p53 REVIEW ARTICLE

Significance of TP53 Mutations in Human Cancer: A Critical Analysis of Mutations at CpG Dinucleotides

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A detailed analysis of p53 (TP53) mutations involving the 42 CpG dinucleotides was performed to gain greater insight into the mutational mechanism leading to specific selection of these mutations. Although the majority of these CpG dinucleotides have been found to be mutated in cancer cells, the heterogeneous frequency of mutational events suggests that some mutations are not true mutations, but neutral changes that have been co-selected during oncogenic transformation. Among the 1,400 variants found in the 15,000 mutations of the p53 database, 5% have only been described once, indicating that either the mutational event is rare, or the mutation phenotype is very mild, or both. Overall, these data indicate that great caution is required when analyzing the significance of p53 mutations. Hum Mutat 21:192–200, 2003. © 2003 Wiley-Liss, Inc.

INTRODUCTION

Using protein sequencing, Vernon Ingram was the first to discover that a small change in the hemoglobin protein could lead to human sickle-cell anemia [Ingram, 1956]. Since this pioneer work was done, it has largely been demonstrated that gene mutations are the basis for most genetic diseases. The introduction of rapid DNA sequencing technologies constituted a second revolution by replacing the tedious task of protein sequencing. Over recent years, progress has been made in cloning the genes involved in both monogenic and polygenic disorders, including complex diseases such as cancer [Collins, 1995]. For each of these genes, numerous and various types of alterations have also been described, ranging from point mutations to large deletions. The future development of new methods for the detection of point mutations, such as CHIPS technology, will lead to an enormous increase in the detection of new mutations [Cotton, 2002]. It is difficult to evaluate the number of mutations reported in the literature to date, but it is also impossible to predict how many new mutations will be detected in the next 10 years. Nevertheless, a number of points can be predicted: 1) the rate of de novo somatic or germline mutations will never slow down; 2) changes in our environment will lead to a change in the mutational events that modify our genome; 3) knowledge of these mutations will be important for treatment decisions. The reporting and analysis of these mutations will therefore be a major challenge for the future [Claustres et al., 2002; Paalman et al., 2000].

Numerous and various types of alterations have been described for each gene involved in disease, ranging from point mutations to large deletions. With the development of our knowledge in gene alterations leading to human diseases, it has become clear that identification of these mutations should play a critical role not only in diagnosis and prognosis, but also in

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research. First, it is clear from all studies performed to date that mutations are generally not randomly distributed. Hot spot regions have been demonstrated, corresponding to either a DNA region highly susceptible to mutations (such as CpG dinucleotides), or a codon encoding a key residue in the biological function of the protein, or both. Identification of these hot spot regions and natural mutants is essential to define critical regions in an unknown protein. In large genes, such as NF1 (59 exons, 2,818 amino acids; MIM# 162200), RB1 (27 exons, 928 amino acids; MIM# 180200), APC (15 exons, 2,843 amino acids; MIM# 175100), BRCA1 (24 exons, 1,863 amino acids, MIM# 113705), and TTN (363 exons, about 25,000 amino acids; MIM# 188840), detection of point mutations by direct sequencing analysis is fairly difficult due to the size of the target gene. Identification of a hot spot region allows analysis to be focused on this region, keeping in mind that a negative result should be viewed with caution.

Second, it has now been clearly demonstrated that alterations in a single gene can cause various types of disorders. For example, mutations of the RET gene (MIM# 164761) have been associated with multiple endocrine neoplasia types IIA [Mulligan et al., 1993] and IIB [Hofstra et al., 1994], familial medullary thyroid carcinoma [Xue et al., 1994], and a non-cancerous disorder known as Hirschsprung's disease [Edery et al., 1994; Romeo et al., 1994]. Mutations appear to be localized in specific domains of the protein for each of these disorders. The site of specific alterations at various positions in a given gene has also been shown to be associated with specific clinical features, as is the case of colon cancer and mutations in the APC gene. A mutation in the C-terminus of the protein has been specifically associated with a secondary abnormality, congenital hypertrophy of the retinal pigment epithelium [Olschwang et al., 1993], whereas mutations in the N-terminus are associated with an attenuated phenotype [Spirio et al., 1993].

Third, analysis of mutations can lead to the definition of risk factors. For instance, in the VHL (MIM# 193300) families presenting mutations leading to truncated proteins, at least one member developed an RCC in 83% of cases versus only 54% in families presenting missense mutations [Gallou et al., 1999].

Fourth, in diseases characterized by considerable variation in the clinical phenotype between families and also within the same family, such as Marfan syndrome (MIM# 154700), it is very important to confirm or formally exclude the diagnosis in high-risk family members as early as possible because of the potentially fatal cardiovascular complications of the disease.

Finally, the analysis of the p53 (TP53; MIM# 191170) database that contains a very large number of point mutations has led to the development of a new field, i.e., molecular epidemiology, in which analysis of the mutational spectrum reveals a direct causal effect between carcinogen exposure and a specific cancer [Hussain et al., 2000].

All of these examples show that, in the future, mutation databases must not be simply repositories of locus-specific mutations, but must constitute dynamic databases linked to various computerized tools for analysis that can be searched directly on-line. Many reviews have been published about the basic features of p53, the clinical implications of p53 mutations, and the analysis of p53 mutations in relation to carcinogen exposure [Michael and Oren, 2002; Soussi and Béroud, 2001; Vogelstein et al., 2000; Wallace-Brodeur and Lowe, 1999]. The purpose of this review is not to repeat what has already been described and discussed many times before, but to perform a critical analysis of the significance of the various mutations described in the p53 gene.

TP53 GENE MUTATIONS IN HUMAN CANCER

During their life, normal cells are constantly exposed to various endogenous and exogenous stresses that alter their normal behavior. Genetic insults that can lead to mutations are particularly harmful, as their transmission to daughter cells can lead to neoplasia. To ensure rigorous homeostasis, mammalian cells have selected for key regulators that control normal cell growth [Levine, 1997]. The p53 tumor suppressor gene was initially identified as being essential for the DNA damage checkpoint, but it was subsequently found to have a broader function after cellular stress, such as oncogene activation or hypoxia [Vousden, 2002]. The p53 protein functions as a tetrameric transcription factor found at very low levels in normal unstressed cells. After stress, different pathways lead to post-translational modification of the protein and its stabilization. This accumulation activates the transcription of a wide range of genes involved in various activities, including cell cycle inhibition and apoptosis depending on the cellular context, the extent of damage, or other unknown parameters. p53 mutations are the most common genetic alteration found in human cancer [Soussi and Béroud, 2001]. These alterations are usually missense point mutations that are scattered along the p53 gene. In some cancer types, p53 function is indirectly abolished either by nuclear exclusion (neuroblastoma), interaction with viral protein (cervical cancer), or via its interaction with overexpressed mdm2 protein (sarcoma). No p53 mutations are observed in testis cancer and melanoma, but, in melanoma, the apoptotic pathway induced by p53 in response to chemotherapeutic agents is affected by alterations in the Apaf gene, acting downstream to p53.

Over the last 10 years, several p53 mutation databases have been developed [Hainaut and Hollstein, 2000; Soussi and Béroud, 2001]. Analysis

FIGURE 1.   **Distribution of p53 mutations at codons 175, 248, and 273. Wild-type at each codon=Arg. A: distribution of the various mutational events at each codon for all types of cancer. B: frequency of various mutational events at codons 175, 248, and 273 in various types of cancer.**

of p53 mutations in relation to exposure to various carcinogens has already been described in numerous papers and will not be discussed in the present review [Hussain and Harris, 1999; Hussain et al., 2000]. One of the major challenges for the future concerns the relationship between p53 mutations and clinical features, such as response to treatment or survival. The implication of p53 (and p53 family members such as p63 and p73) [Bénard et al., 2003] in apoptosis induced by various agents used in cancer therapy, such as radiotherapy or chemotherapy, suggests that inactivation of p53 could be associated with resistance to treatment. Although cellular and animal models have provided very convincing results in favor of such an association [Parant and Lozano, 2003], clinical data are more controversial (reviewed in the articles of the p53 special issue of *Human Mutation* published March 2003; see Soussi [2003] for overview). This situation is made even more complex by the finding that p53 mutants are highly heterogeneous in terms of loss of function, leading to various phenotypes that could be cell-specific.

In the present review, we analyzed the latest version of the UMD-p53 database [Béroud and Soussi, 2003] (15,078 mutations as of May 2002) to gain a better insight into the significance of the various p53 mutations described in the literature. More than 70% of p53 mutations are not hot spot mutations and about 4.4% of these mutations have been reported only once and their significance needs to be analyzed.

TP53 MUTATIONS AT CODONS 175, 248, AND 273

Alterations at codons 175, 248, and 273 correspond to 19% of all mutations described in the database and are usually considered to be hot spot mutations.

However, these mutations must be interpreted very cautiously, as they present several levels of heterogeneity: 1) several different variants are observed for each locus (Fig. 1A and B); 2) the frequency of these variants differs from one cancer to another; and 3) some of these variants induce only a partial defect in biological properties. $G>T$ transversion is clearly different between the various cancers with a predominance in the two cancers related to tobacco smoking: lung cancer and head and neck cancer. Figure 5 indicates the rank of each p53 variant in the various types of cancer. The most frequent variant in the entire p53 database is $G>A$ at codon 175, which accounts for 5% of all mutations. It is also the most frequent mutation in colon and breast cancer, but is only in sixth position for lung cancer. These tables highlight the fact that the majority of mutations in colon and breast cancer are CpG dinucleotide transitions, while the mutations in lung cancer are predominantly $G>T$ transversions. The two first rank positions in lung cancer, codons 157 and 158, were already mentioned in the first analysis of the p53 database in 1992 [Caron de Fromentel and Soussi, 1992]. More recently, it has been shown that these codons are a preferential target for benzo(α)pyrene adducts, a carcinogen associated with tobacco smoking [Denissenko et al., 1996]. Although GC$>$AT transitions at codons 175, 248, and 273 are frequent, each one accounts for $<$ 5% of all mutations, which is not really compatible with a clear-cut notion of hot spot.

TP53 MUTATIONS AT CPG DINUCLEOTIDES

Analysis of all point mutations in the p53 gene shows that 51% are G:C$>$A:T transitions, and 59%

of these mutations affect a CpG dinucleotide. In mammalian cells, the cytosine in this dinucleotide is very often methylated and it has been shown that the 42 CpG sites of the p53 gene are methylated in normal tissue [Tornaletti and Pfeifer, 1995]. It is generally assumed that the higher deamination rate of 5-methylcytosine leading to a T/G mismatch that is not efficiently repaired could lead to this high rate of transversion in the p53 gene. Deamination of cytosine leads to a U/G mismatch that could be removed more efficiently. Although attractive, this hypothesis has not been formally demonstrated and several lines of evidence suggest that other models should also be investigated. Various studies have demonstrated that exogenous carcinogens, such as BPDE or UV sunlight, have a greater affinity for methylated CpG dinucleotides than their unmethylated counterparts [Denissenko et al., 1997; You et al., 1999]. It is conceivable that endogenous mutagens, derived from an altered cell metabolism, could also target methylated CpG dinucleotide leading to a high rate of transition.

A detailed analysis of p53 mutations at the 42 CpG dinucleotide was performed in order to gain greater insight into the mutational mechanisms leading to specific selection of these mutations. These CpG can occur in three forms in the coding sequence of the gene: CGN, NCG, or NNC GNN, that will be called type I, II and III, respectively. Transition at type I CpG always leads to amino acid substitution whether it is the first or the second base that is changed (specific targeting of the first C will lead to a C to T transition on the transcribed strand; targeting of the second C, on the opposite strand, will lead to a similar event that will be translated as a G>A transition on the transcribed stranded). Transition at type II CpG will only lead to mutation when the C is modified, as mutation of the G residue does not change the amino acid residue due to degeneration of the genetic code. Similarly, transition at type III CpG will only lead to a change of the amino residue when the G is modified. Examination of transitions at the 42 CpG of the p53 gene clearly shows a high degree of heterogeneity both for the frequency of mutations and also for the pattern of mutation between the two residues (Fig. 2). Targeting of the two C residues in both strands of a CpG dinucleotide is expected to occur and to be repaired at a similar rate. Examination of the frequency of transition at the two hot spot codons, 248 and 273 (type I CpG), confirms this expectation as there is roughly the same number of C>T and G>T substitutions (Figs. 1 and 2). On the other hand, examination of the two other hot spot codons, 175 and 282, shows a marked disequilibrium in the distribution of mutations. Similar findings are observed for codons 196, 213, and 306 that present a lower frequency of mutation. It is essential to keep in mind that these mutations are not a true representation of the rate of p53 gene mutation, as only
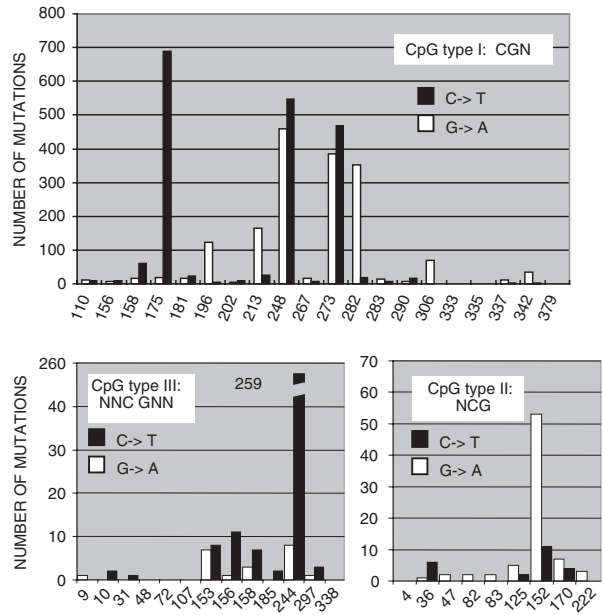

FIGURE 2. Distribution of GC>AT transitions at each CpG dinucleotide of the p53 gene. Open and closed bars correspond to G>A and C>T transition, respectively.

alterations that lead to a growth advantage for tumor progression will be selected. It is likely that some transitions at specific CpG dinucleotides will not lead to a selectable alteration. In 1994, we already reported this disequilibrium for codon 175, as it was already obvious in a mutation database composed of only a few hundred mutations [Caron de Fromentel and Soussi, 1992; Ory et al., 1994]. To analyze the function and/or loss of function of p53 at this codon, we constructed a library of 15 different p53 mutations at position 175 [Ory et al., 1994]. The G>A transition leads to an arginine substituted for an histidine. The biochemical and biological function of this mutant, which is the most frequent in the p53 mutation database, is totally impaired. It also appears to be associated with a gain of function activity leading to resistance to chemotherapy [Blandino et al., 1999]. The C>T transition leads to an arginine substituted for a cysteine. In all studies performed to date, this substitution does not inactivate p53 activity [Ory et al., 1994], indicating that only inactivating mutations are selected during the transformation process. It is noteworthy that some mutations analyzed in this study induced p53 inactivation despite the fact that they were not found in human tumors. All these mutations are double mutations such as CGC>TTC (Arg to Phe) suggesting a predominance for single-base mutations in the mechanisms leading to p53 inactivation (excluding skin cancer, in which tandem mutations are linked to UV exposure). Mutations at other CpG dinucleotides, such as 282, 196, or 213, have not been extensively studied, but it is reasonable to suppose that they are
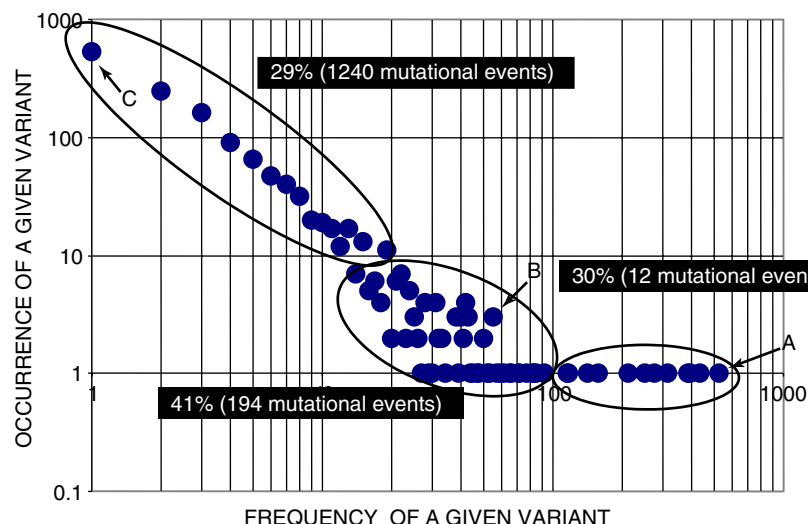
FIGURE 3. Frequency of p53 variants in the database: the X-axis represents the frequency at which a given variant is found mutated in the database. theY-axis represents the number of each individual variant. **A:**The unique variant 175G>A is the only one found 688 times in the database. **B:**Variants 245G>T, 278C>T, and 242G>T are found 55 times in the database. **C:** Five-hundred and eighty-five variants are only found once in the database. [Color figure can be viewed in the online issue at www. interscience.wiley.com.]

similar to those described for codon 175. This disequilibrium does not lead to a null result for the underrepresented mutant except for codon 306. For type I CpG codons, such as 175, 196, 213 and 282, 18, 5, 25, and 18 mutants have been identified at the underrepresented position, respectively. Seven of the 18 G>A mutants at position 282 are found in tumors containing more than one p53 mutation. In each case, these second mutations correspond to well-known inactivating mutations. Multiple mutations in a single tumor is an uncommon event for the p53 gene, as the most frequent mechanism appears to be a single mutation in one allele and loss of the second allele due to partial or complete deletion of chromosome 17. Less than 0.5% of tumors harbor multiple mutations. These observations strongly suggest that G>A mutants at position 282 are not true inactivating mutations *per se*, but neutral mutations that could have been co-selected with a second mutation constituting the true driving force for p53 inactivation. For the remaining 11 cases, it is possible that the true mutation may not have been detected, as most of these studies only examined exons 5 to 8. Similar comments may apply to codons 175, 196, and 213.

An analogous argument is also possible for type II CpG. The frequency of p53 mutations at these CpG is very low, except for codon 152. Although the C>T transition leads to an inactivating mutation, the G>A transition does not change the identity of the proline residue. Nevertheless, 11 tumors harbor this neutral mutation. Five of them are found in tumors containing more than one p53 mutation. In each case, these second mutations correspond to well-known inactivating mutations. A similar situation is observed for type III CpG, with a high frequency of double

mutations associated with the C>T transition at codon 153 or 244 (Fig. 2).

Taken together, these observations suggest that there is a high rate of spontaneous deamination in tumor cells leading to GC>TA transitions, many of which are not selected because they either occur in non-essential DNA regions or do not alter the growth property of the cell. The occurrence of a true inactivating p53 mutation in this background of neutral mutations could lead to the co-selection of two events such as those described above.

HETEROGENEOUS DISTRIBUTION OF TP53 MUTATIONS

As indicated above, the p53 mutation database contains 15,000 mutations distributed among 1,500 different variants. The frequency of these variants is very different. The most common variants are 175 G>A, which is found 688 times; 248G>A, found 548 times; and 273 G>A, found 468 times (Fig. 3). At the other end of the spectrum, 585 variants are only found once, 265 variants are found twice, and 156 variants are found three times. It should be noted that only 12 variants are found more than 100 times, 194 variants are found between 11 and 99 times, and 1,240 variants are found less than 10 times. This series of rare variants also correspond to 29% of all mutations of the database (Fig. 3).

Common variants are clearly real p53 mutants. Biochemical and biological studies have demonstrated that these p53 proteins have impaired DNA binding activity leading to a protein that is inactive for transactivation. They have also lost their growth
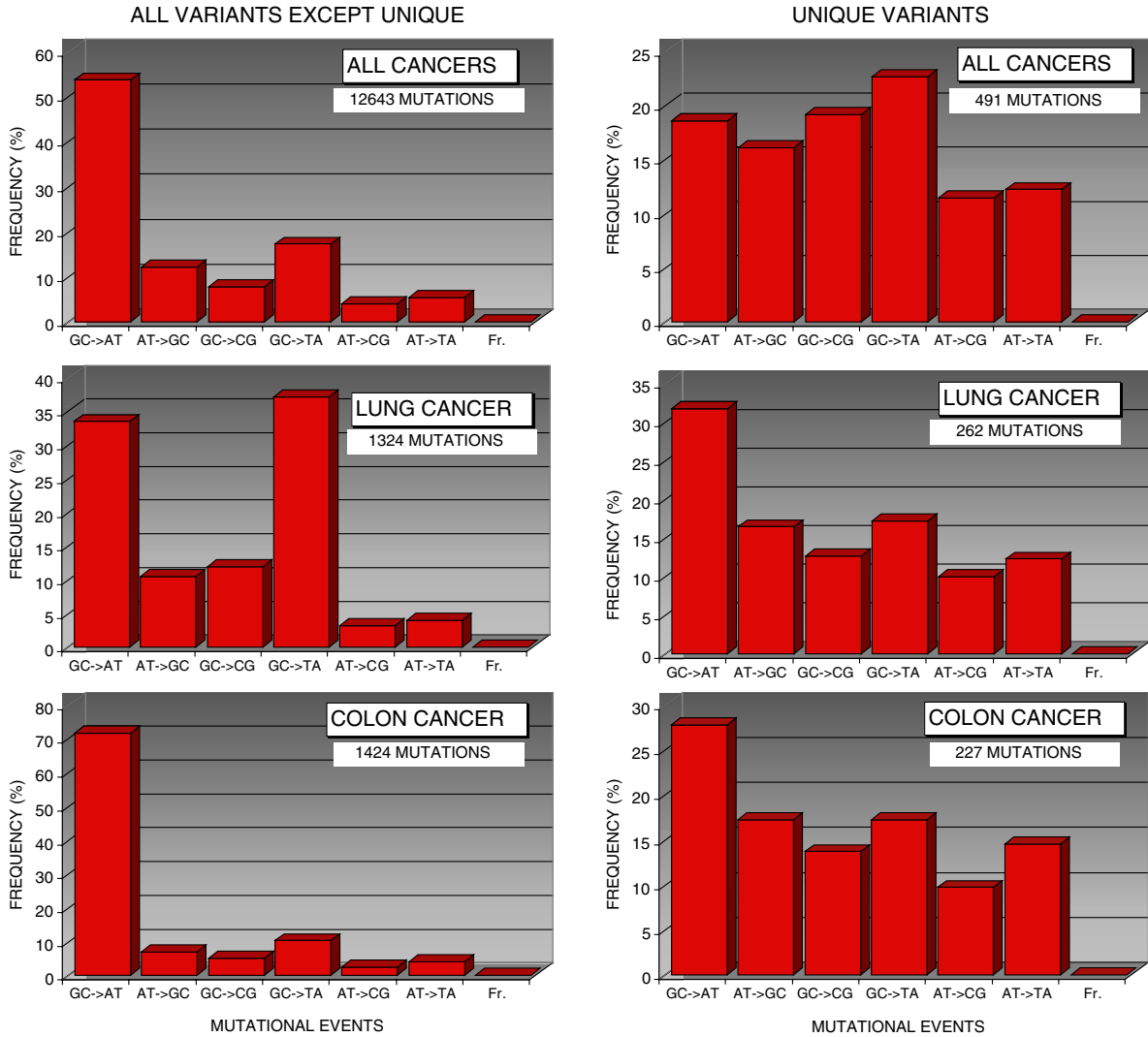
FIGURE 4. Distribution of mutational events in the UMD-p53 database. Comparison of the entire database versus variants that are only represented once. [Color figure can be viewed in the online issue at www.interscience.wiley.com.]

arrest or pro-apoptotic properties [Forrester et al., 1995; Hinds et al., 1990; Ory et al., 1994].

On the other hand, it is more difficult to determine the biological significance of rare variants. Only 1.7% of these variants occur at CpG dinucleotides, indicating that they are not associated with any specific event at this nucleotide sequence. There are several explanations for the rare occurrence of these mutations, none of which are exclusive. The most prosaic explanation is a laboratory artifact ranging from PCR or sequencing errors to typing errors or error in codon assignment. In a recent analysis of the 1,500 articles describing p53, we have observed typing errors in the identity of the wild-type codon in about 5% of publications with a notable increase in recent years with several publications presenting multiple errors. Although these errors are easily detected, as the sequence of wild-type p53 is well known, it is impossible to detect such errors in the description of

mutations, but we can assume that they occur at a similar frequency. When corresponding authors are contacted to provide corrections, only very few reply. We estimate that at least 2% to 5% of p53 mutations in the database are incorrect, but there is no way of identifying them. The pattern of mutational events that inactivates the p53 gene is specific from one cancer to another. In colon cancer, there is a high rate of transitions at CpG dinucleotides, whereas in lung cancer, GC>TA transversion is the leading mutational event. These differences are due to the heterogeneity of the mutagenic process that inactivates p53 in these two cancers. This feature has already been largely described and discussed over the last 10 years. Analysis of the mutational event of the unique variants reveals two important findings: 1) they are identical from one cancer to another (Fig. 4 and data not shown) and 2) there is no specific mutational event compared to the analysis performed

ALL CANCERS

Rk	Pos	WT	Mut.	Evt	Freq.
1	175	CGC	CAC	G->A	5.13 %
2	248	CGG	CAG	G->A	4.09 %
3	273	CGT	CAT	G->A	3.49 %
4	248	CGG	TGG	C->T	3.42 %
5	273	CGT	TGT	C->T	2.86 %
6	282	CGG	TGG	C->T	2.63 %
7	249	AGG	AGT	G->T	2.05 %
8	245	GGC	AGC	G->A	1.93 %
9	220	TAT	TGT	A->G	1.40 %
10	213	CGA	TGA	C->T	1.23 %

COLON CANCER

Rk	Pos	WT	Mut.	Evt	Freq.
1	175	CGC	CAC	G->A	12.62 %
2	248	CGG	CAG	G->A	7.58 %
3	248	CGG	TGG	C->T	7.25 %
4	282	CGG	TGG	C->T	5.64 %
5	273	CGT	CAT	G->A	5.30 %
6	245	GGC	AGC	G->A	4.30 %
7	273	CGT	TGT	C->T	3.49 %
8	213	CGA	TGA	C->T	2.08 %
9	245	GGC	GAC	G->A	1.48 %
10	196	CGA	TGA	C->T	1.48 %

BREAST CANCER

Rk	Pos	WT	Mut.	Evt	Freq.
1	175	CGC	CAC	G->A	5.40 %
2	248	CGG	CAG	G->A	5.20 %
3	273	CGT	CAT	G->A	4.40 %
4	248	CGG	TGG	C->T	3.60 %
5	245	GGC	AGC	G->A	1.87 %
6	213	CGA	TGA	C->T	1.73 %
7	220	TAT	TGT	A->G	1.67 %
8	273	CGT	TGT	C->T	1.47 %
9	282	CGG	TGG	C->T	1.33 %
10	285	GAG	AAG	G->A	1.33 %

LUNG CANCER

Rk	Pos	WT	Mut.	Evt	Freq.
1	157	GTC	TTC	G->T	2.42 %
2	158	CGC	CTC	G->T	2.36 %
3	273	CGT	CTT	G->T	2.36 %
4	248	CGG	CTG	G->T	2.24 %
5	249	AGG	AGT	G->T	2.06 %
6	175	CGC	CAC	G->A	1.94 %
7	245	GGC	TGC	G->T	1.82 %
8	248	CGG	TGG	C->T	1.70 %
9	273	CGT	CAT	G->A	1.64 %
10	249	AGG	ATG	G->T	1.64 %

FIGURE 5.   **Rank of the 10 most frequent p53 mutations. G>T transversions associated with tobacco smoking are shown in black. Transitions at CpG dinucleotides are shown in gray. Other events are shown in white.**

for frequent variants in the same cancer. Once again, these data suggest that the majority of unique variants are probably laboratory artifacts, although it is impossible to exclude that some of these rare variants are true p53 inactivating mutations and their rarity could be due either to the difficulty of the mutational event to occur in vivo or the leakiness of the p53 mutation that could need a specific genetic background to be effective. Both hypotheses could also be simultaneously true. Analysis of the 175P mutant p53 is a good example of the ambiguity of this situation [Friedlander et al., 1996; Rowan et al., 1996]. This mutant has only been reported four times in the literature. It was initially reported as a wild-type p53, as it retains growth arrest activity and transactivation of the WAF1 gene, but it was subsequently demonstrated that this mutant was defective for apoptosis demonstrating for the first time that mutant p53 may be only partially inactivated. Nevertheless, its rarity in the UMD-p53 database suggests that it does not play an important role during the transforming process. Mutation at the last residue of codon 125 is also a good example of a common mistake (375 G>A

(codon 125 ACG>ACA; Thr>Thr)) [Holmila et al., 2003]. This mutation was found three times in the latest version of the p53 database. This finding in solid tumor tissue is considered to be neutral, as it is not associated with any amino-acid change. More recently, using an RNA- and DNA-based analysis of Li-Fraumeni patients, Varley et al. [1999] demonstrated that this mutation, localized at the 3′ end of exon 4, leads to abnormal splicing. A more thorough review of the literature shows that this abnormal splicing was already described more than 10 years ago in a leukemia cell line [Soudon et al., 1991]. (Fig. 5)

CONCLUSIONS AND PROSPECTS

This raises the question of the cut-off value that should be used to ensure that each variant corresponds to a true mutation, but this question is difficult to answer at the present time. Functional analysis of these variants does not provide any clues. The high fragility of the DNA binding domain of p53 implies that the majority of mutations occurring between codons 100 and 300 will lead to p53 inactivation.

Many of them will not occur in human cancers for multiple reasons. As stated above, the construction of artificial mutants (i.e., not found in human cancer) at position 175 give rise to inactive p53 with the same properties as mutants found in cancer. In view of the difficulty of evaluating these rare variants, we believe that the p53 mutation database should be analyzed very cautiously. Although the main messages from previous analyses, such as p53 mutations and carcinogen exposure, cannot be denied, more detailed analysis with cancer subtype or in specific populations must be performed very cautiously.

Several studies have demonstrated that p53 mutations may be found in rheumatoid arthritis patients [Sun and Cheung, 2002; Yamanishi et al., 2002]. Many of these mutations are transitions and it is suggested they could be caused by DNA damage associated with oxidative stress. Analysis of these mutations indicates that only a few of them are found at hot spot regions and some of them have never been described in the p53 database. These observations could suggest that different types of p53 mutants are involved in synovial tissue leading to a milder p53 defect. Analysis of these mutant p53 in their specific cellular context would be very useful to provide greater insight into the significance of these mutations.

All these data indicate that the shaping of p53 mutants is a very complex problem and that both upstream and downstream elements are involved. Although upstream pathways appear to be well defined and involved an enormous heterogeneity of endogenous or exogenous carcinogen exposure, downstream pathways are more vague and could be highly cell type specific.

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NOTE ADDED IN PROOF

While this paper was in press, we have been aware that Rodin et al. have previously reported that p53 mutation at CpG dinucleotide could be co-selected during the transformational process (see Rodin et al., [1998] CpG transition strand asymetry and hitchhiking mutations as measures of tumorigenic selection in shaping the p53 mutation spectrum. Int J Mol Med 1:191–199).

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