

## DATABASES

The UMD TP53 Database and Website:  
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Mutation of the p53 gene is the most frequent genetic alteration found in human cancer, but it is also the most frequently reported with more than 22,000 mutations published in 2,000 papers. In 1991, we developed a database and software to handle and analyze all this information. The database has been widely used for clinical analysis and molecular epidemiology. We have expanded the scope of the database by integrating structural, phylogenetic and biological information on wild-type (wt) and mutant TP53. Integration of the TP53 mutant activity database provides unique information that will be useful to both clinicians and scientists. All of this information is available from a new website ([www.umd.be:2072/](http://www.umd.be:2072/)) that will generate a detailed informative page for every TP53 mutant in the database. New tools to check TP53 mutations and minimize errors found in the literature are also available. *Hum Mutat* 27(1), 14–20, 2006. © 2005 Wiley-Liss, Inc.

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## INTRODUCTION

Mutations in the p53 gene (TP53; MIM# 191170) are found in approximately 50% of human cancers [Soussi and Bérout, 2001]. Apart from the fact that tumor cells must select for inactivation of the TP53 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 [Greenblatt et al., 1994; Soussi, 1996]. The TP53 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 [Oren, 2003; Vogelstein et al., 2000; Vousden and Lu, 2002]. The unique feature of TP53 compared to other tumor suppressor genes (TSGs) is its mode of inactivation. While most TSGs are inactivated by mutations leading to absence of protein synthesis (or production of a truncated product), more than 80% of TP53 alterations are missense mutations that lead to the synthesis of a stable full-length protein [Soussi and Bérout, 2001]. This selection to maintain mutant TP53 in tumor cells is believed to be required for both a dominant negative activity to inhibit wild-type (wt) TP53 expressed by the remaining allele, and for a gain of function that transforms mutant TP53 into a dominant oncogene [Dittmer et al., 1993; Lane and Benchemol, 1990; Soussi, 2003]. An important feature of the TP53 protein is the extreme flexibility and fragility of the DNA binding domain (residues 100–300) [Milner, 1995]. Every residue of this domain has been found to be modified at least three times and several residues can sustain multiple different alterations (Fig. 1).

One of the most puzzling aspects of mutant TP53 proteins is their structural, biochemical and biological heterogeneity. Several studies have revealed that specific TP53 mutations are associated with either a poorer prognosis or a poor response to treatment. In breast [Berns et al., 1998; Borresen et al., 1995; Kucera et al., 1999] and colon cancer [Borresen Dale et al., 1998; Goh et al., 1995], 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*<sup>-/-</sup> (*Trp53* is the mouse gene encoding TP53) mice differs from that of mice harboring point mutations [Liu et al., 2004; Olive et al., 2004].

Recently, several large-scale analyses have demonstrated this marked heterogeneity of TP53 mutants. Using an inducible system, Resnick and Inga [2003] showed that the level of mutant TP53 protein has a profound impact on transactivation. Kato et al.

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|            | TD<br>1-42 | PRO<br>62-92 | DBD<br>100-300 | TET<br>325-363 | REG<br>364-393 |
|------------|------------|--------------|----------------|----------------|----------------|
| Total      | 61         | 206          | 20544          | 282            | 23             |
| Missense   | 37 (60%)   | 101 (49%)    | 17131 (83%)    | 129 (46%)      | 16 (70%)       |
| Nonsense   | 4 (7%)     | 26 (13%)     | 1345 (7%)      | 102 (36%)      | 1 (4%)         |
| Frameshift | 20 (33%)   | 79 (38%)     | 2068 (10%)     | 51 (18%)       | 6 (26%)        |

FIGURE 1. Distribution of p53 mutations in the various functional domains of p53. TD, Transactivation domain; PRO, proline-rich domain; DBD, DNA-binding domain; TET, oligomerization domain; REG, regulatory domain. The higher frequency of frameshift mutations in the TD and PRO domains is statistically significant ( $p < 0.0001$ ). The frequency of p53 mutations outside the DBD is biased, as the majority of studies focus on exons 5 to 8 [Soussi and Bérout, 2001].

TABLE 1. Bias in TP53 Mutation Literature

|                     | Problems   | Resolution   |
|---------------------|--|--|
| Imprecise data      | Only amino acid changes are indicated. Only a single base change is indicated in the codon with two similar bases.                               | Only unambiguous mutations are included.                   |
| Inaccurate data     | Wt position or codon is not correctly assigned, translation errors between codon and aa residues.  | These data are not included in the database.               |
| Duplication of data | Multiple publications of the same data with a Materials and Methods section describing them as a new set of patients.                            | Publications are carefully checked to minimize redundancy. |
| Dubious data        | Data with a high frequency of multiple mutations in each tumour and/or a high frequency of silent mutations and/or an unusual mutation hot spot. | See text.  |

[2003] constructed a library of 2,314 TP53 mutants that have been analyzed for their transactivation properties toward a panel of eight transcription promoters. This study experimentally confirmed the notion of a wide variety of TP53 mutants with different behaviors.

The Universal Mutation Database (UMD) TP53 mutation database was initially created in 1990 as a repository of published TP53 mutations. Due to the correlation between the hot spot for TP53 mutation and the highly conserved domain of the protein, studies have focussed on these regions leading to the ultimate discovery of the specific DNA binding activity of TP53.

It was subsequently shown that the TP53 gene could be used for “molecular archaeology” to study cancer etiology [Hussain et al., 2000]. These studies demonstrate a link between exposure to various types of carcinogens and the development of specific cancers. The most striking example is that of tandem mutations, specifically induced by ultraviolet radiation, which are only observed in skin cancers. The relationships between G>T transversion and lung cancer in smokers or mutation of codon 249 observed in aflatoxin B1-induced liver cancers are also very demonstrative.

The UMD software and the TP53 mutation database have recently been the subject of several major revisions: 1) increased number of reported mutations (21,717); 2) thorough curation in order to remove many duplicates entries that are still very difficult to detect; 3) merging the TP53 mutation database and the TP53 mutant activity database, which provides functional information on more than 80% of TP53 missense mutations; and 4) development of structural, functional and phylogenetic tools for each TP53 residue [Bérout et al., 2005]. These updates were developed not only for clinicians interested in TP53 mutations, but also for basic scientists as a central database gathering information disseminated in thousands of publications.

### Update of TP53 Mutations and Curation of the TP53 Mutation Database (2005 Build 01)

TP53 mutations are the commonest genetic alteration reported to date. Among the 60,000 mutations reported in various genes, 30% correspond to TP53 alterations. This high frequency of reports is associated with a large number of problems that were difficult to detect several years ago (Table 1). Approximately 10 to 20% of reports of TP53 mutations present at least one of the problems described in Table 1. Careful curation has been performed to ensure the highest quality of data, but we cannot exclude a background of either duplicated or wrong data originating either from experimental errors or typing errors (Table 1).

Concerning imprecise data, corresponding authors have been systematically contacted by email requesting correct data (until the end of 2004). As the response rate was very low (less than 5%), this procedure was not pursued.

About 5 to 10% of publications contain inaccurate data in the table of mutations. It can be either a single mistake due to typing errors, but publications with errors that could affect several or all samples are also encountered. In the majority of these cases, use of a genetic code and the wt TP53 sequence could eliminate most of these errors. As for imprecise data, contacting corresponding authors is inefficient and incorrect data are not included. Reports with more than 50% of incorrect data have been totally discarded.

To circumvent this problem, several tools are now available that could be used before publication. First, the “check new TP53 mutation” function on the website has been improved (Fig. 2). Entry of the position of the mutation (either with the protein or cDNA nomenclature) leads to a page listing every TP53 mutant of

the database at this position. This first level of information indicates whether the newfound mutation is frequent or infrequent. Furthermore, choosing a specific mutant opens a second page that includes the transactivating activities of the TP53 mutant, its distribution in various types of cancer and their

references (Fig. 2). This function will be useful to detect TP53 mutations that have never been previously detected. In order to circumvent typing or translation errors, an Excel (Microsoft; www.microsoft.com) spreadsheet is also available for download. Using a genetic code and the wt TP53 sequence, it allows the

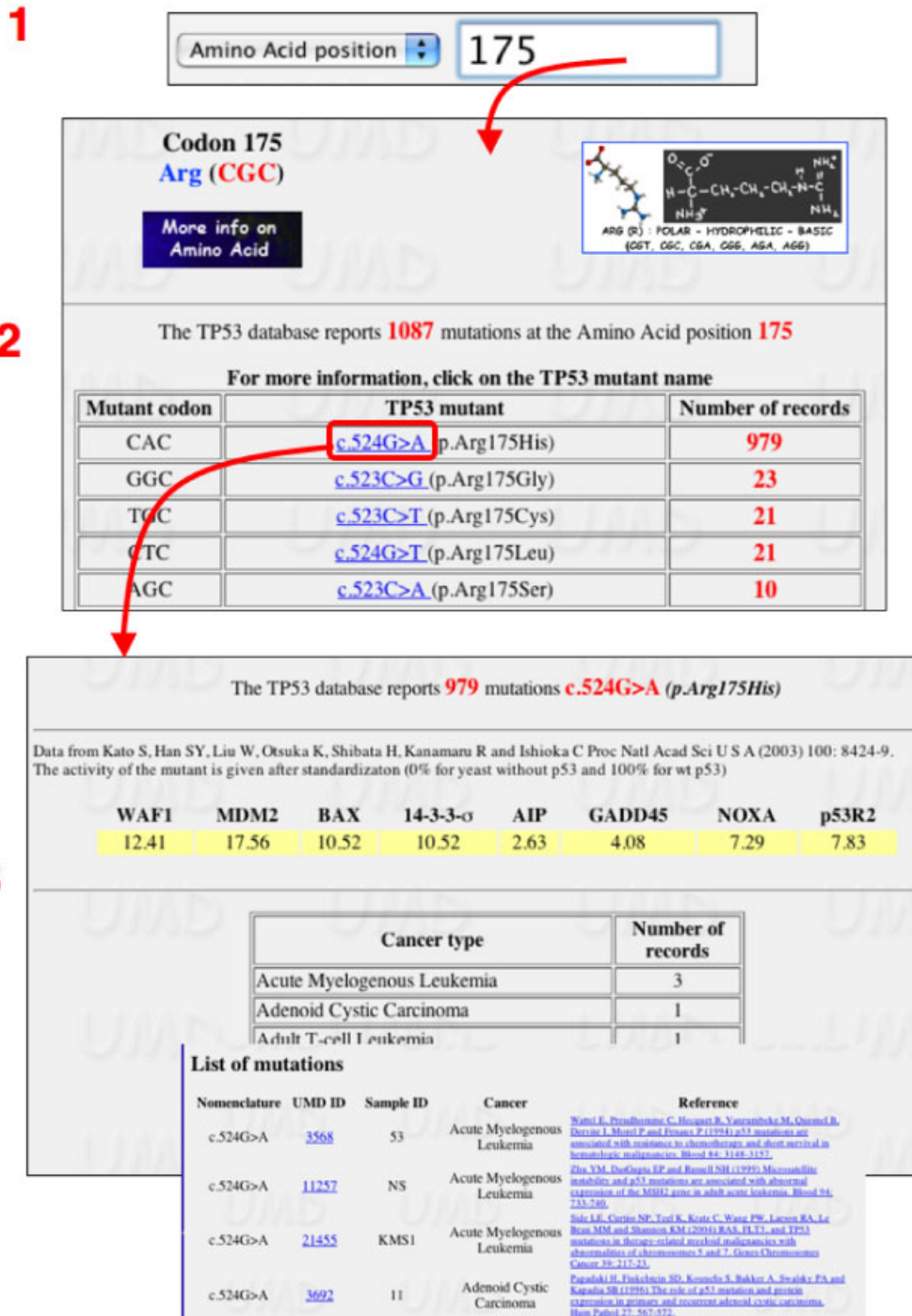


FIGURE 2. Checking a new TP53 mutation. Entering the position of the mutation (aa or nucleotide) in the entry page (1) opens a second page (2) that lists all mutations found at this position and their various mutational events. Choosing a particular genetic alteration opens a third page (3) that displays the mutant activity and the list of cancers and publications related to this mutation. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

automatic creation of a table that correctly describes the various mutations using the official nomenclature. This table can be used in any word processor for publication.

Duplication of data is the major problem. This can be due to simultaneous publication of the same set of TP53 mutations in a different journal, in a low-ranked journal followed by a higher-ranked journal over several years or several other combinations. Sometimes some new mutations are added to the old set, but in every case, there is no information in the publication indicating that extraction of the genetic material and sequencing have already been published elsewhere. When publications are performed over several years, the first publication is not even mentioned in the second paper. The main problem for inclusion in the database is that sample numbering is usually different from one publication to another for the same set of patients. In order to solve this problem, we used patient data such as age, gender, tumor stage, and position of the mutation to remove duplicates. Nevertheless, we cannot be sure that all duplicates have been eliminated.

This problem, as well as the problem of inaccurate data, raises important concerns that go far beyond the scope of this article. We believe that reviewers and editors should generate new guidelines to minimize these errors.

The last problem concerns dubious data. It highlights the difficulty of the curator's task. It also demonstrates that only a field specialist can curate data with a minimum of errors. Dubious reports on TP53 mutations were previously selected empirically

based on the unusual pattern of data. Until 2004, all these publications were not included in the UMD-TP53 database. Following integration of the TP53 mutation database with the activity database, we have now a tool to check whether all these dubious data behave like the remaining entries and all these publications were therefore added to the 2005 version of the database. The meta-analysis of TP53 mutation activity confirms that mutants described in these reports have behaviors that are statistically different from other mutants (Soussi et al., in press, b). Although we have kept them in the database, a warning flag has been added because they may bias the analyses.

The new TP53 mutation database contains 22,717 mutations. Activity analysis of the entire database indicates that, apart from a few dubious reports, the majority of TP53 mutations present the same range of loss of activity. Analysis of mutation profile and activity will be published elsewhere (Soussi et al., in press, a).

**Update of TP53 Data**

The organization of the information regarding TP53 mutations is summarized in Figure 3. It includes the most informative data concerning the TP53 protein: 1) structural data and TP53 folding; 2) evolutionary conservation of the protein; 3) posttranslational modifications of the protein; and 4) mutant protein activity (also see Supplementary Figs. S1 to S6; available online at <http://www.interscience.wiley.com/jpages/1059-7794/suppmat>).

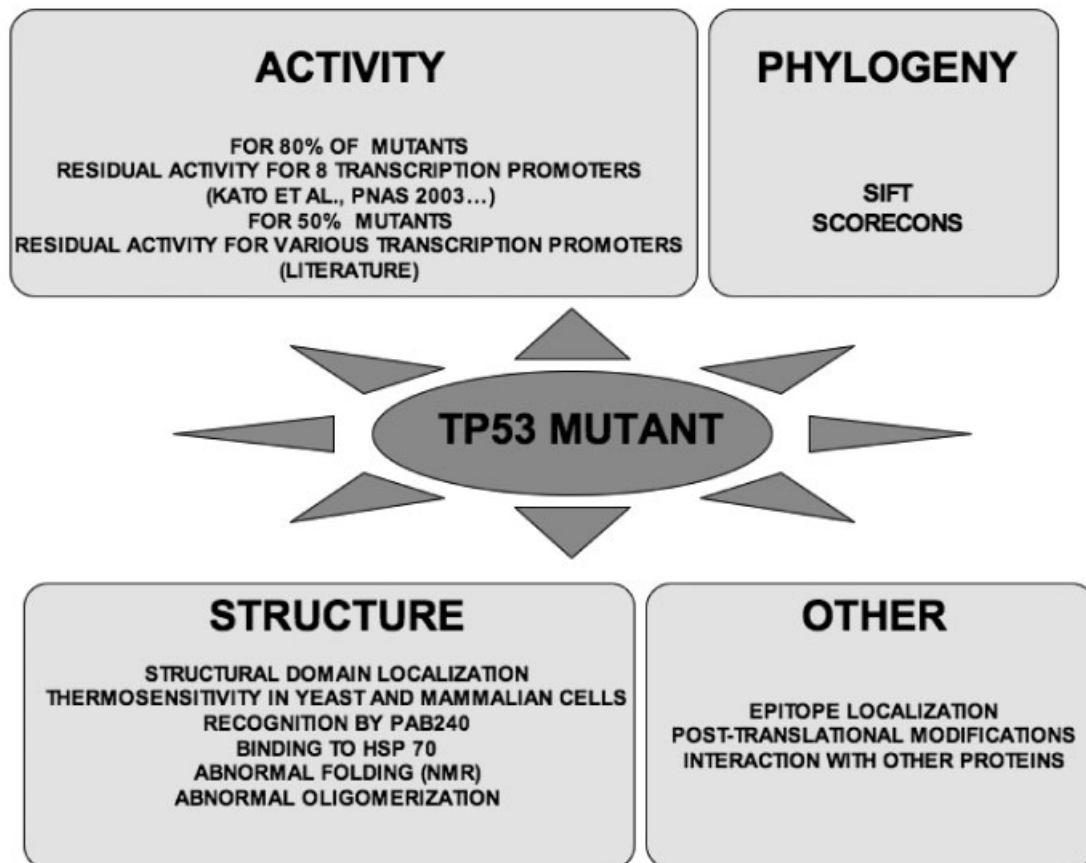


FIGURE 3. Organization of the information related to TP53 mutants. All of this information is available for the 393 residues of the TP53 protein.

## Phylogeny

The entire sequence of the TP53 protein from 33 vertebrate species is now available. Predictions regarding missense mutations can be supported by comparative evolutionary analysis to establish whether mutations are situated in conserved regions. Several programs have been developed to perform this type of analysis. "Sorting intolerant from tolerant" (SIFT) is a sequence homology-based tool that sorts intolerant from tolerant amino acid substitutions and predicts whether an amino acid substitution in a protein will have a phenotypic effect. SIFT is based on the premise that protein evolution is correlated with protein function. Positions important for function should be conserved in an alignment of the protein family, whereas unimportant positions should appear diverse in an alignment. SCORECONS is a program that quantifies residue conservation in a multiple sequence alignment. Given a multiple sequence alignment file, it calculates the degree of amino acid variability in each column of the alignment. The algorithms used by these two programs are slightly different leading to complementary information. A more detailed analysis of these two programs in relation to mutant TP53 activity will be published elsewhere (Soussi et al., unpublished results). SIFT and SCORECONS analysis is available for each TP53 amino acid in the UMD site (Supplementary Fig. S1; [www.umd.be:2072/](http://www.umd.be:2072/)). TP53 sequences and alignment are also available for viewing and downloading at the TP53 website.

## Activity

In a recent study, Kato et al. [2003] described the construction and characterization of 2,314 TP53 mutations distributed at every position of the protein. The biological activity of each mutant was evaluated in vitro in a yeast system using eight different transcription promoters. Among these missense 2,314 mutants, 1,250 correspond to natural mutants occurring in neoplasia. Nonsense mutations and frameshift mutations were not constructed as they are assumed to be inactive. These data have been incorporated into the UMD TP53 database and are now available at our website. This is the first time that activity and mutation databases can be linked in eukaryotes. To our knowledge, it is only available for the LacI gene and has been invaluable for structure-function relationship analysis. We believe that this novel integrated database should provide a foundation for many studies on TP53, but also constitutes a model for other databases of genes with a major clinical impact. Although the original work was performed in yeast, more recent analyses of the TP53 mutant library have been conducted in mammalian cells. This information has been added to the website that also includes other studies performed by the scientific community.

## Structural Information

Although wt TP53 is usually considered to be a TSG, mutant TP53 should be considered to be a dominant oncogene. Furthermore, the diversity of TP53 mutants can underlie a marked heterogeneity in tumor behavior. The protein contains several domains that have been extensively studied by in vitro mutagenesis: 1) the transactivation domain; 2) the proline-rich domain; 3) the specific DNA-binding domain (DBD); and 4) the tetramerization domain and a nonspecific DNA-binding domain that could be involved in DNA damage recognition. Several nuclear export or localization signals have also been identified in various region of the protein. Furthermore, the TP53 protein is the subject of extensive posttranslational modifications that are important for its activation or its degradation. Twenty-two residues

of the protein are subject to phosphorylation, ubiquitination, acetylation, sumoylation, methylation, or neddylation [Bode and Dong, 2004]. Finally, the protein can interact with a plethora of other proteins and some of these complexes have been analyzed by crystallography [Cho et al., 1994; Gorina and Pavletich, 1996; Kussie et al., 1996]. The flexibility of the TP53 protein was initially identified using monoclonal antibodies (mAbs) able to discriminate mutations that change TP53 folding [Gannon et al., 1990; Legros et al., 1994]. Two classes of mutations have been distinguished on the basis of various in vitro assays and the three-dimensional structure of the protein [Cho et al., 1994]: class I mutations, exemplified by mutants at codon 248 (7.6% in the TP53 database, <http://p53.free.fr/>), affect amino acids directly involved in the protein-DNA interaction. They have a wt conformation as probed by conformational mAbs and they do not bind to the chaperone hsp70 [Hinds et al., 1990; Ory et al., 1994]. Class II mutations, exemplified by mutants at codon 175 (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. This biochemical and biological heterogeneity has been confirmed and refined by structural studies. For example, nuclear magnetic resonance (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 [Bullock et al., 2000; Wong et al., 1999]. Recent analyses using more sophisticated biophysical techniques have revealed that the central region of the TP53 protein can adopt at least five thermodynamic states [Bullock and Fersht, 2001]. The marked flexibility of TP53 mutants is also highlighted by the discovery that more than 100 TP53 mutants are thermosensitive; i.e., wt activity at 30°C and mutant at 37°C. All of this structural heterogeneity results in a marked variability in terms of loss of DNA binding activity and transactivation of TP53 mutants. The DNA-binding site recognized by TP53 is highly degenerated and the affinity of TP53 for the various biological sites is variable [El-Deiry et al., 1992]. 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 [Friedlander et al., 1996; Rowan et al., 1996]. All these data have been collected from the literature and are included in the database for each mutant (Fig. 3).

## TP53 Mutant Analysis

Several TP53 mutants will be analyzed to illustrate the various features of the website.

Codon 302 (Glycine) is not a mutation hot spot in human cancer (23 mutations in the database). These mutations include seven deletions, 11 missense mutations (p.Gly302Arg (one), p.Gly302Glu (seven), p.Gly302Ala (two), and p.Gly302Val (one)), and five mutations that do not change the amino acid residue (Supplementary Fig. S2). This residue is not localized in a key structural region of the protein; SCORECONS analysis indicates that it is moderately conserved throughout evolution in mammals and poorly conserved in all vertebrates. SIFT score predicts that substitutions at this position will not have any phenotypic effect except for p.Gly302Val, which displays a borderline score, as the transactivation activity of all of these mutants, as reported by Kato et al. [2003], indicates that they have a similar activity to that of wt TP53.

Codon 175 (Arginine) is a mutation hot spot (Supplementary Fig. S3). The high selection for this mutant in human cancer is due to the combination of its essential function for the folding

of the DNA-binding domain of TP53 protein and the high mutability of the methylated CpG dinucleotide in the wt codon (CGA). It is localized in the L2 loop of the protein. A total of 90% of mutations at this position are p.Arg175His, a TP53 totally devoid of TP53 activity. Furthermore, this mutant has an altered conformation as shown by its interaction with hsp70 or the conformational antibody PAb240. This information is of importance in the design of new molecules that could restore TP53 DNA-binding activity. It is known that mutants with an unfolded structure are less prone to reactivation than mutants affecting residues involved in the DNA-binding domain. Among other alterations at this position, the p.Arg175Pro mutant has an interesting behavior. It has a normal cell cycle arrest and gene p21 induction behavior [Ory et al., 1994], but is deficient for apoptotic activity and does not transactivate bax or PIG3 genes (Supplementary Fig. S4). The reasons for this heterogeneity are unknown at the present time, but could be related to a difference of interaction with various coactivating molecules.

The DBD domain is also the binding site for the p53BP2/ASPP1 protein. Crystallographic analysis of the complex between the two proteins demonstrates a marked homology between the TP53 residues involved in this interaction and those interacting with DNA. Most of the TP53 hotspot mutations are also unable to interact with this protein. p53BP2/ASPP1 and a second protein, ASPP2, are important cofactors in the transactivational activity of TP53 in relation to apoptotic genes. The mechanisms leading to this specific activation are unknown at the present time and it is still too early to say whether loss of this interaction participates in neoplasia. There is relatively limited information concerning the interaction between ASPP proteins and mutant TP53 at the present time, but these data will be added to the database as they become available.

Codon 213 (Arginine) is a good example of other features available in the database (Supplementary Fig. S5). First, this mutant displays a thermosensitive behavior, which has been analyzed both in a yeast assay and in mammalian cells (Supplementary Fig. S5). All data on thermosensitive TP53 mutants have been compiled in the database. It is noteworthy that many mutants in this region (211–217; Sheet S7) have a thermosensitive behavior, suggesting that it is important for TP53 folding. Another available feature is disruption of the epitope for mAbs. A database of all mAbs specific for the TP53 protein has been available for a long time (<http://p53.free.fr>). The precise epitopes for all of these mAbs have been localized and this information has been linked to the TP53 mutation database. Mutations at codon 213 change the sequence of the epitope of PAB240 (Supplementary Fig. S5), as analysis of the RAJI cell line, which expresses a mutation at codon 213, shows that the PAB240 epitope is lost.

Codon 155 (Threonine) is found in the TP53 of 23 vertebrate species, while this residue is a Serine in the remaining 10 species, including the 4 monkey TP53 proteins. In humans, this residue has been shown to be phosphorylated by the COP9 signalosome complex to target TP53 protein for degradation. Among the 22 residues of TP53 that are modified after translation, codon 155, is the only one that is the target for a significant number of mutations (Supplementary Fig. S6). Two hypotheses can explain this lack of mutation at the other sites. Either alterations of these other sites are lethal for the cell or, on the contrary, they do not induce any harmful effects. Several arguments, including the lack of any phenotype of mice expressing mutant TP53 at several phosphorylation sites, suggest that the second hypothesis is more likely. Nine of the 98 missense mutations found at codon 155 are

“neutral mutations” that do not change the residue. Interestingly, six of these mutations are associated with another mutation in the same tumor, suggesting that these neutral mutations could be hitchhiking mutations coselected with a true mutation. Among the 89 remaining missense mutations at position 155, only eight are associated with another mutation ( $P < 0.001$ ; Fisher test).

Four of the 98 missense mutations at codon 155 lead to the synthesis of a serine residue that is not predicted to be detrimental by SIFT, as confirmed by the potent transactivation activity of this p.Thr155Ser mutant. The transcriptional activity of other mutants at this position is severely compromised. Paradoxically, it has been shown that impaired phosphorylation at this residue leads to TP53 stabilization and an increase of WAF1 activity, a situation that should not be selected for cellular transformation. Other properties could also be linked to this residue and further studies are necessary to resolve this apparent contradiction.

The observation that weak or “neutral” mutations can be found in a single tumor indicates that stopping TP53 sequence analysis once a mutation has been found, as suggested by some authors, could be detrimental to accurate analysis.

## FUTURE CONSIDERATIONS

The first release of this integrated TP53 database (2005 Build 1) is available for the entire scientific community and will have a wide audience for both clinicians and basic scientists. Its open structure also allows rapid update when new information is published. UMD software has been used to create up to 50 LSDBs. This particular development performed for TP53 can therefore be applied to other mutation databases when more data are available. We believe that, with the increasing volume of data available from high throughput technologies, these integrated databases will be pivotal for the design or development of various types of studies.

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