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p53 mutations as fingerprints of environmental carcinogens*

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Abstract: Mutations of the p53 tumor suppressor gene occur in a great majority of human cancers. The protein product of p53 gene is involved in DNA damage response. Consequently, p53 gene may be a preferred target for environmental carcinogens, which also act as DNA-damaging agents. This is probably why p53 mutations are frequent in cancers linked to environmental carcinogens. Moreover, these carcinogens leave molecular fingerprints on the p53 gene. Thus, the study of p53 mutation spectra has been a useful approach to implicate suspected carcinogens to different human cancers. This review provides further insight into the significance of p53 mutation spectra in ten common human malignancies (skin, liver, lung, bladder, breast, head and neck, esophagus, stomach and colorectal cancers, and hematological malignancies), in relation with environmental carcinogens.

p53 GENE AND ITS CELLULAR FUNCTIONS

The human p53 gene is located on the short arm of chromosome 17 (17p13.1) and spans 16–20 kb DNA. The gene has 11 exons coding for an mRNA of 2.2–2.5 kb and a protein of approximately 53 kDa of 393 amino acids. Both exon-intron organization of the gene and amino acid sequence of the protein is conserved between species [1]. p53 is a DNA-binding protein with transcription regulatory activities, and can be divided into three domains, encompassing the amino-terminal domain containing the activation domain, the central core containing its sequence-specific DNA-binding domain, and multifunctional carboxy-terminal domain. p53 protein is present at very low levels in normal cells. Under certain stress, cells are able to upregulate their p53 levels by a post-transcriptional mechanism. The major factors that induce p53 mutations have in common the ability to cause DNA damage. There are two major p53-dependent responses. a) Cell cycle arrest: p53 has a clearly defined role in G1/S arrest following DNA damage. p53-mediated cell cycle arrest at the G1/S boundary involves the activation of $p21^{cip1/kip1}$ protein. This protein acts as an inhibitor of cyclin-dependent kinases (CDK), including CDK2 which allows the G1/S transition when complexed with cyclin E. b) Programmed cell death or apoptosis. In some cell types, the induction of p53 by DNA damage leads to a p53-dependent apoptosis rather than cell cycle arrest (reviewed in ref. 2). It was recently reported that p53 is also involved in DNA repair through activation of ribonucleotide reductase gene [3].

p53 AND CANCER

Since the first documentation of human p53 mutations in colorectal cancers in 1990, extensive studies by many different laboratories on various human cancers showed that this gene is mutated or inactivated in a great majority of human cancers, independent of tissue origin and etiology. Presently, it is estimated that about 40% of human cancers display mutations on p53 gene. Almost all of the major cancers (cancers of the skin, lung, liver, breast, gastrointestinal tract, bladder, haematological) display mutations scattered at the DNA-binding domain. There are five hotspots (codons 175, 248, 249, 273, and 282)

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which were found to be more frequently affected by mutations. These residues are involved either directly or indirectly in specific binding of p53 to its target DNA sequences. The frequency of p53 mutations is low in some tumors, for example, cervical cancers are etiologically linked to infection with human papillomaviruses 16 and 18. These viruses encode a protein (E6) which is able to inactivate wild-type p53 by inducing a rapid degradation. (For a recent review, see ref. 4.)

p53 mutation spectra in common malignancies

The mutations of p53 gene have been extensively studied in many human cancers for the last decade. Therefore, there is a large number of mutation data on p53 gene available on several databases. We used two of these databases [5,6], to analyze the patterns of p53 mutations in ten common human malignancies (Fig. 1).

First, we calculated the overall frequencies of different types of mutations (base substitutions, insertions and deletions). As shown in Fig.1, G:C→A:T transitions were observed as the most frequent type of mutations (42.6%). The G:C→T:A transversions were the second most frequent mutations (16.8%). The order of frequencies of other mutations was as follows: A:T→G:C transitions (10.7%), deletions (8.9%), G:C→C:G transversions (8%), A:T→T:A transversions (5.6%), A:T→C:G transversions (3.7%), insertions (2.6%), and CC:GG→TT:AA double transitions (0.7%), respectively. This overall distribution pattern in all cancers was taken as a reference pattern, and the types of mutations that showed higher frequencies were considered as mutations induced by etiologically suspected environmental carcinogens for a given site of malignancy.

Liver cancers (hepatocellular carcinomas)

These tumors displayed a higher frequency for G:C→T:A and A:T→ T:A transversions. Moreover, G:C→T:A substitutions were the most frequent (39.1%) type of mutations in hepatocellular carcinoma (HCC). This type of mutation initially described in HCC from southern Africa and China has been related to aflatoxins [7,8]. Aflatoxins are powerful mutagens inducing preferentially G:C→T:A transversions in microorganisms. This type of mutation (mostly occurring at codon 249 of p53) was observed in HCC from patients at high risk of aflatoxin exposure, but not in other HCC [9]. These observations strongly suggested that G:C→T:A transitions in HCC are induced by environmental aflatoxins which are produced by some Aspergillus species contaminating food in different geographical locations in the world. Many other studies have confirmed this interesting association between G:C→T:A transitions in HCC and high risk of aflatoxin exposure [10]. The higher frequency of A:T→T:A transversions in HCC has no explanation for the time being. However, this mutation is apparently unrelated to aflatoxin exposure. Thus, there is probably another, unknown, environmental carcinogen which causes this type of mutation in HCC.

Lung cancers

Similar to HCC, in lung cancers, G:C→T:A transversions were the most frequently observed mutations (32.4%). In addition, an excess of G:C→C:G transversions were also seen in lung cancers. Both epidemiological and experimental evidences demonstrated that tobacco smoking causes lung cancer. High rate of G:C→T:A transversions observed in lung cancers strongly suggests that benzo-a-pyrene present in tobacco smoke is one of the major lung carcinogens in humans. The benzo-a-pyrene, upon activation, is able to bind G residues. Preferential formation of benzo-a-pyrene adducts at lung cancer mutational hotspots in p53 have been demonstrated [11]. This carcinogen was also shown to induce principally G:C→T:A transversions under experimental conditions [12]. The excess of G:C→C:G transversions in lung cancers may be related to other carcinogens to which tobacco smokers are exposed. An aromatic amine, namely 4-aminobiphenyl, was shown to induce this type of transitions [13]. Thus, benzo-a-pyrene, and 4-aminobiphenyl to a lesser degree, are the major carcinogens related to tobacco smoking in lung cancer patients.

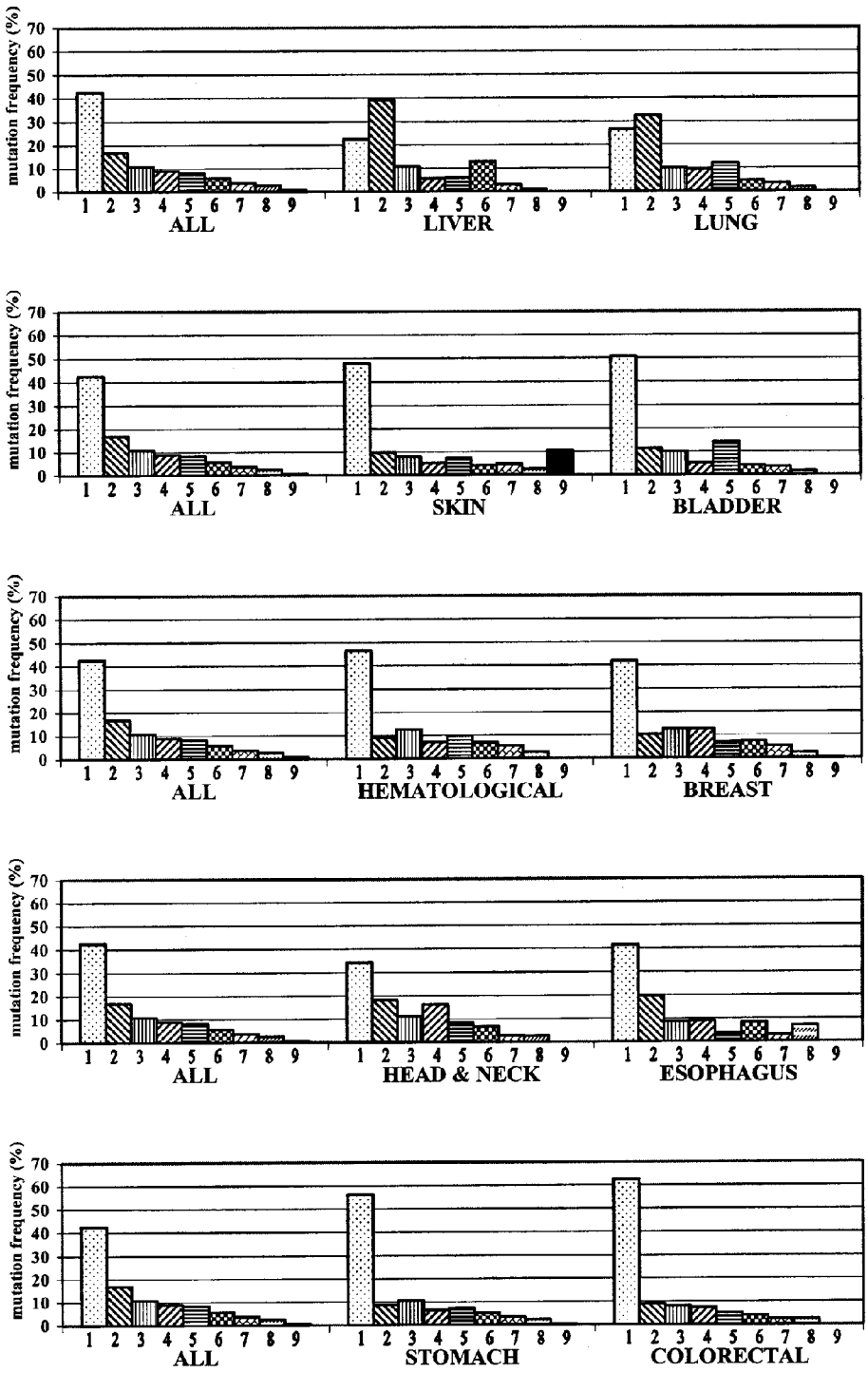

Fig. 1 Frequencies in percentages of different types of p53 mutations in skin, liver, lung, bladder, breast, head and neck, esophagus, stomach and colorectal cancers, and hematological malignancies as compared to overall frequencies observed in these cancers. (1) G:C→A:T, (2) G:C→T:A, (3) A:T→G:C, (4) deletions, (5) G:C→C:G, (6) A:T→T:A, (7) A:T→C:G, (8) insertions, and CC:GG→TT:AA (9).

Non-melanoma skin cancers

An excess of CC→TT double mutations, as well as a slight excess of G:C→A:T transitions were observed in non-melanoma skin cancers. There is strong epidemiological evidence that skin cancer is related to excessive sunlight exposure. This risk is also linked to exposure to UV from sunlight (for a review, see ref. 14). There is a plethora of data about UV-induced DNA damage and UV-induced C→T transitions. The CC→TT tandem transition is considered to be a highly specific of UV-induced mutation. Thus, both CC→TT and G:C→A:T mutations which show an excess of frequency in non-melanoma skin cancers are induced by UV from sunlight [4].

Bladder cancers

Bladder cancers display an excess of G:C→A:T transitions and G:C→C:G transversions. These mutations may be induced as a result of exposure to different etiological agents involved in bladder cancers. Tobacco smoking and occupational exposure to certain chemical dyes, as well as inflammatory reactions are known to be major risk factors for these cancers [4]. The lack of G:C→T:A excess in these cancers suggests that benzo-a-pyrene of tobacco smoke is not the principal factor linking bladder cancer to smoking. On the other hand, G:C→C:G transversions are known to be preferentially induced by 4-aminobiphenyl [13]. Both smokers and chemical dye workers are exposed to this arylamine. Thus, the excess of G:C→C:G transversions observed in bladder cancers may indicate that these mutations are linked to both tobacco smoking and chemical dyes. The excess of G:C→A:T transitions may also be related to the same type of carcinogens, and/or inflammatory reactions.

Hematological malignancies

The majority of p53 mutations in hematological malignancies are consistent with the overall pattern of p53 mutations. A slight excess of A:T→G:C, G:C→C:G, and A:T→T:A transversions could be observed. These findings are not significant enough to implicate potential carcinogens in these malignancies.

Breast cancer

The pattern of the breast cancer mutation spectrum did not differ strongly from the overall pattern of p53 mutations. However, there was a slight excess in deletions and mutations affecting A:T base-pairs (A:T→G:C, A:T→C:G, and A:T→T:A). These observations suggest that at least a fraction of breast cancers may be related to environmental carcinogens. However, it is presently unknown what type of potential carcinogens can induce deletions and/or A:T base-pair substitutions in these cancers.

Head and neck cancers

An excess of deletion-type mutations as well as a slight excess of G:C→T:A transversions were observed in these tumors. For the time being, there is no clue about deletion-type mutations and environmental carcinogens involved in head and neck cancers. On the other hand, the slight excess of G:C→T:A transversions may indicate that these tumors are related to benzo-a-pyrene exposure by tobacco smoking.

Esophageal cancer

We noticed an excess of insertions, A:T→T:A transversions, and a slight excess of G:C→T:A transversions in esophagus cancers. There is no clear indication about the causes of excessive insertions and A:T→T:A transversions in these tumors. In contrast, the slight excess in G:C→T:A transversions may be related to benzo-a-pyrene or similarly acting carcinogens.

Stomach and colorectal cancers

These two cancers displayed a similar pattern of p53 mutation spectra: an excess of G:C→A:T transitions. This type of mutation, which is the most dominant form in all cancers, was assumed to be a spontaneous mutation as a result of methylcytosine deamination [4]. Thus, it is unclear whether their excess in stomach and colorectal cancers is related to a specific carcinogen.

CONCLUSION

The p53 mutation spectra allowed to link several environmental carcinogens to human cancers. The frequency of p53 mutations in most cancers is high. In addition, there are now more than 10 000 p53 mutations described in different human cancers, available in commonly accessible databases. The systematic study of these mutations may help to find additional links between suspected carcinogens and different human cancers.

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