HUMAN MUTATION 21:217–228 (2003)

p53 REVIEW ARTICLE

TP53 Mutations in Human Skin Cancers

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For the p53 Special Issue

The *p53* gene (*TP53*) is mutated in numerous human cancers. We have used it as a molecular target to characterize the induction of mutations in human skin cancers. About 50% of all skin cancers in normal individuals exhibit *p53* mutations. This frequency rises to 90% in skin cancers of patients with the DNA-repair deficiency known as xeroderma pigmentosum (XP). These mutations are characterized by a specific signature, attributed to the ultraviolet uvB part of the solar spectrum. In this review, we will describe different *p53* mutation spectra, in relation to the various histopathological types of skin cancers such as basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and malignant melanoma as well as to the DNA repair efficiency of the patients. In particular, different mutational hot spots are found among the various spectra. We have tried to elucidate them in terms of induced DNA lesion hot spots, as well as speed of local nucleotide excision repair (NER) or sequence effects. The molecular analysis of these mutagenic characteristics should help in the understanding of the origin of human skin cancers in the general population. Hum Mutat 21:217–228, 2003. © 2003 Wiley-Liss, Inc.

KEY WORDS: p53; TP53; tumor; carcinogen; cancer; DNA repair; environment; radiation, UV; skin; ultraviolet; xeroderma pigmentosum; XP; melanoma

DATABASES:
TP53 – OMIM: 191170; GenBank: NM_000546 (mRNA)
http://p53.curie.fr/ (p53 Web Site at Institut Curie)
www.iarc.fr/p53 (IARC p53 Mutation Database)

INTRODUCTION

Among all human tumors, skin cancers represent the most frequent human malignancies. Basal cell carcinomas, squamous cell carcinomas (non-melanoma skin cancers), and melanomas mainly represent them. Skin cancers are the ultimate response to repeated sun exposure, especially in light-skinned individuals, and their frequencies are increasing constantly in the last couple of decades [Black et al., 1997]. Non-melanoma skin cancers are relatively frequent but are easily cured; in contrast, melanomas are very aggressive tumors that metastasize very quickly.

Skin cancers are due to a complex sequence of random events caused and promoted by UV radiation (uvB and uvA, and perhaps visible light and infrared). The uvB radiation mainly produces DNA lesions between adjacent pyrimidines (TT; CT; TC; CC). Two types of lesions are essentially produced: the cyclobutane pyrimidine dimers (CPD) and the (6-4) photoproducts (6-4PP) [Mullenders et al., 1993]. These lesions are normally repaired by the nucleotide excision repair (NER) system. NER repairs the damaged strand of DNA in a "cut and paste" manner that consists of mainly five steps: recognition of the lesion, opening of the DNA helix, demarcation of the lesion, dual incision of the damaged DNA strand, error-free resynthesis of the gap left by the dual incision, and ligation of the newly synthesized DNA strand [Hoeijmakers, 2001; Stary and Sarasin, 2002]. A schematic model for NER is shown in Figure 1.

Recognition Step

Two subpathways of repair have been demonstrated: the slow global genome repair (GGR) (Fig. 1A) and the rapid transcription-coupled repair (TCR) (Fig. 1B). TCR repairs a subset of lesions, including UV-lesions, on the transcribed strand of active genes while GGR repairs those lesions on the rest of the genome [Bohr et al., 1985; Le Page et al., 2000; Mellon et al., 1987]. Mainly, the mechanism of

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DOI 10.1002/humu.10179
Published online in Wiley InterScience (www.interscience.wiley.com).

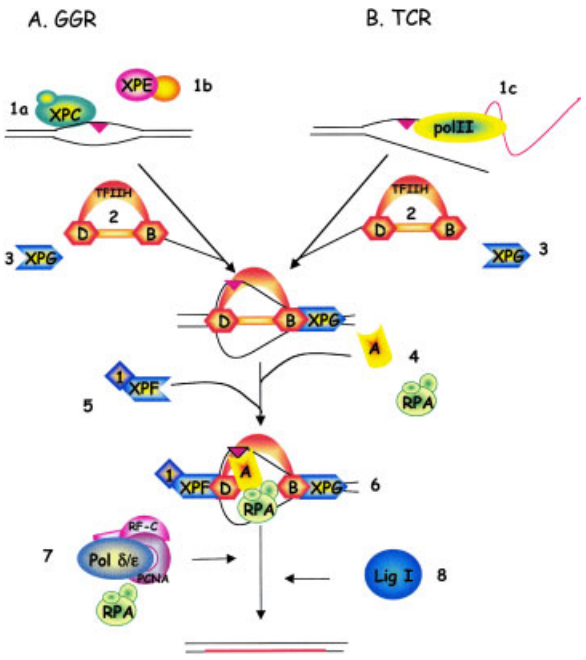

FIGURE 1. **Schematic pathway of nucleotide excision repair. More details are included in the text. [Color figure can be viewed in the online issue at www.interscience.wiley.com.]**

these two pathways is the same with the exception of the recognition step. In TCR, the signal for the recognition of the lesion is insured by the blockage of the RNA pol II at the level of the damage [Mu and Sancar, 1997] (Fig. 1B). In GGR, the protein XPC complexed with the homologous protein of Rad 23 in humans (HHR23B) (Fig. 1A), recognizes the distortion of the DNA helix caused by a bulky lesion, and triggers the signal for the rest of the repair machinery on the damaged DNA [Sugasawa et al., 1998]. Recently, it has been shown that the XPC protein can easily recognize 6-4PP but can hardly recognize CPDs, which are less distorting lesions [Wang and Taylor, 1991]. To be able to recognize CPD, the XPC protein would need the help of another protein, such as the XPE-DDB (Fig. 1A), or a local opening of the helix (2–4 bases) in the region of the lesion [Sugasawa et al., 2001].

Opening of the Helix

The presence of the XPC protein on the lesion or the blockage of the RNA pol II are both signals for the arrival of the multi-complex TFIIH. The helicases XPB and XPD, which are part of the transcription factor TFIIH [Schaeffer et al., 1994; Schaeffer et al., 1993], open the double strand of DNA around the lesion [Evans et al., 1997] (Figs. 1, 2). The presence of TFIIH on a lesion triggers the binding of the endonuclease XPG (Figs. 1, 3), although the incision by XPG is strictly dependent on the presence of XPA [Volker et al., 2001].

Demarcation of the Lesion

The proteins XPA and RPA (replication protein A) may organize the repair machinery around the lesion (Figs. 1, 4). XPA is a protein known to bind damaged DNA and to physically interact with the TFIIH complex and the complex ERCC1-XPF [Li et al., 1994; Park et al., 1995; Park and Sancar, 1994] (Figs. 1, 5). RPA also helps the local unwinding of the DNA by its high affinity to single-strand DNA [de Laat et al., 1998].

Dual Incision

Once the damaged strand has been detected and separated from the undamaged one, an oligonucleotide of 24–32 bases is excised by the concerted activities of two endonucleases. XPG cuts at the 3′ of the lesion [O'Donovan et al., 1994] and the complex ERCC1-XPF cuts at the 5′ [Sijbers et al., 1996] (Figs. 1, 6). The TFIIH complex could be involved in the positioning of XPG, RPA, and PCNA (proliferating cell nuclear antigen), and could be responsible for the polarity of this cut [Iyer et al., 1996].

Gap Filling

Finally the gap left by the excision of the damaged fragment is filled in by DNA synthesis. Pol δ and/or pol ε together with RPA, PCNA, RF-C (replication factor C), resynthesize the novel strand [Shivji et al., 1995] (Figs. 1, 7) and the reaction is completed by the ligation of the newly synthesized strand by the DNA ligase I [Barnes et al., 1992] (Figs. 1, 8).

Unrepaired lesions are at the origin of a temporary UV-induced stress response such as the p53 protein stabilization or RNA synthesis inhibition and they may give rise to mutations, after one or two rounds of replication, all along the genomic DNA including tumor suppressor genes (such as the *p53* gene TP53; MIM# 191170) or proto-oncogenes (such as the *ras* family genes) and therefore can lead to carcinogenesis [Dumaz et al., 1994]. The general dogma in carcinogenesis is the hypothesis of clonal expansion of damaged cells due to activation or inhibition of crucial genes implicated in the control of cell cycle, maintenance of genetic integrity, proliferation, and differentiation. It appears clearly that DNA repair systems play a crucial role in maintaining the genetic integrity against genotoxic attacks and in protecting us from massive tumoral development. This major role is demonstrated by the high incidence of skin cancers in NER-deficient syndromes such xeroderma pigmentosum (XP). In this syndrome, numerous unrepaired UV-lesions lead to mutations on key genes and those will start a cellular process of immortalization followed by cell transformation. Ultraviolet light leaves a molecular signature on these genes in all skin cancers (melanomas and non-melanoma skin cancers) in the

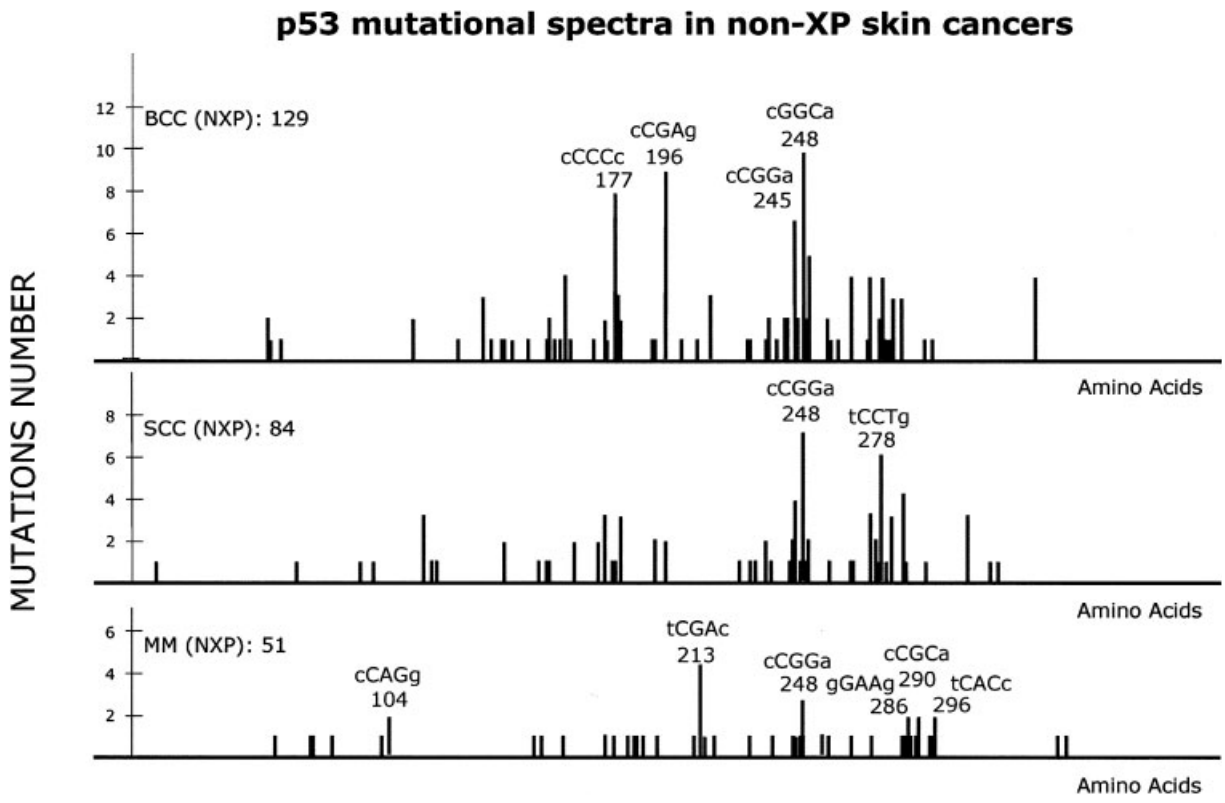

FIGURE 2. ***TP53*** **mutational spectra in skin cancers from non-XP patients in accordance with the nature of skin malignancy. Numbers on the spectra represent the number of the mutated codons. Only mutational hot spots are represented including the sequences of the codons.**

form of specific mutations, which can help us to understand the pathological history of these tumors.

Even if other factors such as immunological responses, genetic predisposition, skin phototypes, viruses, etc., are to be taken into account for the development of skin cancer, in this review we will only focus on the nature of mutations and their positions along the *p53* gene. It is our aim to generate mutational spectra of the three main types of skin cancers issued from normal individuals and NER-deficient XP patients. We will try to highlight the differences between those spectra, since these diversities could reflect a different molecular mechanism in the origin of the distinct pathologies.

Data from skin cancers of normal individuals come from the Institut Curie's p53 database (http://p53.curie.fr/) [Beroud and Soussi, 2003], while data from skin cancers of XP patients come from our own laboratory completed by data from the literature [D'Errico et al., 2000; Dumaz et al., 1993; Giglia et al., 1998; Matsumura et al., 1995; Sato et al., 1993; Soufir et al., 2000; Spatz et al., 2001]. We must point out that because XP patients, due to their repair deficiency, develop numerous skin cancers, some of the tumors we have analyzed belonged to the same patient. In this case, to avoid including in our analysis mutations coming from metastasis that could complicate the analysis, we have only taken into consideration samples which, after histological analysis, were judged as primary tumors.

Moreover it is also important to underline that in our study we considered CC to TT tandem mutations as a unique event of translesion synthesis and not as two distinct transitions C to T. For this reason, we reviewed the list of mutations for skin cancers of normal individuals in the database and compiled a new list that underscored CC to TT tandem mutations.

HUMAN SKIN CANCERS

Two classes of malignancies represent mainly the majority of skin cancers: malignant melanomas (MM) deriving from melanocytes, and non-melanoma skin cancers (NMSC) deriving from basal or suprabasal keratinocytes. The role of UV irradiation in the skin carcinogenesis has been clearly demonstrated by the fact that skin cancers (essentially NMSC) are mainly located on sun-exposed parts of the body, are more frequent on light-skinned individuals, and increase with low latitude exposures. Mutations found in the *p53* gene are mainly C to T transitions located on dipyrimidine sites considered as the UV molecular signature [Ziegler et al., 1994; Dumaz et al., 1994].

Malignant Melanomas

Malignant melanomas represent only 3–5% of total skin cancers, however, they are very aggressive and produce metastasis very rapidly. The role of UV irradiation in the induction of MM is not clear. They are not always located on sun-exposed parts of the body and there is no substantially increased number of MM in individuals who are more sun-exposed, except for the elderly [Rivers, 1996]. They are often associated with a loss of function of the INK4a locus [Hussussian et al., 1994], less frequently with a *p53* mutation (10%) [Papp et al., 1996]. However, XP patients develop MM that are mainly located on sun-exposed parts of the body, they are not as aggressive as the non-XP MM and they show a very high frequency of *p53* mutations (60%); all of these mutations are typically UV-induced ones [Spatz et al., 2001].

Basal Cell Carcinomas

Basal cell carcinomas (BCC) are the most frequent malignancy in humans, but very rarely lead to metastasis (1 in 10,000). This tumor appears mainly on exposed parts of the body of light-skinned individuals [Goldberg, 1996]. An inherited disease, the Gorlin syndrome, is characterized by a predisposition in developing BCC. The gene that causes this syndrome is the PTCH tumor suppressor gene [Hahn et al., 1996; Johnson et al., 1996]. In BCC from XP and non-XP patients, PTCH and *p53* are found mutated with a high frequency and the mutations are clearly UV-induced ones [Bodak et al., 1999].

Squamous Cell Carcinomas

Squamous cell carcinomas (SCC) are not as frequent as BCC in DNA-proficient individuals, but they eventually metastasized. SCCs are mainly located on chronically sun-exposed parts of the body and often on pre-existing clinical lesions such as scarves. The pre-malignant lesion is the actinic keratosis (AK). One AK in over 1,000 evolutes toward a SCC, the others may regress spontaneously if they are not exposed to more sun [Marks, 1996]. *P53* mutations are found frequently in SCC and also in AK, indicating that they can be one of the first events in the genesis of SCC. These mutations are also C to T transitions located on dipyrimidine sites [Ziegler et al., 1994].

TP53 MUTATIONAL SPECTRA FROM SKIN CANCERS OF NORMAL INDIVIDUALS

Mutation Spectra

We statistically analyzed the mutational spectra of the *p53* gene of the three types of malignancies found in normal individuals by using the HyperG program developed by Cariello et al. [1994]. The three spectra (shown in Fig. 2) are statistically different (HyperG p values: BCC-SCC $p<0.005$; BCC-MM $p<0.001$; SCC-MM $p<0.001$). To calculate the hot spots of mutation we applied the law of Poisson as already described [Stary et al., 2002]; the calculated hot spots and the corresponding sequences are shown on the spectra.

Only one hot spot is found in common among all three pathologies: the Arg 248. This is probably due to three factors: 1) the presence of a bipyrimidine site, target for UV-induced lesions, at the level of this codon **c.C**GG.a (the capital letters among two points show the sequence of the codon and the boldface indicates the mutated bases); 2) the presence of a CpG sequence, the cytosine in this sequence has been shown to be methylated [Magewu and Jones, 1994; Tornaletti and Pfeifer, 1995], and to undertake a rapid deamination into thymine [Tu et al., 1998]; 3) the Arg 248 is an amino acid in direct contact with the DNA [Cho et al., 1994], hence when mutated it disrupts the DNA binding functions of the P53 protein and therefore its transactivation properties. This would lead to a selective advantage for cells having the 248 mutation.

Apart from the codon 248, the other mutational hot spots seem to be characteristic of the tumoral types.

BCC Hot Spots

Mutational hot spots typical of BCC are codons 177, 196, and 245. Codon 177 is not frequently mutated in other malignancies and seems to be specific only for skin BCC. Very few data are known about this codon but it is interesting to notice that 177 is a sequence that is very slowly repaired after UV-irradiation [Tornaletti and Pfeifer, 1994]. Codon 196 and 245 are both also found mutated in breast and colon cancers and codon 245 in other types of cancers, such as lung carcinoma, head and neck, ovary, stomach, and esophagus malignancies, including also the germinal mutations found in the Li-Fraumeni syndrome. We can hypothesize that modification of the corresponding amino acid should give a strong selective advantage to the mutated cells.

SCC Hot Spots

The hot spot at codon 278 seems to be specific of skin SCC. It is mutated also in other types of internal cancers although at lower frequency. At the level of this codon, UV-induced lesions are normally repaired between 24 and 48 hr: before 24 hr no UV-induced lesions are repaired whereas after 48 hr 88% of the lesions are repaired [Tornaletti and Pfeifer, 1994].

MM Hot Spots

Mutational hot spots in MM are the codon 104 (rarely mutated in other types of cancers); codon 213;

and codons 286, 290, and 296 (which are all grouped in a region of the *p53* gene infrequently modified). UV-lesions at the level of the codon 213 are slowly repaired [Tornaletti and Pfeifer, 1994]. Codon 213 is not found modified in NMSC but it is frequently mutated in breast and colon cancers.

The differences found between BCC and SCC could be explained in two equivalent ways. The first is based on the hypothesis that BCC and SCC could derive from two different skin stem cells in which P53 could play a different role in the genome safeguarding. For instance, P53 could activate or inhibit certain specific genes in the stem cell that will originate SCC and other genes in the stem cell that will originate BCC. The selection will therefore be different on these two cell lines. In favor of this hypothesis is also the fact that PTCH gene is exclusively found mutated in BCC but not in SCC. There are more mutation hotspots containing CpG sequences in BCC than in SCC. This may indicate a difference in the time of the corresponding stem cells to enter the cell cycle or to carry out replication after UV-exposure, leading to different levels of 5meC deamination during this time.

The second possibility is based on the hypothesis that BCC and SCC derive from a unique stem cell but that certain particular mutations of P53 produced randomly could orientate more to the BCC phenotype while others more to the SCC.

The differences found between MM and NMSC could be rationally explained by the fact that MM derive from melanocytes and NMSC from keratinocytes. The presence of melanines in the former cell type could play a role in filtering UV rays, therefore changing the quantity and quality of UV damage to the genome. Moreover, some melanines could increase the production of free oxygen radicals in the presence of UV light. In melanocytes the nature of the damaging agents could hence be different from the ones in keratinocytes. It is interesting to notice that *p53* is not frequently mutated in MM (10%), while the gene *p16* (*INK4a*) is often modified. This confirms that P53-independent pathway can also control the cell cycle and hence would be involved in the carcinogenesis of MM.

Mutation Type

We have also analyzed the type of mutations found in the three different skin malignancies. We have graphically represented the result of this analysis through a series of histograms shown on Figure 3 (non-XP histograms). We have statistically compared the different distributions two-by-two by using the X^2 test. The distribution of the types of mutations is statistically different between MM and SCC/BCC ($p < 5 \times 10^{-3}$), but not between SCC and BCC ($p < 0.3$). MM are characterized by a higher frequency of A.T→G.C, probably caused by mutagens other than uvB. For this kind of substitution a role for free oxygen radicals could be seen. Although there is no statistical difference between the distribution of the types of mutations between BCC and SCC, we would like to point out that there is indeed a higher frequency of C.G→A.T in SCC than in BCC. This difference deserves to be investigated in more detail.

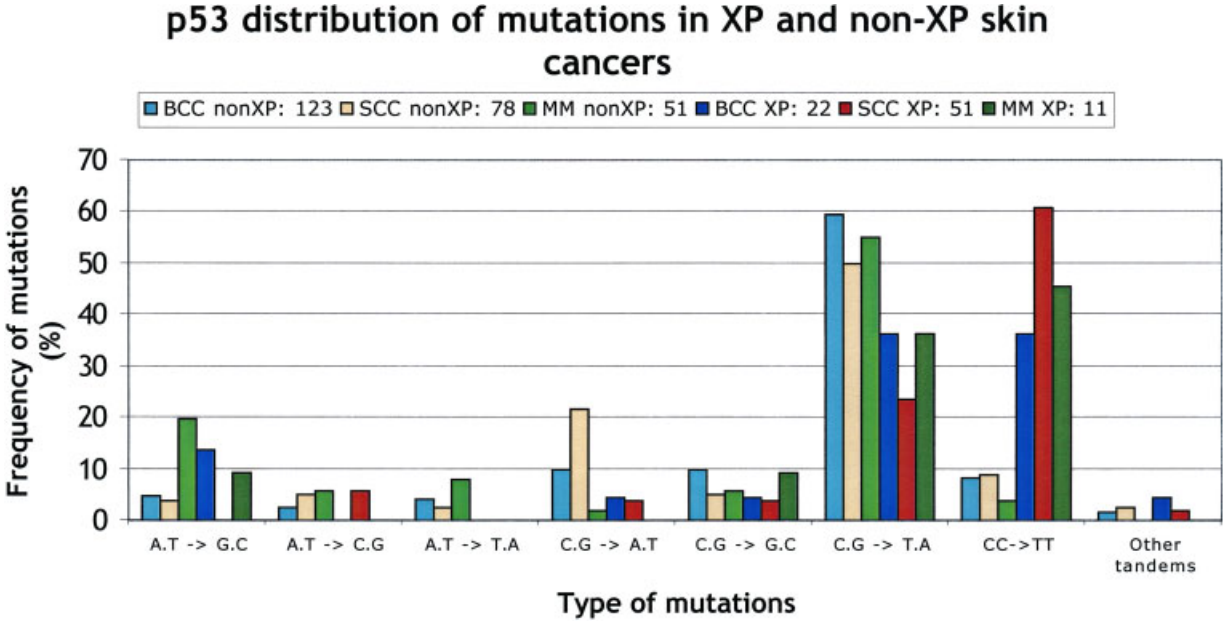

FIGURE 3. **Distribution of the different types of base substitutions found on *p53* gene in XP and non-XP skin cancers in accordance with the nature of skin malignancy. [Color figure can be viewed in the online issue at www.interscience.wiley.com.]**

TP53 MUTATIONAL SPECTRA FROM SKIN CANCERS OF XP INDIVIDUALS

The most striking difference between NMSC from XP and from non-XP patients is their number and the age of appearance. While non-XP NMSC appear when the patients are usually older than 50–60 yr, XP NMSC appear very early in life (about 3–5 yr old) and with a very high frequency, corresponding roughly to a 4,000-increase compared to the normal population [Kraemer et al., 1987]. Usually, these malignancies are less aggressive than the ones found in non-XP patients, at least for melanomas [Kraemer et al., 1994]. At the molecular level another striking difference is the kind of mutations found on different key genes such as the *p53* gene, the PTCH gene, or the INK4a locus [Soufir et al., 2000]. For both XP and non-XP skin cancers, mutations are located on dipyrimidine sequences, but for non-XP they are mainly C to T transitions while for XP tumors they are essentially CC to TT tandem mutations. Similarly, the distribution of these mutations on the *p53* gene is not identical for the two types of patients for reasons that are still unclear.

Mutation Spectra

We statistically analyze the mutational spectra of the gene *p53* in skin cancers from XP patients. The three spectra (shown in Fig. 4) are statistically different (HyperG p values: BCC-SCC $p<0.005$; BCC-MM $p<0.01$; SCC-MM $p<0.001$). To calculate the hot spots of mutation we applied the Poisson distribution. These calculated hot spots and the corresponding sequences are shown on the spectra (Fig. 4).

Only one hot spot is found in common among all three pathologies: His 179. This codon is not a mutational hot spot in non-XP skin cancers. We can suppose that the codon 179 could be a sensitive site for UV-lesions quickly repaired in repair-proficient cells. Unfortunately, no data on the speed of repair of this sequence are available.

Another striking feature is the mutational hot-spot Arg 282, which seems to be specific of XP SCC, although it has been detected in other XP and non-XP skin cancers. The reasons for this specificity are still unknown. Indeed it will be interesting to study the properties of the 282 P53 mutant to determine whether or not this mutant could induce a higher cell proliferation, in a repair-deficient background, of stem cells leading to SCC.

We also compared the mutational spectra of XP skin cancers and non-XP skin cancer in accordance with the type of tumor. The XP and non-XP spectra are statistically different (hyperG values: XP BCC vs. non-XP BCC $p<0.001$; XP SCC vs. non-XP SCC $p<0.005$; XP MM vs. non-XP MM $p<0.001$).

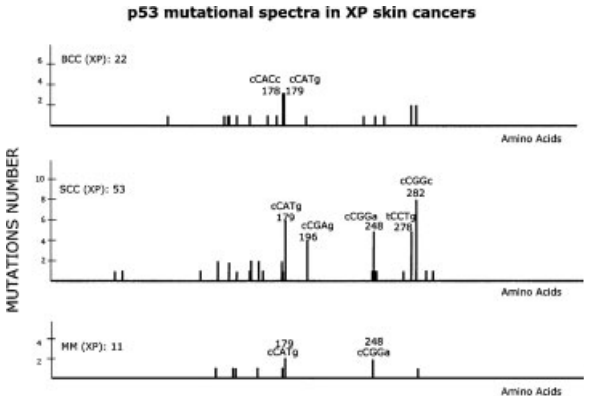

FIGURE 4. ***TP53* mutational spectra in skin cancers from XP patients in accordance with the nature of skin malignancy. Numbers on the spectra represent the number of the mutated codons. Only mutational hot spots are represented including the sequences of the codons.**

It is obvious that in non-XP cells the competition between the speed of repair at a given site after UV-exposure and the speed of replication will partly determine the location of induced mutations. This is obviously not true for XP cells that cannot repair UV-induced lesions. Therefore, one can conclude that the statistical differences between XP and non-XP for a given class of skin tumors are basically due to low repair or high repair rates in the original damaged stem cells.

Mutation Type

We have also analyzed the type of mutations found in the three different skin malignancies in XP individuals. We have graphically represented the result of this analysis through a series of histograms shown on Figure 3 (XP histograms). We have statistically compared the different distributions two by two by using the X^2 test. The distribution of the types of mutations is statistically different between the XP BCC vs. non-XP BCC $p<0.02$; XP SCC vs. non-XP SCC $p<10^{-4}$; XP MM vs. non-XP MM $p<0.02$.

The striking difference between XP and non-XP skin cancers is the great majority of CC to TT tandem mutations in XP malignancies. Thirty-six percent of the mutations found in XP BCC, 45% in XP MM, and 61% in XP SCC are CC to TT tandem mutations, whereas this type of substitution is found very rarely (<10%) in non-XP skin cancers. On the contrary, there are fewer C:G→T:A transitions (always located on dipyrimidine sequences) in XP skin cancers than in non-XP. This difference is not due to a selective advantage of the mutated proteins bearing the CC to TT tandem mutation versus C to T transition, because in most cases the two mutations cause strictly the same amino-acid change in P53, producing hence the same mutant P53 protein. The same tendency is observed in genes other than *p53* such as *PTCH*

[Bodak et al., 1999] and *INK4a* [Soufir et al., 2000]. CC to TT tandem mutations are found independently from the type of tumors, although we observe a diminished frequency in BCC, the reason for this may probably be due to the fact that many BCC we have studied belonged to XPV patients (see later for more explanations).

CC toTT Tandem Mutations at CpG Sites

Both for XP and non-XP skin cancers, CC to TT tandem mutations are found more frequently on CpG sequences, known to be methylated at the level of the cytosines, at least in the *p53* gene [Magewu and Jones, 1994; Tornaletti and Pfeifer, 1995; reviewed by Soussi and Beroud, 2003]. On the contrary, C to T transitions are more frequently found on non-methylated cytosines [Giglia et al., 1998]. This high frequency of CC to TT tandem mutations in NER-deficient cells and the role of the methylation in the origin of these mutations remain obscure. We propose a model to explain the origin of CC to TT tandem mutations, as depicted in Figure 5.

The UV-lesion on CC*pG site (the C* indicates the 5meC) could be submitted to a spontaneous or UV-induced deamination of the cytosines, the first one to deaminate would be the 3′ one, because of a high molecular instability of methylated cytosines. The longer the lesion stays in the cell before replication and without being repaired, the more the 5′ cytosine is likely to be also deaminated. Deamination of a 5meC will give rise to a thymine, while deamination of a C will lead to a uracil. If replication occurs before repair, the replicative DNA polymerase will insert an adenine oppo-site a T or a U and this will be the cause of the mutations. In a NER-deficient cell, UV-lesions stay longer on the DNA, hence, the chance for a second deamination is higher than in a NER-proficient cell. According to this reasoning it is likely to find more CC to TT tandem mutations in UV-damaged cells in XP skin.

TP53 MUTATIONAL SPECTRA FROM SKIN CANCERS OF XPC AND XPV

Under the term xeroderma pigmentosum is commonly designed a phenotype that has several features, such as sun sensitivity, photophobia, early skin cancers and in some cases, neurodegeneration. Nevertheless, seven complementation groups (from XPA to XPG), therefore seven genes, are implicated in this phenotype. All XP complementation groups are deficient in GGR and TCR, apart from XPC and XPE, which are only deficient in GGR.

A variant form of xeroderma pigmentosum, called XPV, has also been described. Cells from XPV are proficient in NER but are deficient in the translesion synthesis (TLS) through UV-induced lesions [Cordonnier et al., 1999]. XPV cells present a high level of UV-induced mutagenesis [Wang et al., 1993]. The gene mutated in XPV was cloned in 1999 by two different groups [Johnson et al., 1999; Masutani et al., 1999], it is the gene of the pol η, or hRAD30A, the human homolog of the protein Rad 30 in *S. cerevisiae*. Pol η is a polymerase that can synthesize opposite a cyclobutane thymine dimer in a relative error-free manner [Masutani et al., 2000]. When polη is mutated as in the case of XPV, other DNA polymerases, probably more error-prone, will synthesize through the lesions inducing more mutations. This increased mutagenesis, albeit with a normal NER pathway, is at the origin of the cancer-proneness of XPV patients.

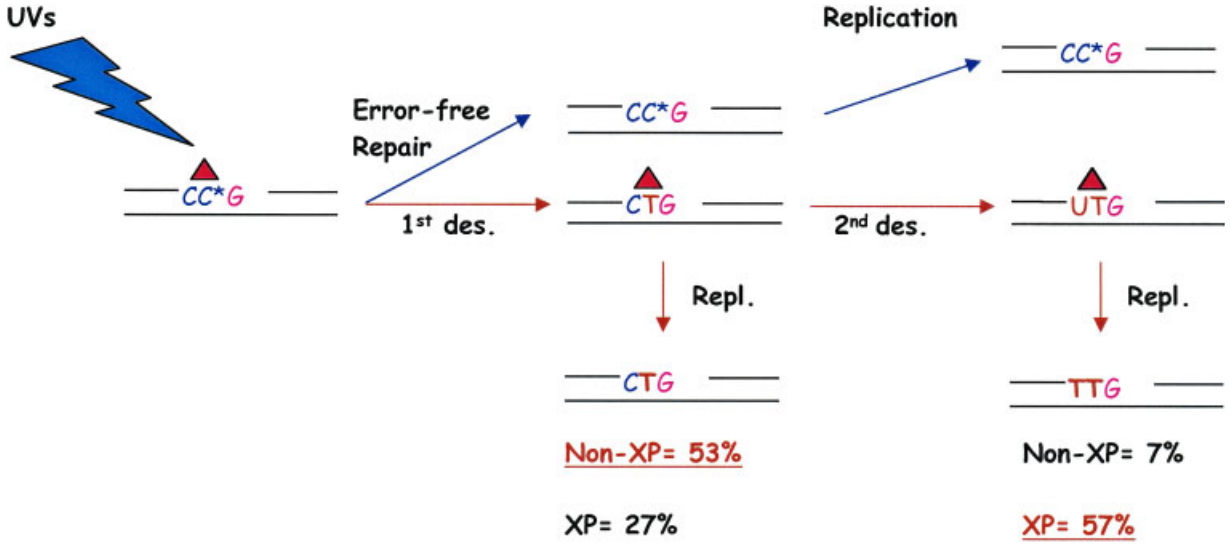

FIGURE 5. **Schematic model of time-dependent double deamination of UV-induced lesions on C5meC photolesion. [Color figure can be viewed in the online issue at www.interscience.wiley.com.]**

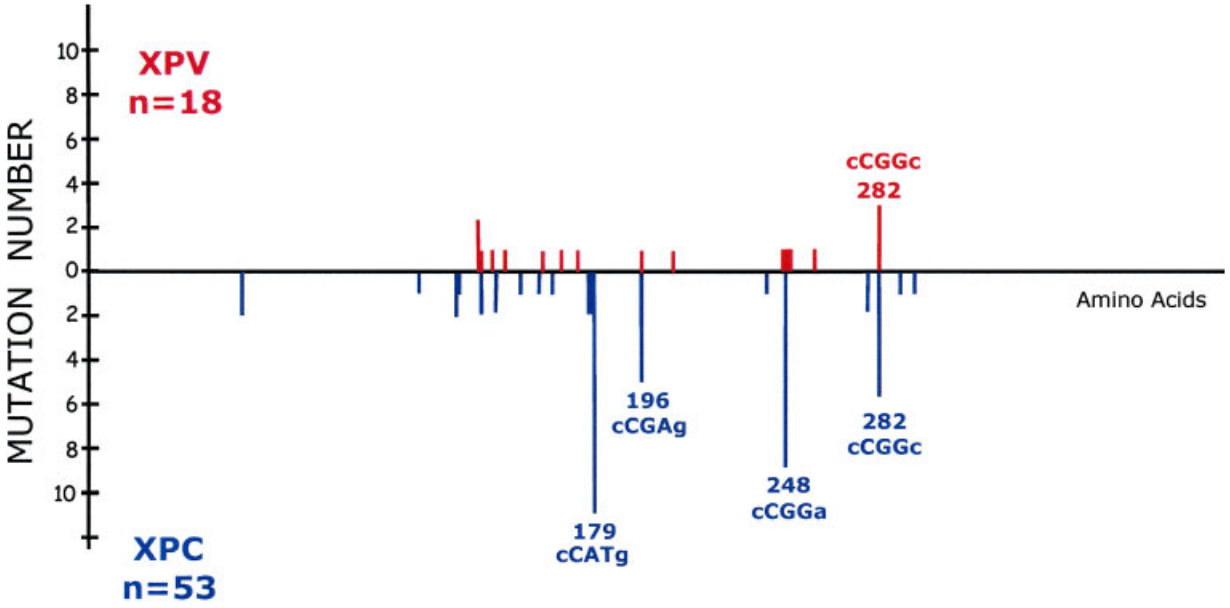

FIGURE 6. ***TP53* mutational spectra in skin cancers from XPC and XPV patients. Numbers on the spectra represent the number of the mutated codons. Only mutational hot spots are represented. The sequences of the codons are also shown. [Color figure can be viewed in the online issue at www.interscience.wiley.com.]**

Mutation Spectra

In our analysis the great majority of XP skin cancers belonged to XPC and XPV patients. We wanted to define the typical *p53* mutational spectra of these two groups of patients. The two spectra are depicted in Figure 6. They are statistically different (HyperG values: XPV vs. XPC $p<0.05$). Even though the number of mutations in the XPV group is small, a mutational hot spot could be identified, the amino acid Arg 282. The UV-induced DNA lesions on this codon at CC is located on the non-transcribed strand of the p53 gene. This explains why it is often mutated in XPC tumors because these patients are GGR-deficient. In the case of non-XP patients and XPV patients, this lesion should be repaired because they are GGR-proficient but still at a lower rate than lesions on the transcribed strands. If replication occurs before a complete repair, the DNA lesion will be replicated by the less error-prone DNA polymerase η in normal cell but by another more-error prone translesion polymerase in XPV. This may explain the existence of the 282 hotspot in XP-C and XPV tumors but not in tumors from normal individuals.

Mutation Type

We wanted to focus on the type of mutations found in XPC and XPV skin cancers and to compare these two distributions with the non-XP one. The result of this analysis is shown in Figure 7. The distribution for XPC is statistically different from the other two (X^2 values: XPC vs. XPV $p=5\times10^{-3}$; XPC vs. non-XP $p<10^{-4}$), although no statistical difference could be found between XPV and non-XP (X^2 values: XPV vs. non-XP $p<0.33$), probably because the absolute number of mutations in the two groups is very different (252 mutations in non-XP and 18 mutations in XPV skin cancers).

CC to TT tandem mutations represent the great majority (76%) of mutations in XPC skin cancers, on the contrary, C to T transitions represent just 16% of the total mutations. It seems that CC to TT tandem mutations are specific of the XPC complementation group. This may be due, according to the model depicted in Figure 5, to the fact that XPC cells are much more resistant to UV-induced apoptosis than the other complementation groups [Queille et al., 2001]. In this case, UV-irradiated XPC cells will not die after UV-irradiation and will have enough time to carry out the cytosine deamination.

In XPV skin cancers C to T transitions represent the majority of mutations (39%). CC to TT tandem mutations are just 22% of the total mutations. This value, although smaller than the one observed for XPC patients, is intermediate between XPC and non-XP. In the light of the model of the time-dependent double deamination of two cytosines we proposed in the previous section, it was puzzling how to explain this intermediate frequency. The answer is still unclear but we can hazard a hypothesis helped by recently published results [Broughton et al., 2002]. In this work the authors identify two different groups of mutated pol η: pol η molecules which have lost the signal of nuclear localization and are still proficient for trans-lesion synthesis (TLS) but not present in the nucleus at the moment of the

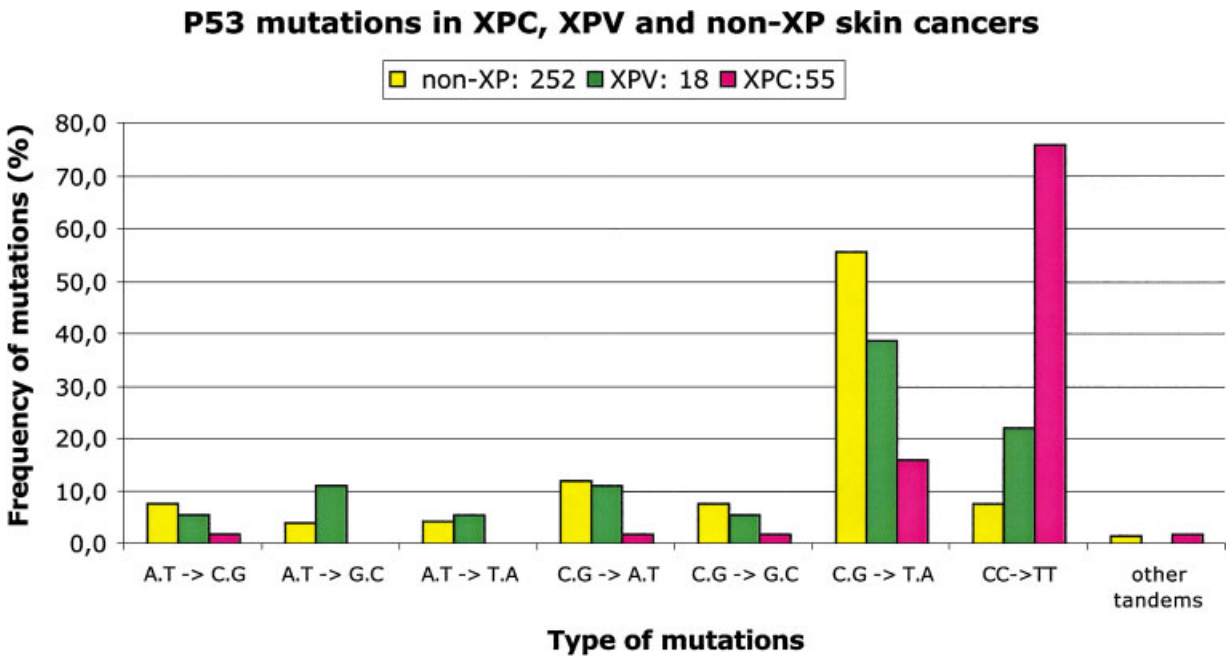

FIGURE 7. **Distribution of the different types of base substitutions found on *p53* gene in XPC, XPV, and non-XP skin cancers. [Color figure can be viewed in the online issue at www.interscience.wiley.com.]**

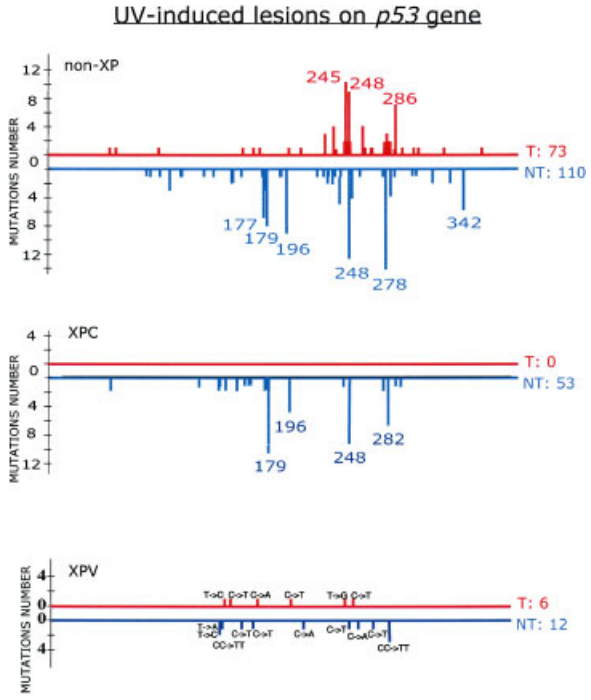

FIGURE 8. **Distribution of the UV-induced lesions along the transcribed and non-transcribed strand of the *p53* gene in non-XP, XPC, and XPV skin cancers. Numbers on the spectra represent the number of the mutated codons. In the spectrum from XPV skin cancers the types of mutations are depicted close to the corresponding mutated codons. [Color figure can be viewed in the online issue at www.interscience.wiley.-**

trans-lesion synthesis; and pol η molecules which have lost their trans-lesion functions and have no polymerase activity.

We have detected four CC to TT tandem mutations (which represent the 22% of the total mutations). Three of these mutations belong to two patients (cells XP62VI and XP127VI) from which we know that pol η is truncated, therefore cytoplasmic. The fourth CC to TT tandem mutation belongs to a patient from which we do not have the sequence data of pol η. It could be possible that the fact of having no pol η in the nucleus but all the polymerase molecules present in the cytoplasm at the moment of the TLS could slow down, in a way that we ignore, the reaction of TLS by another DNA polymerase. Cytosines, in UV-induced lesions, could therefore have more time to be deaminated and even more rapidly because they are in a single-strand sequence [Frederico et al., 1990]. In fact, it has been known for a long time that full-size DNA synthesis takes much more time to be completed in XPV than in NER-proficient cells.

Transcribed and Non-Transcribed Strands

UV-lesions have the property to be targeted on bipyrimidine sequences. This property can be used to determine whether the lesions that are at the origin of mutations were on the transcribed or non-transcribed strand of the *p53* gene. We have therefore positioned the mutations along the *p53* gene in accordance with the position of the UV-induced lesion. The result of this analysis is shown in Figure 8.

In non-XP skin cancers, lesions at the origin of mutations are almost equally distributed between the two strands of the *p53* gene. On the contrary, in XPC skin cancers UV-induced lesions responsible for

producing mutations are found only on the non-transcribed strand, since XPC cells are deficient in GGR but repair normally the transcribed strand of active genes. In XPV, skin cancers lesions are equally distributed, like in non-XP skin cancers, on both strands of the *p53* gene. We have also indicated the type of mutations that every lesion has caused. In some mutagenesis work done on XPV cells [Wang et al., 1993] or XPV cellular extracts [McGregor et al., 1999], the authors found a strand bias in the distribution of C to A transversions, more frequently found on the leading strand, and C to T transitions, more frequently found on the lagging strand. We cannot confirm these results. As shown in Figure 8, C to A and C to T mutations are equally distributed on both strands, only the CC to TT tandem mutations show a strand bias, and are only found on the non-transcribed strand of DNA. This fully agrees with the hypothesis we proposed for the codon 282 in XP tumors. We do not know which strand of the gene *p53* is the leading (or lagging) strand and we cannot exclude that both strands could be at the same time leading and lagging if two replication forks are active at 5′ and 3′ of the *p53* gene. However, from the striking difference between the two strands of the p53 gene in XPC tumors, it is obvious that the TCR pathway plays the major role in determining repair and therefore mutation induction.

CONCLUSIONS

The *p53* gene is up to now the most studied gene in terms of mutation characteristics. Thanks to update databases, one can have information on more than 15,000 mutated *p53* molecules from independent origins. The vast majority of these mutants are point mutations that give much more mechanistic information than deletions or insertions. The database we have used in this study is available at the web site: http://p53.curie.fr/.

The extraordinarily high level of information that we have, not only on these *p53* mutants, but also on the structure and the pleiotropic role of this protein in normal cell cycle regulation and in the carcinogenesis process can help researchers to dissect the origins of the carcinogenic events. A strong disadvantage, however, lies in the fact that all *p53* mutants are not biologically identical and there is a strong selective advantage for some hot spots versus other point mutations. This should be kept in mind while comparing mutation hot spots between different classes of tumors or types of organs.

In this paper, we have reviewed only skin cancers, the etiology of which have essentially implicated sun exposure. The analysis of *p53* mutations in all skin cancers analyzed from DNA repair-proficient patients shows that in vivo in normal human individuals the majority of mutations correspond to C to T transitions almost exclusively at dipyrimidine sites known to be hotspots of DNA lesions following UV-exposure. This in vivo result confirms nicely what has been found in the past, either in vitro using purified templates and DNA polymerases or ex vivo using bacteria, shuttle vectors, or cultured human cells. This C to T UV-signature in DNA repair proficient patients has been replaced by a more characteristic CC to TT tandem mutations in the DNA repair-deficient XP patients that represent more than 60% of mutations in this type of skin cancers. Although CC to TT mutations were found with in vitro systems, this high level was never obtained probably because of the lack of enough time to allow CC deamination as hypothesized in Figure 5. Indeed, in the various in vitro systems the time between UV-irradiation and replication of damaged templates is usually shorter than 24–48 hr. That is probably not enough for a full deamination of the two cytosines, whereas this time can be as long as several weeks in irradiated human skin.

Another interesting confirmation is the existence of preferential repair (transcription-coupled repair) in vivo in XP-C patients. This pathway has been discovered in cultured cells but the extraordinary distribution of all DNA lesions giving rise to mutations present only on the non-transcribed strand of the *p53* gene (see Fig. 8) is a nice demonstration that the TCR process does exist in XP-C patients in vivo.

The recent discovery of the polη gene, as well as numerous mutagenic DNA polymerases, have given new interest in the study of the XP variants. Although the number of *p53* mutations is low because these patients are rare, it is clear that the mutation spectra is different between XPV skin tumors and classical XP patients. Again the *p53* mutation spectra can tell us some in vivo indications about the mutagenic specificity of some of these error-prone polymerases.

Our data also show differences in *p53* mutation spectra among the three major UV-linked skin cancers. The interpretation of these differences is quite difficult to approach. This can be due to either different types of UV-induced DNA lesions in the various stem cells that will give rise to skin cancers or to different efficiency of repair of these lesions in the stem cells. It is also quite possible that each type of pathway leading to a given tumor needs specific p53 mutations. However, the comprehension of the role of each *p53* mutant in the carcinogenic process should help us to better understand the etiology and the processing of skin tumors.

In conclusion, the *p53* databases have been very helpful in correlating mutagenic and carcinogenic processes. Other mutation databases should also be developed, specifically to certain classes of proteins, in order to confirm all these data found with the *p53* gene.

ACKNOWLEDGMENTS

The authors thank Dr. Simone Benhamou (EMI 00 06 - INSERM, Evry, France) for her help in statistical analysis and Dr. Anne Stary for her helpful discussion on mutagenesis mechanism. Giuseppina Giglia-Mari is a fellow of the Association pour la Recherche sur le Cancer (Villejuif, France).

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