involuntary cloning of mutant p53, this ambiguity is also due to our propensity to over-categorize in order to satisfy our Cartesian and oversimplistic view of science.

The idea that some p53 mutations can actively participate in cellular transformation was originally postulated in 1990 and several arguments favor such a model [8,11]. First of all, the mode of "inactivation" of wild-type p53. Unlike most other tumor suppressor genes that are inactivated by frameshift or nonsense mutations leading to disappearance or aberrant synthesis of the gene product, almost 80% of p53 gene mutations are missense mutations leading to the synthesis of a protein, lacking its specific DNA binding function and accumulating in the nucleus of tumor cells [1]. This particular selection for accumulation of p53 mutations in tumor cells can have two consequences: (i) a dominant negative role by hetero-oligomerization with wild-type p53 expressed by the second allele or (ii) a specific gain of function of mutant p53 (Fig. 1). Many studies have tried to distinguish between these two hypotheses, with no clear-cut conclusions [10,12]. This task is further complicated by the fact that not all p53 mutations appear to be equivalent and present a marked heterogeneity of activities. Transfection of various p53 mutations into cells devoid of endogenous p53 leads to an increase in their carcinogenicity, which varies according to the type of mutation [9,13,14]. Even in mouse embryo fibroblasts heterozygous for the mouse equivalent of the human R175H mutation, mutant p53 exhibits increased proliferative capacity and transformation potential [15]. This research into the oncogenic potential of certain p53 mutations is not purely theoretical, but has obvious clinical implications, as it could explain the marked disparity of the results of studies trying to demonstrate a relationship between the presence of a p53 gene mutation and various clinical parameters, such as survival or response to treatment. In breast cancer patients, the response to adriamycin is very strongly correlated with the presence of a mutation specifically localized in the loop domains L2 or L3 of the p53 protein [16]. In vitro, the expression of p53 mutations in position 175 (R175H) specifically induces resistance of cells to etoposides compared to other p53 mutations [17].

In addition to activities of mutant p53, p53 has two homologues that might also contribute to its oncogenic potential. p63 and p73, discovered 6 years ago, express many isoforms due to alternating use of transcription promoters and alternative splicing [18]. Long isoforms (TA-p73 or TA-p63) are able to transactivate the same target genes as p53 and induce apoptosis, while short forms (DN-p63 or DN-73) have an opposite activity via dominant negative mechanisms. p63 and p73 are able to cooperate with p53 to induce apoptosis, suggesting the existence of a complex network of interactions between the products of these three genes [19]. Although

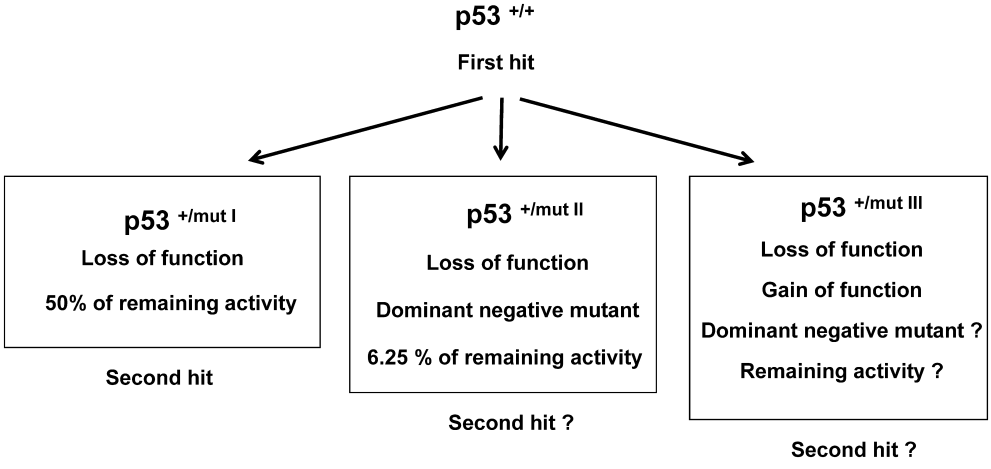

Fig. 1. p53 and cancer: in the classical situation (left, mut I), the first hit leads to inactivation of p53 without affecting the activity of the second allele. However, this loss of function may have certain consequences for the cell. The second hit leads to complete loss of function of p53. In the case of a dominant negative mutant (middle, mut II), an estimated 1/16 of the tetramers theoretically have 4 wild-type monomers if the two proteins are expressed in identical quantities and tetramerization is not affected by the mutation. If the dominant negative effect of a single monomer is sufficient to inactivate p53, then the remaining activity will be 6.25%. In the case of partial penetrance of the dominant negative effect, the activity gradient will be between 6% and 50%. Loss of the second allele may not be mandatory, depending on the remaining activity. In the case of a mutant with a gain of function (right, mut III), the situation is more complex with an important combinatorial effect. Loss of the second allele may also be unnecessary in this case. This particular situation of the p53 gene results from: (i) its particular mode of inactivation by missense mutations and (ii) its tetrameric structure.

wt p53 does not interact with p73 or p63, some mutant p53 proteins bind strongly to the two p53 homologues via their DNA binding domains (see below). This interaction leads to inactivation of p73 and p63 function [20–23].

It is therefore likely that although the *wild-type* p53 gene is effectively a tumor suppressor gene, some *mutant* p53 proteins can be considered oncogenes (Fig. 1). The distinction between oncogene and tumor suppressor gene, although simple and able to account for the mechanisms of activation or inactivation of these various genes, is probably imperfect and does not allow characterization of all genes involved in the processes of carcinogenesis.

The genes involved can also be classified as a function of the various roles that they play in malignant transformation of a cell when they are altered. Three types of genes are distinguished at the present time [24,25]. Gatekeeper genes regulate cellular homeostasis and the cell cycle by controlling the entry of the cell in the various phases of the cell cycle (Rb, VHL, or APC). Caretaker genes participate in maintenance of the integrity of the genome and allow the cell to transmit an identical genome during successive cell divisions; they act as caretakers of the genome (MLH1, MSH2, or gene XP). Finally, landscaper genes maintain the integrity and equilibrium of the various cellular components of a tissue (PTEN, Smad4). Once again, the situation is more ambiguous for the p53 gene, which can be classified as both a gatekeeper and a caretaker gene. Its apoptotic and antiproliferative activity makes it an important gatekeeper and re-introduction of a wild-type p53 gene into a tumor cell effectively restores the cell cycle control properties. On the other hand, its activity in the control of chromosomal stability would classify p53 as a caretaker gene, whose phenotype cannot be corrected by functional supplementation. p53 probably has a very heterogeneous role in tumor processes as a function of the tissue considered, the chronology of the p53 alteration (early or late), the p53 function targeted (cell cycle or apoptosis) and finally, but importantly, the nature of the other alterations already present in the cell.

Heterogeneity of p53 mutations in human cancers

The selection to maintain mutant *TP53* in tumor cells is believed to be required for both a dominant negative activity of wt *TP53* expressed by the remaining allele as discussed previously. An important feature of the *TP53* protein is the extreme flexibility and fragility of the DNA binding domain (residues 90–300) [10], as more than 200 of the 393 residues have been found to be modified and several residues have sustained multiple alterations. Most *TP53* mutations are localized in the DNA binding domain of the protein (residues 100–300) leading to a bias of *TP53* mutation analysis, as more than 80% of *TP53* mutation studies focus on exons 5–8 (residues 126–306) [1]. One of the most puzzling aspects of mutant *TP53* proteins is their structural, biochemical, and biological heterogeneity. The structural difference between the various mutant *TP53* was initially identified using monoclonal antibodies able to discriminate mutations that change *TP53* folding and mutations in the residues involved in DNA recognition [26,27]. Two classes of mutations have been distinguished on the basis of various in vitro assays and the three-dimensional structure of the protein [28]: class I mutations, exemplified by mutants at codon 248 (7.6% in the p53 database, http://p53.free.fr/), affect amino acids directly involved in the protein–DNA interaction. They have a wild-type conformation as probed by conformational monoclonal antibodies and they do not bind to the chaperone hsp70 [29,30]. Class II mutations, exemplified by the R175H mutant (4.9% in the database), have an altered conformation with intense binding to hsp70. The amino acids altered in this class of mutants are involved in stabilizing the tertiary structure of the protein. Class II mutations are associated with a more severe phenotype in vitro than class I mutations [29]. Due to an irreversible change of conformation, class II mutants cannot be restored to the wild-type conformation by activating antibodies or peptides [31]. Such heterogeneity can also lie in the nature of the resulting residue. Mutant R273H has a wild type conformation whereas mutant R273P is denatured [29]. This biochemical and biological heterogeneity has been confirmed and refined by structural studies. For example, NMR spectroscopy suggests that mutations in the L3 domain can induce either limited or extensive conformational changes, depending on their position or the type of substitution [32,33].

Recent analyses using more sophisticated biophysical techniques have revealed that the central region of the *TP53* protein can adopt at least five thermodynamic states [34]. Biochemical analyses have shown that *TP53* mutant proteins can be heterogeneous in terms of loss of DNA binding activity and transactivation. The DNA binding site recognized by *TP53* is highly degenerated and the affinity of *TP53* for the various biological sites is variable [35]. Some mutant *TP53* display only partial loss of their DNA binding activity allowing the mutant to bind only to a subset of *TP53* response elements [36,37]. This feature is linked to a differential transactivation activity. Biologically, these mutants have lost their apoptotic properties, but their cell cycle arrest activity remains similar to that of the wt protein. The biological significance of one of these mutants has been elucidated in a mouse model [38]. Mice homozygous for the R172P mutation (equivalent to the R175P alteration) are defective in p53-dependent apoptosis, but retain a partial cell cycle checkpoint function. Importantly, these mice exhibit a delayed tumor phenotype strongly indicating that the cell cycle checkpoint func-

tion of p53 is as important as apoptosis in tumor suppression (Table 1).

Do these differences in structure and function of the various p53 mutants have clinical implications? Several studies have revealed that specific p53 mutations are associated with either a poorer prognosis or a poor response to treatment. In breast [39–41] and colon cancer [42,43], there is a strong association between mutations in the L2/L3 loop and shorter survival or poor response to treatment. These data are also emphasized by the observation that the distribution of tumors in *Trp53*$^{-/-}$ (*Trp53* is the mouse gene encoding p53) mice differs from that of mice harboring point mutations [38,44] (Table 1).

Various pathways leading to an oncogene

Many heterogeneous mechanisms can lead to a gain of function of mutant p53 [45,46]. Most of these

Table 1
Heterogeneity of phenotype in mice expressing different p53 mutants

Mutation[a]	Mutant properties	Mouse status	Phenotype	Reference
P53 gene knockout	Total deletion of the gene	Heterozygote	High susceptibility to spontaneous tumours Mean survival times of 15–16 months High frequency of T-cell lymphoma and sarcoma with Frequent aneuploidy Rare metastasis	[68,69]
		Homozygote	High susceptibility to early spontaneous tumours Mean survival times of 4–5 months High frequency of T-cell lymphoma and sarcoma with Frequent aneuploidy Rare metastasis	
Knockin R172P (R175P)	Mutant deficient for apoptosis only	Heterozygote	No data available	[38]
		Homozygote	High susceptibility to spontaneous tumours Mean survival times longer than for p53$^{-/-}$ mice (12 months) High frequency of T-cell lymphoma and sarcoma The majority of tumours are diploid	
Knockin R172H (R175H) (C57BL/6)	Hot spot mutant found in human tumors	Heterozygote	High susceptibility to spontaneous tumours Mean survival times of 15–16 months High frequency of T-cell lymphoma and sarcoma with frequent aneuploidy High frequency of metastasis	[15]
		Homozygote	High susceptibility to early spontaneous tumours Mean survival times of 4–5 months High frequency of T-cell lymphoma and sarcoma with Frequent aneuploidy Rare metastasis (Similar to p53$^{-/-}$ phenotype so far)	
Knockin R172H (R175H) 129S4/SVJae	Hot spot mutant found in human tumors	Heterozygote	High susceptibility to spontaneous tumours Mean survival times of 15-16 months High frequency of metastatic sarcomas	[44]
		R172H/KO[b]	High susceptibility to early spontaneous tumours Mean survival times of 4-5 months High frequency of metastatic carcinomas	
Knockin R270H (R273H) 129S4/SVJae	Hot spot mutant found in human tumors	Heterozygote	High susceptibility to spontaneous tumours Mean survival times of 15–16 months High frequency of metastatic carcinomas	
		R172H/KO[b]	High susceptibility to early spontaneous tumours Mean survival times of 4–5 months High frequency of metastatic carcinomas	

As the genetic background of the mouse strain has a profound influence on the phenotype of mouse with similar p53 mutations, we have individualized each study and indicated mouse strains that were used.
This table summarizes only mouse models expressing mutant p53 found in human cancer. Other mouse models with mutations in regulatory domains are not described.

[a] The position of the mutation corresponds to the mouse p53 gene and for human p53 inside the parentheses.

[b] The p53 mutant is expressed on a p53 null background leading to the expression of one mutant p53 allele without wt p53.

mechanisms have been analyzed in cell systems and their existence in vivo in human tumors remains to be demonstrated. These mechanisms can be classified into two types: those involving an interaction between mutant p53 and cellular proteins, and those involving an interaction with DNA, although these two types of mechanisms are not mutually exclusive.

The DNA mechanism was initially considered to be predominant with the discovery of genes specifically transactivated by certain p53 mutants [45,47–49] for a complete list of these genes. These genes are generally involved in proliferation or cell survival. Analysis of the DNA sequences recognized by these mutants shows that they are completely different from the consensus site found in genes regulated by wild-type p53. Furthermore, no nucleotide consensus has been defined by comparing these sequences. The work conducted by Deppert et al. [50–52] suggests that this interaction between mutant p53 and DNA could be related to the conformation and structure of the DNA and not to its sequence. This model is based on the observation that various types of mutant p53 (but not wild-type p53) can recognize the MAR (''matrix attachment region'') sequence. These DNA sequences anchor chromatin fibers to the nuclear matrix and generate domains that can have either a transcriptionally active or inactive structure. MARs are polymorphic and appear to be distributed throughout the genome. There is no known consensus sequence that is characteristic of a MAR. Due to the known structural flexibility of MAR elements, in vitro binding of mutp53 to MAR elements is independent of the presence of any consensus motif, but is greatly dependent on the length of DNA. Whereas sequence-specific recognition of linear (duplex) DNA is mediated solely by the p53 core DNA binding domain, binding to MAR is more complex, involving either solely the C-terminal DNA binding domain, or the p53 core domain and the p53 C-terminus.

More recently, Zalcenstein et al. [53] showed that the hot spot R175H mutant was able to strongly inhibit transcription of the Fas pro-apoptotic gene. Other mutants (codons 248 and 273) have a similar property, but less pronounced. As wild-type p53 activates transcription of the Fas gene, there may be a mixed regulation phenomenon on this gene, according to the p53 gene status. The authors showed that this inhibition of transcription by mutant p53 required binding of the protein to a different promoter site from that recognized by wild-type p53.

Wild-type p53 also interacts with a large number of cellular proteins. Some of these proteins are important for regulation of p53 transcriptional activity (p300, sp1, and TBP), but are also associated with all cellular transcription processes. As the quantity of mutant p53 in a tumor cell is very much greater than that of wild-type p53, a ''squelching'' effect probably occurs, leading to quantitative or qualitative transcriptional abnormalities.

Recent studies have shown that resistance to anticancer agents involves inactivation of the apoptotic function of the p73 protein by mutant p53 [54]. As already discussed, the two homologous genes of p53, p63, and p73 have been identified. As the p73 gene is only rarely genetically or epigenetically altered in human tumors, is it possible to consider another indirect mechanism leading to p73 inactivation? This is a fundamental question, as p73 inactivation could explain certain mechanisms of resistance to chemotherapy. The answer to this question can be found in studies of protein interactions between various members of the p53 family. Although it has now been fairly clearly established that wild-type p53 cannot form stable heteroligomers with p73 or p63, this is not the case for mutant p53. A strong interaction involving the DNA binding domain of the two partners has been characterized between certain p53 mutations and p73 or p63 [21,22,55]. This interaction also leads to inactivation of the transactivation functions of p73 and p63. This has now also clearly been shown in a mouse model with the R172H (R175H in humans) mutation. Mouse embryo fibroblasts and tumor cells from mice expressing only the mutant protein functionally inactivate p63 and p73 [15]. Moreover, downmodulation of p63 and p73 increases the transformation potential of p53-null cells to a level similar to that of p53 mutant cells.

Various types of p53 mutations can interact with p73, depending on the author and experimental conditions, but recent studies show that a change of conformation of the central domain of mutant p53 is the essential component in this interaction with p73 [56]. On the other hand, several groups have shown that p53 polymorphism in codon 72 (Arg or Pro) has an important influence on this interaction, as it is only detected in mutant p53 with Arg polymorphism [22]. This polymorphism in codon 72 is not neutral, either structurally or functionally, and recent studies suggest that the Arg form of wild-type p53 has a greater apoptotic activity than the Pro isoform. Using isogenic cell lines expressing a great diversity of p53 mutations either in the Arg form or the Pro form, Bergamaschi et al. [54] showed that only those mutants expressing the Arg form are resistant to cytotoxic agents. Furthermore, analysis of a homogeneous population of patients with head and neck cancer presenting identical p53 mutations to those studied in vitro demonstrated that the majority of patients expressing mutant p53 associated with Arg polymorphism have a poor response to chemotherapy and a shorter survival. This is the first large-scale study combining both basic research and clinical data in order to more accurately evaluate the role of the p53 signaling pathway in human tumors.

The majority of p53 mutations are localized in the DNA binding domain in the central part of p53. This domain is also the binding site for the p53BP2/ASPP1 protein [57]. Crystallographic analysis of the complex between the two proteins demonstrates a marked homology between the p53 residues involved in this interaction and those interacting with DNA [58]. Most of the hot spot p53 mutations are also unable to interact with this protein. p53BP2/ASPP1 and a second protein, ASPP2, are important cofactors in the transactivational activity of p53 in relation to apoptotic genes [59]. The mechanisms leading to this specific activation are unknown at the present time and it is still too early to know whether loss of this interaction participates in neoplasia.

Mechanistically, the stability of mutant p53 proteins would enhance interactions with cellular proteins and contribute to its oncogenic properties. The stability of mutant p53 proteins as compared to wild type p53 is well known and has been used as a means of detecting mutant p53 in human cancers by immunohistochemistry. However, the inherent stability of mutant p53 has come into question in the analysis of mice with two different p53 mutations [15,44]. Normal tissues from knock-kin mice heterozygous for the R172H (equivalent to human R175H) and the R270H (equivalent to human R273H) mutations do not have stable mutant p53. Stable p53 was seen in some, but not all, tumors in heterozygous mice. Mutant p53 instability was not due to the presence of wild-type p53 as loss of wild type p53 was not a common event in the genesis of tumors in these mice. Moreover, mice engineered with one mutant and one p53 null allele also had unstable mutant p53. The instability of mutant p53 seen in tumors indicates that secondary events in the genesis of the tumors contribute to mutant p53 stability and potentially to the gain of function phenotype.

Apoptosis or control of the cell cycle: are both targets in human cancers?

p53 is certainly essential to the cellular response after stress by inducing a series of signals leading to DNA repair, cell cycle arrest or apoptosis. On the other hand, the way in which p53 activates this response is not as simple as previously thought. Historically, the antiproliferative function of p53 was considered to be the most important function following the discovery of its first target, the p21Waf1 gene. This was followed by demonstration of the apoptotic function of p53 with the characterization of multiple pro-apoptotic target genes (bax, puma, and noxa, see [60] for review). By 1995, it appeared that the apoptotic activity of p53 was not exclusively related to its transactivation activity and that it could be very dependent on the cell type used. p53 also possesses a transcriptional repression activity, the mechanisms of which have not been fully elucidated. The Bcl-2 anti-apoptotic gene is one of the targets of this activity. As indicated below, p53 also probably has a direct apoptotic activity on the mitochondrion.

This new pro-apoptotic function of p53, independent of transcription, has been recently discovered. Following a genotoxic stress, a small quantity of p53 is detected on mitochondria [61]. Mitochondrial p53 interacts with the anti-apoptotic protein Bcl-XL, induces bak oligomerization, and leads to release of cytochrome *c* after permeabilization of the mitochondrial membrane [62]. This is a very rapid response (30 min), which precedes the transcriptional response (at least 2 h) [63]. This response is also tissue-specific and appears to be limited to radiosensitive tissues. It is noteworthy that the mutants found in human cancers (and therefore presenting defective transcriptional activity) are also unable to interact with Bcl-XL and are consequently unable to induce apoptosis. If these results are confirmed, these mutations could be considered to be ''double hit'' mutation able to simultaneously or differentially inactivate various biological properties of p53.

It has been generally accepted that the apoptotic activity of p53 is the main target of p53 gene mutations. The absence of this apoptotic activity could therefore not only account for tumor progression according to the established rules of multi-hit carcinogenesis, but could also explain treatment resistance phenomena. Nevertheless, recent data once again suggest that this model is still far too simple and needs to be reviewed.

Analysis of the various types of mutant p53 has distinguished the antiproliferative activity of p53 from its anti-apoptotic activity. Most of these mutants are exclusively defective for their anti-apoptotic activity and the capacity to transactivate apoptotic genes. Clear evidence for the importance of the antiproliferative capacity of p53 in tumor suppression has been elucidated in the mouse as indicated above [38].

More recently, a particular region of p53 (proline-rich region, 62–96) has also been specifically associated with the apoptotic activity of p53 [64,65]. This region has been relatively preserved throughout evolution. It presents a homology with the SH3 domain of sarc and could therefore constitute an auxiliary protein binding site. Deletion of this region leads to complete loss of the apoptotic activity of p53 associated with absence of transactivation activity on pro-apoptotic genes such as BAX or PIG3 [66]. This region could be necessary for cellular cofactors specifically involved in the apoptotic activity of p53. No p53 mutant active for apoptosis and deficient for regulation of the cell cycle has been demonstrated at the present time.

The p53 mutation database contains 21,000 mutations corresponding to 1300 variants identified in various types of human tumors [67]. These are therefore variants whose loss of function was selected during the neoplastic

process. In most cases, it is difficult to evaluate the target of this loss of function because each residue of the central part of p53 can be associated with various functions, as described above. Nevertheless, detailed analysis of this database shows several biases that are difficult to explain at the present time. First of all, mutants with a deficient apoptotic activity but a preserved antiproliferative activity are very under-represented compared to other mutants [67]. Although these mutants have been the subject of detailed studies, they are very largely under-represented in human tumors suggesting a low carcinogenic property. This decreased carcinogenic activity has been corroborated in a mouse model with an apoptotic defective p53 mutant that retained antiproliferative function [38]. Analysis of the p53 mutation database also reveals that, among the residues that are highly phylogenetically conserved in the various p53 family members, codon 98 is totally spared from mutation. This codon is located at the junction of the proline-rich region and the DNA binding domain. Mutagenesis analysis of this codon revealed that it is essential for the transcriptional activation of genes involved in apoptosis via the mitochondrial pathway and for p53 stability induced by the JNK pathway. All mutants at position 98 are specifically impaired for apoptosis, but retain cell cycle arrest activity (K. Bensaad and T. Soussi, unpublished). These results suggest that this codon mediates an important function of the p53 protein that could determine the selection of apoptosis versus cell cycle arrest and that it cannot be abolished in human cancer.

Continued progress in the p53 arena highlights the enigma of p53 function. Not only is the loss of p53 critical to the development of human cancers, but the presence of mutant p53 provides additional growth advantages to the tumor cells. The mechanism resulting in stability of mutant p53 remains unknown. Additionally, data indicate that the apoptotic and antiproliferative activities of p53 are both important in tumor suppression.

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