HUMAN MUTATION 15:105–113 (2000)

MDI SPECIAL ARTICLE

p53 Website and Analysis of p53 Gene Mutations in Human Cancer: Forging a Link Between Epidemiology and Carcinogenesis

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The p53 tumor suppressor gene has proven to be one of the genes most often mutated in human cancers. It involves mainly point mutations leading to amino acid substitutions in the central region of the protein which impairs normal functions. Analysis of the mutational events that target the p53 gene has revealed evidence for both exogenous and endogenous mutational mechanisms. For example, the p53 mutational spectrum reveals evidence for a direct causal effect of ultraviolet radiation in skin cancer, of aflatoxin B1 in liver cancer, and of tobacco smoke in lung cancer. This novel field, molecular epidemiology of human cancer risk, has added a new dimension to classical associative epidemiology by providing a direct link between human cancer and carcinogen exposure. For such analysis, we devised a generic software called UMD (Universal Mutation Database). It was developed as a generic software to create locus-specific databases (LSDBs) with the 4th Dimension® package from ACI. This software includes an optimized structure to assist and secure data entry and to allow the input of various clinical data. Hum Mutat 15:105–113, 2000. © 2000 Wiley-Liss, Inc.

KEY WORDS: **MDI; p53; PT53; mutation database; locus-specific database; cancer; carcinogenesis; epidemiology; UMD**

INTRODUCTION

Exposure to environmental chemicals has been recognized as an etiological factor in human carcinogenesis since the 18th century, when the carcinogenic effects of soot were observed in the scrota of chimney sweeps [for review, see IARC, 1990]. In 1981, it was estimated that about 80% of all cancers in the USA were due to environmental factors, and would thus be preventable [Doll and Peto, 1981]. Classical descriptive epidemiology has identified high-risk populations such as cigarette smokers and certain industrial workers. The use of animal models, and later, cell cultures, has revealed a large number of chemical compounds to be highly carcinogenic [Harris, 1991]. Yet until recently no direct link between exposure to carcinogens, genetic alteration, and human cancer could be drawn. This can now be addressed using DNA technology and the study of genes altered in human cancers, in a new field of molecular medicine: molecular epidemiology. Among the plethora of genes that are altered in human cancers, the tumor suppressor gene p53 occupies a special place because it is the most frequent target for abnormalities in every type of cancer [Caron de Fromentel and Soussi, 1992; Hollstein et al., 1991].

p53 PROTEIN AND GENOME STABILITY

The tumor suppressor gene p53 is a single copy gene localized on the short arm of chromosome 17. It codes for a phosphoprotein expressed at very low levels in the nucleus of normal cells [for review, see Soussi, 1995]. Upon physical or chemical DNA damage, the functional p53 can either arrest cell cycle progression in the late G1 phase [Kastan et al., 1991; Smith et al., 1994], thus allowing the DNA to be repaired prior to its replication, or induce apoptosis, leading to cell death

Received 7 September 1999; accepted revised manuscript 7 October 1999.

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Contract grant sponsors: ARC; Fondation de France.

[Lowe et al., 1993; Yonish-Rouach et al., 1991]. Growth arrest function is achieved by the transactivational properties of p53, which activate a series of genes involved in cell cycle arrest [Pietenpol et al., 1994], whereas the apoptotic pathway of p53 is undefined. More recent studies have shown that p53 may also be directly related to the recognition of DNA damage [Jayaraman and Prives, 1995; Lee et al., 1995] and DNA repair [Wang et al., 1995]. In cells lacking functional p53, i.e., tumor cells, the various pathways described above are not functional, resulting in inefficient DNA repair and the emergence of genetically unstable cells [Livingstone et al., 1992; Yin et al., 1992]. All these studies have led to the proposal that p53 is a key element in the control of genome stability [Lane, 1992].

ASSOCIATION BETWEEN p53 MUTATION AND HUMAN CANCER

Point mutations in the p53 gene have been found in most human cancers [Caron de Fromentel and Soussi, 1992; Hollstein et al., 1991]. More than 10 000 different tumors from various types have been analyzed for p53 alteration and have led to the identification of mutations in many cancer types [Greenblatt et al., 1994]. Their frequency varies from one type to another, but it is on the order of 45–50% for all cancers types [Greenblatt et al., 1994] (Fig. 1). In general, these mutations are associated with a loss of the second allele of the gene. In certain specific types of cancer, p53 inactivation can be achieved through an epigenetic mechanism [Soussi, 1995, for review]. In cervical carcinoma, where the frequency of the p53 mutation is very low, p53 inactivation is considered to be due to the association of this cancer with human papillomavirus, which induces p53 protein degradation. In soft tissue sarcoma, overexpression of the cellular protein mdm-2 leads to functional inactivation of p53 via the formation of a p53-mdm-2 inactive complex.

Further analysis reveals interesting differences with respect to the site of mutation on the gene and the type of mutational event that caused them. The relationship between the presence of a mutation in the p53 gene and different clinical parameters shows that these mutations have a bearing on the clinical prognosis, e.g., in colon [Hamelin et al., 1994] and breast cancer [Bergh et al., 1995].

As of September 1999, there have been over 1,500 reports of mutations in the p53 gene in human cancer [Béroud and Soussi, 1998]. A database of these mutations is now available via the European Bioinformatics Institute and contains data on mutations from 11,000 tumors [Béroud and Soussi, 1998]. Analysis may be carried out by using the position of the amino acid mutated in the p53 protein and by examining the mutational events that altered the gene itself.

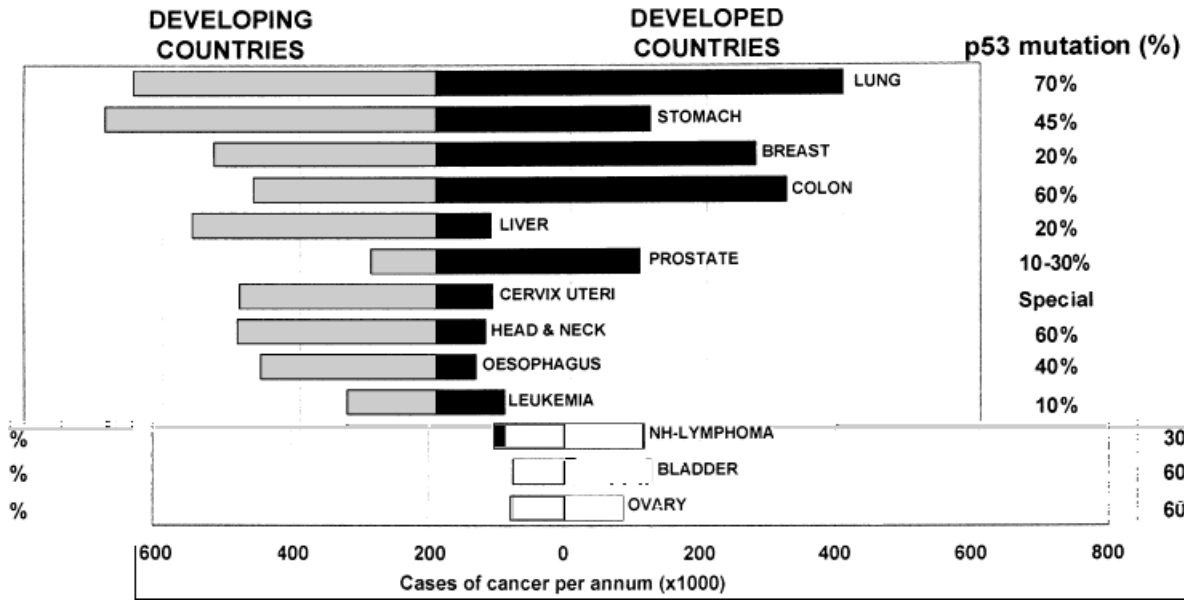

FIGURE 1. **Frequency of cancers worldwide and relationship to p53 mutation rate.**

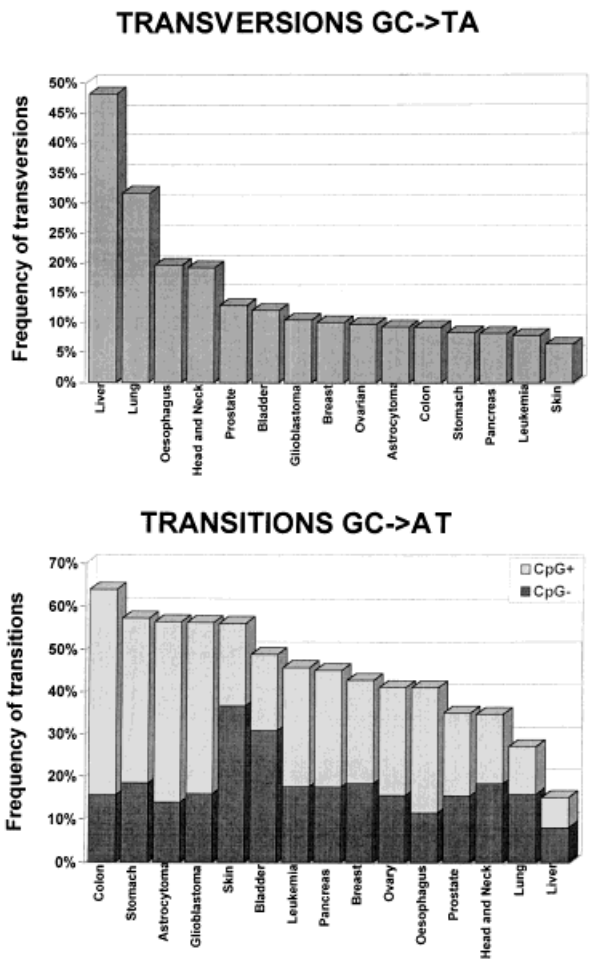

FIGURE 2. **Frequency of GC→AT transitions and GC→TA transversions. Transitions at a CpG dinucleotide are depicted in gray.**

p53 WEBSITE AND THE WEB VERSION OF p53 UMD SOFTWARE

A new p53 Website was created in 1997 (http://perso.curie.fr/Thierry.Soussi/). This site contains extensive information on various aspects on p53 and also links to other p53 sites. The description of all the different features of this site is beyond the scope of this review, but the most important aspect is that we have developed several specific pages dedicated to molecular epidemiology studies. A p53 mutation analysis page offers the opportunity to study and download specific information on a given type of cancer. For each cancer type, a file of p53 mutations specific for this cancer can be downloaded. Other useful features can be found by navigating through this site.

One important novelty of the UMD software is the possibility to perform analyses directly on the Web [Béroud et al., 2000]. The UMD software is now available online on the Web with various features for the analysis of the p53 database (http://www.umd.necker.fr:2001/). The most important feature is called "New mutation?". An essential question following the identification of a mutation in a new tumor sample concerns the occurrence of such mutation in the database. Does the mutation occur at a hot spot codon (if indeed it is a true mutation) or is this a position that has not been found mutated so far, suggesting that one should verify the sequence of the sample. In addition, other analyses tools and listings are available.

p53 GENE AS A PROBE FOR MOLECULAR EPIDEMIOLOGY

Analysis of all types of DNA mutations in different malignant tumors defines the mutational spectra according to the cancer under study. There are generally two types of genetic alteration, endogenous and exogenous, and they result in different forms and locations of mutation. Endogenous mutations result from errors occurring during the various biological processes linked to DNA metabolism, while exogenous mutations arise as the result of environmental factors. The spectra of these mutations can therefore be used to study the etiology of a cancer. It is now feasible to analyze a large number of tumor specimens by polymerase chain reaction (PCR) and DNA sequencing. At present (October, 1999), there have been over 1,500 published reports of p53 mutations in various types of human cancer [Béroud and Soussi, 1998]. A p53 mutation database has been created [see Béroud et al., 2000] containing 11,000 p53 mutations from patients with more than 50 different types of cancer [Béroud and Soussi, 1998]. These mutations may be analyzed at different levels: position of the mutated amino acid in the p53 protein, and mutational events which altered the p53 gene itself.

In order for a particular gene to be used for study of the origin of carcinogenesis in a human population, it must exhibit the following properties: 1) it must be mutated in many types of cancers, 2) the frequency of mutation must be high, 3) the gene must be altered mainly by point mutations, and 4) the gene must be small enough to be relatively easy to analyze. At present, these characteristics are found in two genes, the Ha-ras oncogene and the p53 gene. One of the disadvantages of ras is the small number of codons (only three) that are targets of mutations. In contrast, 300 of the 393 codons in the p53 gene are found to be modified. They all correspond to the p53 domain which is

essential to the activity of the wild-type p53 protein. Moreover, as shown in Figure 1, the p53 gene is mutated in more than 50% of all cancers studied, enabling molecular epidemiological studies to seek specific fingerprints of carcinogens and demonstrate their role in the development of cancer [Dumaz et al., 1994; Greenblatt et al., 1994].

MUTATIONS OR VARIATIONS?

To show a causal relationship between p53 and cancer, it is important to demonstrate that each of these mutations is truly deleterious to biological function, that the mutation inactivates p53 function, and that it is not a neutral polymorphism. Different observations prove that these are truly acquired mutations: 1) they are present only in tumor tissue, but are absent in nonmalignant tissue from the same patient. There is little polymorphism in the human p53 gene (six codons in the coding sequence) and their positions have been carefully documented in order to distinguish them from true mutation. 2) More than 90% of the mutations reported so far are clustered between exons 4 and 9 [Caron de Fromentel and Soussi, 1992; Hollstein et al., 1991]. This region is highly conserved throughout evolution and contains the DNA binding domain of the protein which is essential to p53 functional activity [Cho et al., 1994]. Contrary to the transactivation domain, which is rather resistant to point mutation, the flexible DNA binding domain is highly sensitive to any point mutation. 3) A detailed analysis of the most common p53 mutants shows that they have lost their biological activity [Ory et al., 1994].

Unlike other tumor suppressor genes such as Rb1 or APC, which are totally inactivated either by frameshift mutations or by gene deletion, 90% of p53 alterations are missense mutations which lead to the accumulation of mutant p53 in tumor cells. This selection for point mutations suggests that mutant p53 is not inactive and may have a dominant oncogenic function in the development of the tumor [Harvey et al., 1995].

BIASES OF THE DATABASE

The p53 mutation database contains 12,000 mutations from approximately 11,000 tumors or cell lines. A considerable number of tumors harbor several mutations, explaining the difference between these two figures. Yet it is important to keep in mind that this database contains some biases which might affect the interpretation.

Mutations Not Associated With Cancer

It was recently shown that p53 mutations can be found in healthy skin in cancer-free individuals [Jonason et al., 1996; Ren et al., 1997]. These mutations were found to be the result of ultraviolet radiation and are interesting from a molecular epidemiology standpoint in that their etiology illustrates the importance of ultraviolet radiation in this type of lesion. On the other hand, from a "mechanistic" standpoint this type of mutation should be interpreted with caution, since they are present in the absence of cancer tissue detectable by highly sensitive amplification methods. As these foci probably do not progress to cancer, it is possible that these mutations do not exert a deleterious enough effect to produce a preneoplastic clone. It should be noted that out of the 19 mutations listed to date, six are unique events never before reported in a cancer, eight were reported less than 10 times, and five more than 11 times. As long as a functional analysis of these mutations has not been done, it is impossible to assess their potential to induce cellular transformation. Likewise, the publication of p53 mutations in tissues from patients with rheumatoid arthritis should also be interpreted with caution [Firestein et al., 1997]. The fact that over half of these mutations are only very rarely found in the database might indicate that either they have no biological significance or that the target biological activity in this disease differs from that in neoplastic disease.

Choice of Region Analyzed

More than 70% of the molecular studies focus on the central region of the p53 gene, more specifically, on exons 5 through 8 which encode the DNA binding domain. More recent studies on all the coding exons (exons 2 through 11) show that a considerable number of mutations are found in exons 4 and 10 [Casey et al., 1996; Norberg et al., 1998]. Furthermore, the mutations in exons 5–8 are significantly different from those found in exons 4, 9, and 10, with a predominance of nonsense mutations and deletions in the latter. Twenty-eight of the 41 nonsense mutations found in exon 10 are located in codon 342, which contains a CpG dinucleotide.

The DNA binding domain of p53 encoded by exons 5–8 is very fragile, with a highly ordered three-dimensional conformation [Cho et al., 1994]. Any missense mutation can cause a modification that alters DNA binding specificity. The other regions of the p53 protein (exons 4 or 10)

are less fragile and the effect of missense mutations is less pronounced. It is therefore likely that only those mutations with a more drastic effect on the protein are selected during tumorigenesis.

The biological effect of a total absence of p53 due to a nonsense or frameshift mutation probably differs from that associated with a mutant p53 protein with a negative dominant effect or even a gain in function. It is therefore essential that clinical trials aiming to accurately establish the effect of p53 mutations on all clinical parameters (survival, response, etc.) analyze all the exons in p53.

Another potentially significant bias concerns mutations at splicing sites or introns. At present, this type of mutation is not listed in the databases since most studies have concentrated on coding regions. Furthermore, apart from a few cases, the biological effects of splicing mutations are unclear and it is possible that some intron mutations are rare polymorphisms. However, it is likely that this type of mutation is present in human cancer with a frequency of 3–5% [Felix et al., 1993; Foti et al., 1990; Nakai et al., 1994].

Choice of Genetic Material

Most studies have used tumor DNA, although an appreciable number used RNA. One of the advantages of using RNA is the size of the fragment to be sequenced. p53 has an open reading frame of 1,200 nucleotides if all the coding regions are to be analyzed. This approach is more practical than amplification of multiple exons from a DNA sample. Unfortunately, RNA cannot be used to identify splicing mutants. In a comparative analysis of DNA/RNA on 100 tumors, Williams et al. [1998] showed that the RNA analysis missed all the splicing mutations (3) and one nonsense mutation. Lack of detection of nonsense or frameshift mutations with an RNA sample occurs frequently and has been reported by several authors working with p53 or other genes [Stolzenberg et al., 1994]. It is supposedly due to abnormalities in the transport of RNA with mutations causing the synthesis of truncated proteins.

Choice of Biological Material

A large number of retrospective studies have been conducted with DNA extracted from paraffin-embedded samples. The advantage of this approach is that it provides an abundance of samples which can also be subjected to histological study. The new laser microdissection methods also provide a relatively homogeneous population of tumor, peritumoral, or normal cell populations, but unfortunately the quality of the DNA extracted from this type of sample is highly uneven and depends on fixing and storage procedures which vary not only between centers but also within centers over time. DNA extraction from this type of sample is never very reproducible and the DNA is generally of small size, thus potentially posing difficulties with PCR. Indeed, many PCR artifacts have been described [Leejackson et al., 1993; Shiao et al., 1997]. The use of Bouin by many pathology laboratories in France also presents a serious handicap to this type of study due to the presence of picric acid, which can degrade DNA.

Choice of Strategies and Methods for Identification of Mutations

Prescreening methods such as SCCP [Orita et al., 1989] or DGGE [Lerman and Silverstein, 1987] have the advantage of reducing the number of samples to be sequenced, which can potentially reduce diagnostic costs and experimental times. Nevertheless, without going into a discussion of pros and cons, it is clear that none of these prescreening methods is 100% reproducible. For example, nonoptimal use of SSCP with several migration conditions may lead to underestimation of mutation frequency. DGGE is generally considered to have better reproducibility, but it is a difficult method which depends on the sequence to be analyzed [Gejman et al., 1998]. An examination of the publications relating to detection of p53 mutations shows that SSCP is still the most common method that can take into account a slight bias in p53 mutation frequency. New denaturing HPLC methods appear very promising both in terms of sensitivity and potential for automation. A recent publication of the first use of DNA chips for detection of p53 mutations also opens interesting avenues, as do physicochemical methods such as mass spectrometry.

It should also be kept in mind that neither should direct sequencing be considered the gold standard for detection of mutations. In particular, a high level of contamination by healthy tissue may mask signals due to the mutation, which is especially true for automatic sequencing. In such situations SSCP and DGGE appear to be more sensitive.

While not a molecular approach, the functional test developed by R. Iggo's group is also worthy of mention [Flaman et al., 1995; Ishioka et al., 1993]. It has the advantage of directly detecting the biological activity of the mutant protein. Its low cost and good sensitivity currently make it the method

of choice for routine clinical analysis of p53 mutations. This test cannot be used for retrospective studies because it only works with RNA.

Other Biases and Sources of Error

Approximately 1 in 10 articles contain errors concerning the type or position of mutations. These include typographical errors, errors in sequence reading, or any other errors occurring between sequencing and transcription into the publication. In this respect, it would seem imperative that tables of mutations be examined by the reviewers or editor. As far as the database is concerned, a letter to the corresponding author generally allows rectification of the error. In the absence of information, the mutation is ignored.

Another bias concerns mutations published several times. In a considerable number of cases, this is noted in the publication, thereby enabling the mutation to be entered only once. In other cases, however, this is not mentioned. The UMD program contains a routine to evaluate multiple publications based on the name or identification code of the patients for a given author. In clear-cut cases of duplication, the mutations are only entered once. On the other hand, when the name of the patient is changed, this type of duplication cannot be detected.

A further bias involves specific hot spots of the laboratory. Such studies describe an abnormally high level of recurring mutations for a given cancer which is not found in the literature as a whole. In this type of publication, it is exceedingly difficult to distinguish between accidental contamination, a technical artifact related to the type of sample, and a true mutational hot spot.

In conclusion, it is clear that the mutation database should be analyzed with precaution, especially when considering small subsamples. In particular, specific mutational profiles should be validated from studies by more than one laboratory. We have developed a tool on our Internet site to check the type and frequency of mutations which avoids typographical or position errors (http://www.umd.necker.fr:2001/). By using the option "I found a mutation," it is possible to verify the wild-type coding sequence. By indicating the identity of the mutation, the program gives its frequency in the database. For infrequent or original mutations, it is recommended that the molecular analysis be repeated. See Cotton and Horaitis [2000] for a recent discussion on quality control issues for mutation databases in general.

MUTATIONAL PROFILE OF THE p53 GENE

It is beyond the scope of this article to give an extensive review of all the data that are related to p53 alterations in human cancer. Such an analysis will be published in another issue of *Human Mutation* (T. Soussi et al., in preparation). We will limit the current review to three types of cancer whose analyses are the most demonstrative.

An analysis of all the point mutations in the p53 gene shows that 51% are G:C→A:T transitions, 59% of which affect a CpG dinucleotide (Fig. 2). In mammalian cells, the cytosine in this dinucleotide is very often methylated. Spontaneous deamination of cytosine leads to formation of a U:G mismatch which is efficiently repaired by a uracil DNA glycosylase. In contrast, deamination of a 5-methylcytosine leads to a T:G mismatch whose nonspecific repair can lead to a C→T transition. It has also been shown that the spontaneous deamination rate is greater for 5-methylcytosine than for cytosine. All of the 42 CpG dinucleotides in the p53 coding sequence are methylated, irrespective of the tissue [Tornaletti and Pfeifer, 1995].

Bronchopulmonary Cancers and Smoking

Lung cancer is the commonest cancer in the world today, with the exception of skin cancer [Parkin et al., 1993]. Numerous investigations conducted in different countries on different subsets of the general population and with different designs have consistently reported an increase in the occurrence of lung cancer among smokers in comparison with nonsmokers. All investigations have shown a clear-cut dose–response relationship between the amount smoked daily and the subsequent risk of lung cancer. It is now thought that cigarette smoking is responsible for 80–90% of lung cancers. In experimental animals, cigarette smoke induces malignant tumors of the respiratory tract. This smoke is a complex mixture of several hundred different molecules which include such well-known carcinogens as polycyclic aromatic hydrocarbon (benzo(a)pyrene) or N-nitrosamines. Benzo(a)pyrene remains to this day one of the most highly carcinogenic compounds known. Benzo(a)pyrene from sources such as cigarette smoke and automobile exhaust fumes is highly prevalent in the environment.

p53 mutations are common in lung cancer and range from 33% in adenocarcinomas to 70% in small-cell lung cancers [Chiba et al., 1990; Takahashi et al., 1989]. These mutations are mostly G:C → T:A transversions (Fig. 2), with a minority of transition mutations (less than in other cancers).

A strong correlation has been detected between the frequency of these G:C → T:A transversions and lifetime cigarette consumption. This observation has not been made for other cancers such as colon, breast, ovary, or brain cancer, which are not associated with smoking or other exposure to carcinogens. It is compatible with the role of exogenous carcinogens such as benzo(a)pyrene, present in cigarette smoke. After metabolic activation, one of the derivative products of benzo(a)pyrene binds predominantly to guanine and gives rise to specific G:C → T:A transversions.

Hepatocarcinoma and Aflatoxin B1

A considerable number of epidemiological studies have established a strong specific association between infection with hepatitis B virus and hepatocarcinoma (HCC). More recently, aflatoxin B1 has been considered to be a significant etiological factor for liver cancer in southern Africa and Asia. Aflatoxins are compounds produced by fungal strains (such as *Aspergillus flavis* for aflatoxins B1), which are known to be food contaminants in these countries. It has been known for some time that aflatoxins are highly carcinogenic in experimental animals, producing liver tumors in mice, rats fish, ducks, and monkeys, although clear evidence for a causal association with human cancer has been difficult to obtain.

In 1991, two reports appeared concerning mutations in the p53 gene in HCC, with a predominance of the GC → TA transversion at the third base of codon 249 (Arg → Ser) [Bressac et al., 1991; Hsu et al., 1991]. In one case, the patient series was from Mozambique, while the second was from the Qidong province in China. A worldwide epidemiological study showed that the mutation in codon 249 was strictly specific to countries in which food was contaminated by aflatoxin B1 [Ozturk and Other, 1991]. In Mozambique, for example, more than 50% of the mutations were found in codon 249, while in Transkei, which borders on Mozambique (and which has a similar rate of chronic HBV infection), the mutation rate at codon 249 was less than 10%. In countries which do not consume contaminated food (including Europe and the USA), the rate of p53 mutations in HCC is low.

It has been demonstrated in vitro that this phenomenon is due to the very high sensitivity of codon 249 to the action of aflatoxin B1 [Aguilar et al., 1994; Puisieux et al., 1991]. This observation, along with the fact that the mutation is deleterious for p53 function, explains the existence of this mutational hot spot.

Skin Cancer and Ultraviolet Radiation

Skin tumors, melanomas, and basal or squamous cell carcinomas are the most frequent human cancers. Their number has increased dramatically in the last 10 years, especially for melanoma. Several lines of evidence indicate that these skin tumors are linked to sun exposure. Their frequency increases with the amount of exposure or a decrease in latitude and they are usually found on sun-exposed parts of the body. UV-induced mutations have been studied in various animal models. The majority of these UV-induced mutations are located at dipyrimidine sites (i.e., T-T, C-C, C-T, or T-C) and correspond to a C→T transition. A large percentage of them (more than 20%) correspond to tandem mutation which involve the two nucleotides of the dipyrimides sites (C-C → T-T).

Several human syndromes are associated with DNA repair deficiency. Among them, Xeroderma pigmentosum (XP) is an autosomal, recessively inherited disease with clinical and cellular hypersensitivity to UV radiation resulting in a high incidence of skin cancer. In normal humans, skin tumors ordinarily appear in people over 50 years old, while in XP patients, development of skin tumors begins much earlier.

Analysis of various types of human skin cancer shows that there is a significant predominance of C→T transitions at dipyrimidine sites [Brash et al., 1991; Ziegler et al., 1994]. Furthermore, analysis of more than 4,000 p53 mutations in internal tumors shows that less than 1% are tandem mutations, whereas this number is 14% in non-XP skin cancer and 55% in XP skin cancer, which is typical of UV exposure [Dumaz et al., 1994].

p53 Mutations and Other Cancers

Apart from the three types of cancer discussed above, molecular analysis of p53 mutations has revealed other types of heterogeneity linked either to exposure to carcinogens or to the etiology of the disease. Bladder cancer is characterized by a high frequency of GC→AT transitions, but these do not occur at CpG dinucleotides, suggesting that these mutations have a different origin than those seen in colon cancer or hematologic malignancies. Both esophageal and head and neck cancers have a significant frequency of GC→TA transversions and both are associated with smoking and alcohol consumption. Brennan et al. [1995] showed that

the p53 mutation rate in these cases is linked to smoking and alcohol.

CONCLUSIONS AND PERSPECTIVES

The field of molecular epidemiology has grown with remarkable speed. There is no longer any doubt that human cancers are caused by mutations induced by exogenous carcinogens. Mutational analysis of the p53 gene is a striking example of this "molecular" archeology which enables us to define the origin of mutations. In particular, it is essential that we are able to distinguish between endogenous, and thus unavoidable mutations, and those that are exogenous, or potentially avoidable.

The future development of novel methods for the detection of point mutations such as the chips technology will lead to a huge increase of new mutation detection. The rate of de novo mutation will never slow down or stop, either for somatic or germline mutation. Furthermore, changes in our environment will lead to changes in the mutational events which modify our genome. Thus, the task of reporting and analyzing these mutations will be a major challenge, especially if the presence or the identity of such mutations is linked to therapeutic decision-making. This is particularly important for p53, as such alterations have been linked to short survival or resistance to therapy. Moreover, there is an increasing amount of data suggesting that the behavior of various mutant p53 is heterogeneous, leading to different clinical responses. It will be of great importance to create a connection of mutation databases to structural databases. Such structural databases should contain information related to the behavior of each mutant at several levels: structural, cellular, and clinical.

ACKNOWLEDGMENTS

We thank N. Basset-Seguin, P. Beaune, M. Lebras, D. Marzin, and A. Sarasin for critical reading of the manuscript. Work related to creation of the database, the UMD application, and the Website was funded by ARC and the Fondation de France.

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