p53 Mutations in Human Cancers

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Mutations in the evolutionarily conserved codons of the p53 tumor suppressor gene are common in diverse types of human cancer. The p53 mutational spectrum differs among cancers of the colon, lung, esophagus, breast, liver, brain, reticuloendothelial tissues, and hemopoietic tissues. Analysis of these mutations can provide clues to the etiology of these diverse tumors and to the function of specific regions of p53. Transitions predominate in colon, brain, and lymphoid malignancies, whereas G:C to T:A transversions are the most frequent substitutions observed in cancers of the lung and liver. Mutations at A:T base pairs are seen more frequently in esophageal carcinomas than in other solid tumors. Most transitions in colorectal carcinomas, brain tumors, leukemias, and lymphomas are at CpG dinucleotide mutational hot spots. G to T transversions in lung, breast, and esophageal carcinomas are dispersed among numerous codons. In liver tumors in persons from geographic areas in which both aflatoxin B_1 and hepatitis B virus are cancer risk factors, most mutations are at one nucleotide pair of codon 249. These differences may reflect the etiological contributions of both exogenous and endogenous factors to human carcinogenesis.

WHEREAS ANEUPLOIDY IS ALMOST ALWAYS FOUND IN human cancers (1), the most common cancer-related genetic change known at the gene level is p53 mutation (2). The normal allele of this autosomal gene encodes a 53-kD nuclear phosphoprotein involved in the control of cell proliferation, and various mutant alleles with single base substitutions code for proteins that have altered growth regulatory properties (3–9). In addition to point mutations, allelic loss, rearrangements, and deletions of the p53 gene have been detected in human tumors (10–15). These aberrations, together with alterations of oncogenes and other tumor suppressor genes, make up the mutational network leading to malignancy. In this review, we focus on the patterns of base substitution mutations in the p53 gene observed in human cancers and their etiological implications.

An analysis of base substitution mutations is of interest for two reasons. First, because endogenous and exogenous mutagens generate specific kinds of base substitutions at certain preferred sites

(16–18), the p53 mutational spectrum in tumors may provide information about the origins of the mutations that give rise to human cancers. Second, the positions of tumor mutations in the p53 gene sequence define regions of the p53 protein that are likely to be essential for its biological activities and for its interaction with other cellular and viral proteins.

Location of Mutations Within p53

Biochemical features of the p53 protein that may be relevant to the location of mutations are (i) the presence of several domains well conserved in evolution (19) and (ii) the ability to form complexes with viral and cellular proteins such as the simian virus 40 (SV40) large T antigen, the adenovirus 5 E1b protein, the E6 proteins of human papillomavirus 16 and 18 (20–23), and the 70-kD family of heat shock proteins (hsc70) (24). In addition, some p53 mutants lose transcriptional activation potency and lose their ability to bind DNA (25–27). Sequencing of the p53 gene in mammals, amphibians, birds, and fish has revealed five highly conserved domains, four of which fall within exons 5 through 8 (19): domain ii (codons 117 to 142), domain iii (codons 171 to 181), domain iv (codons 234 to 258), and domain v (codons 270 to 286). Domains iii, iv, and v are included in the two binding regions for SV40 large T antigen (28), whereas mutations at various positions between codons 66 and 228 result in a mutant protein able to bind to hsc70 (3, 29, 30).

Ninety-eight percent of the 280 base substitution mutations in this review fall within a 600–base pair region of the p53 gene product (codons 110 to 307, Fig. 1). This sequence encompasses exons 5 through 8, where most of the evolutionarily conserved amino acids are concentrated. Mutation analyses have been confined principally to these exons. Studies that included outlying exons suggest that tumor mutations outside exons 5 through 8 are rare (31–36); nevertheless, they may be underrepresented in this review.

Uninvolved tissues from the patients were tested in many of the cancer cases with p53 mutations in this listing; only the wild-type p53 sequences were found in normal cells of all cases analyzed (31–34, 36–43). We have assumed that all the tumor mutations referred to in Table 1 and Fig. 1 represent separate, somatic mutations. Germline p53 mutations have been described in families with the Li-Fraumeni cancer syndrome (44, 45), some identical to somatic base substitutions seen in sporadic tumors (Fig. 1). It is anticipated that constitutive p53 mutations may be found in individuals with "sporadic" tumors as well as in families prone to develop cancer in addition to those classified as having the Li-Fraumeni syndrome. However, on the basis of previous studies, it is likely that the great majority (>98%) of p53 gene mutations occurring in human tumors represent somatically acquired mutations.

Mutations are found at highly conserved residues of the p53 protein. The 280 base substitutions are distributed over 90 codons (Fig. 1). The majority of the missense mutations (221 of 254 mutations) are at codons corresponding to amino acids conserved in all seven

organisms considered (human, monkey, rat, mouse, chicken, *Xenopus*, and trout). The numerous amino acids that presumably alter the biological function of the p53 protein when substituted are dispersed among four conserved domains as well as along intervening sequences, suggesting that no single domain is responsible for maintaining p53 tumor suppressor function.

We considered the possibility that p53 mutations outside conserved domains ii through v are likely to be observed at the scattered conserved residues. Of 56 missense mutations at intervening stretches (comprising 121 codons), 33 were at one of the 39 codons of residues identical in the seven species (χ^2, $P < 0.001$). Tumor mutations are thus commonly observed at amino acids that are perfectly conserved during evolution, regardless of the amino acid conservation of surrounding residues.

The importance of residues conserved in evolution is also striking when only the mammalian p53 sequences are considered. Tumor missense mutations are almost exclusively at amino acids that are identical in human, monkey, rat, and mouse. Of the 200 amino acid residues in the midregion of the gene, 169 are the same in all four mammalian species. Of the 252 missense mutations, 248 are at these residues (χ^2, $P < 0.01$).

p53 Mutations by Cancer Type

The location and type of mutations in a specific sequence define a mutational spectrum. When all tumor p53 mutations are grouped together, they identify several codons at which exceptionally high numbers of tumor mutations are clustered (hot spots); some codons are the sites of 4% or more of the 280 mutations (Fig. 1). When mutations are examined separately by cancer type, clear differences in spectra emerge, both with respect to the position of the hot spots and with respect to the frequency of transitions (in which a purine is substituted for a purine or a pyrimidine for a pyrimidine) and transversions (in which a purine is substituted for a pyrimidine or vice versa) (Table 1). Examples of contrasting spectra are noted below.

Tumors of the colon and breast. The epidemiology (geographical distribution and proposed risk factors) of colon and breast cancer have several features in common (46); however, p53 mutation patterns in these cancers are dissimilar. G:C to A:T transitions constitute the majority of colon tumor mutations (79%), and most

of these occur at CpG dinucleotides (31, 32, 47–49). More than half of colon tumor transition mutations are at three CpG hot spots in domains iii through v (codons 175, 248, and 273). In sporadic breast cancers (31, 40, 50–52), only four tumors with p53 gene transitions at CpG sites (13%) were found.

The second disparity between these mutation spectra is the frequency of G to T transversions. This base substitution appears to be exceedingly rare in colorectal cancer (Table 1) yet accounts for one-fourth of the 31 breast tumor mutations.

Lymphomas and leukemias. The pattern of mutations in these cancers is remarkably similar to that of colorectal tumors: transitions at CpG dinucleotides constitute a major fraction of the point mutations and are distributed over the same CpG-containing codons. G to T transversions are uncommon, and A:T to G:C transitions predominate among substitutions at A:T pairs. The spectrum of base substitutions appears to be similar for Burkitt lymphoma, other B cell tumors, and T cell malignancies (36), although the frequency of p53 substitution mutations in Burkitt's lymphoma is higher than in other types of B cell malignancies.

Lung and esophageal cancer: Two tobacco-related diseases (53). G:C to T:A mutations are the most frequent substitution in non–small cell lung cancer (nonSCLC). There is an unmistakable strand bias: in each of the 17 cases of this transversion (Table 1), the guanine residue was located on the nontranscribed strand (all G:C to T:A transversions in breast cancers also showed this orientation). Mutagenesis experiments in mammalian cells show that transcriptionally active genes are more frequently mutated than dormant loci (54, 55), but within transcribed genes the nontranscribed strand is more commonly the site of damage or error, giving rise to the mutation (56). This observation has been attributed to the preferential repair of the transcribed strand (54–56) or increased accessibility of the opposite strand to electrophilic attack. A strand bias is not apparent for p53 transition mutations (rather than transversion mutations) at guanine residues in lung and breast cancers.

Transversions are also exceptionally frequent among esophageal tumor p53 mutations in comparison to base substitution patterns of most other cancers. These occur with similar frequency at G:C and A:T pairs, whereas in other solid tumors including lung cancers sequence changes at A:T pairs are uncommon: one per five esophageal cancers with p53 mutations revealed at transversion at A:T compared to one per 17 cancers of other organ sites (χ^2, $P < 0.01$). Curiously, almost half of all chain-terminating mutations (8 of 19)

Table 1. Nature of p53 gene base substitution mutations by cancer type. Although not included in this table, some families with Li-Fraumeni syndrome, which is characterized by a high risk for certain malignancies including leukemias, sarcomas, breast, and brain tumors, carry germline p53 mutations (44, 45). Five of the six constitutional mutations were transitions at G:C pairs. In one family an A:T to G:C mutation was described.

Cancer location	Total muta-tions	Mutations at G:C			Tran-sitions at CpG	Mutations at A:T			References
		→A:T	→T:A	→C:G		→T:A	→G:C	→C:G	
Lung: SCLC	13	6 (46)*	2 (15)	2 (15)	4 (31)	1 (8)	0 (0)	2 (15)	(12, 31, 35, 92)
non-SCLC	30	6 (20)	17 (57)†	4 (13)	3 (10)	2 (7)	1 (3)	0 (0)	(12, 31, 33, 35, 87, 92)
Breast	31	13 (40)	7 (23)	6 (20)	4 (13)	0 (0)	3 (10)	2 (6)	(31, 40, 50–52)
Colon	39	31 (79)†	0 (0)	1 (3)	26 (67)†	1 (3)	6 (15)	0 (0)	(31, 32, 47, 48)
Esophagus	37	16 (43)	9 (24)	0 (0)	7 (19)	5 (14)†	4 (11)	3 (8)	(38, 89, 90, 93)
Liver	19	3 (16)	14 (74)†	1 (5)	0 (0)†	0 (0)	1 (5)	0 (0)	(39, 43, 49)
Bladder	15	7 (47)	2 (13)	3 (20)	5 (33)	0 (0)	2 (13)	1 (7)	(87)
Ovary	11	4 (36)	1 (9)	3 (27)	0 (0)	0 (0)	1 (9)	2 (18)	(52)
Sarcoma	12	8 (66)	2 (17)	2 (17)	7 (53)	0 (0)	0 (0)	0 (0)	(31, 41, 42, 87, 88, 94)
Brain	20	15 (75)	1 (5)	1 (5)	9 (45)	0 (0)	3 (15)	0 (0)	(31, 87, 95)
All solid tumors	227	109 (48)	55 (24)	23 (10)	65 (29)	9 (4)	21 (9)	10 (4)	
Lymphomas and leukemias	53	30 (57)	2 (4)†	2 (4)	25 (47)†	3 (6)	10 (19)	6 (11)	(36, 91)
All tumors	280	139 (50)	57 (20)	25 (9)	90 (32)	12 (4)	31 (11)	16 (6)	

*Numbers in parentheses are percentage of mutations.   †The difference between this value and corresponding number for all other solid tumors grouped together is significant (χ^2, $P < 0.01$ or less).

in solid tumors were seen in esophageal carcinomas.

Hepatocellular carcinomas (HCCs) in persons from different geographical regions. Eighteen base substitution mutations were found in HCC from three different groups, and one in a human HCC cell line (*39, 43, 49*). Eight of the mutations were detected in a series of 16 tumors in individuals from the high-incidence region of Qidong, China, all at the third base pair position of codon 249, and seven were G to T transversions. The mutation was not present in non-malignant cells of these individuals. Four out of 10 HCCs in individuals from a different population at high risk of liver cancer (southern Africa) also contained G to T transversions, three of which were at codon 249. In 22 HCCs from Japanese patients, however, six different point mutations were reported, none of which was at codon 249 (*49*). Other than the clusters of transitions at rare CpG dinucleotide sites, p53 mutations in most human cancers are dispersed over the midregion of the coding sequence (Fig. 1). The finding that 12 of 26 HCCs examined from two high-risk groups contained a mutation at the same codon and base pair is exceptional.
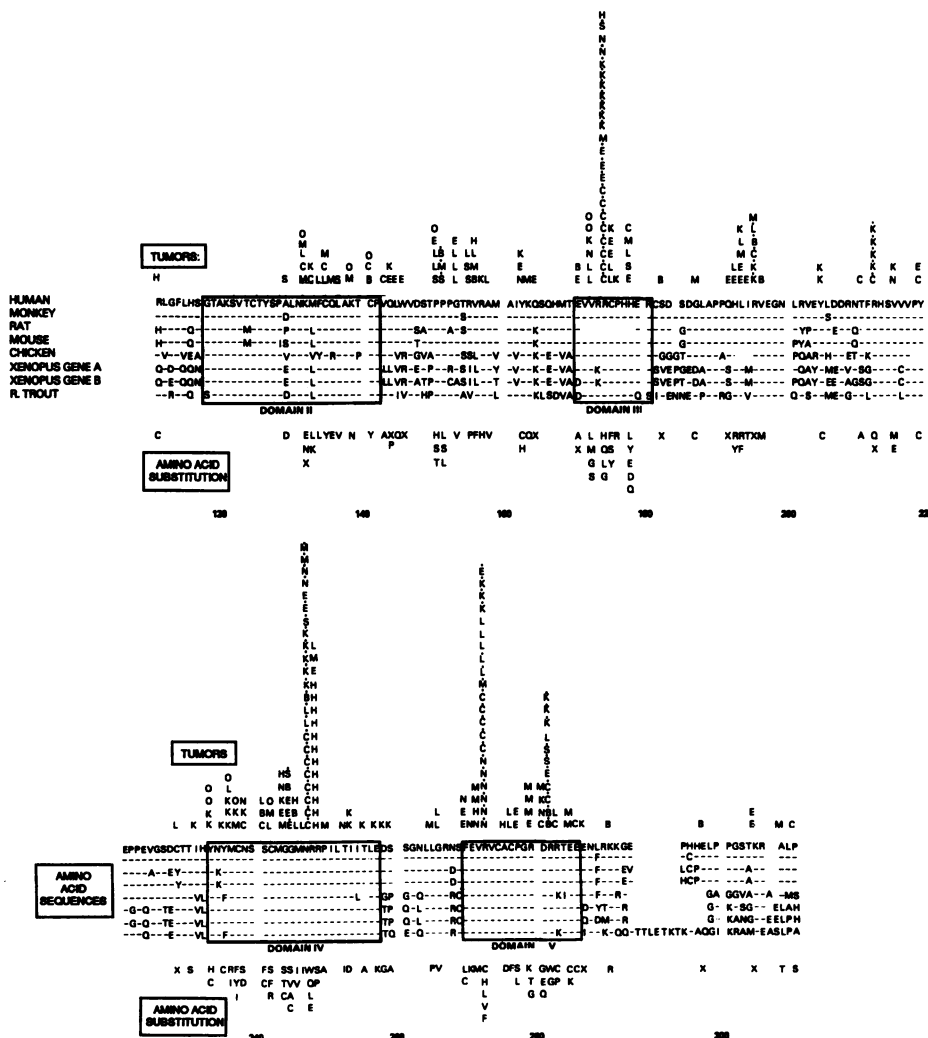
Origins of p53 Mutations

General cellular and environmental factors. One aim of cancer research is to understand the etiology of malignancy, with the long-term objective of reducing cancer incidence. Cancer is the result of an accumulation of genetic changes, and we now know that in many (perhaps the majority of) tumors, one of the genetic lesions is base substitution mutation in the p53 gene. Because p53 mutations are frequent and widespread and occur over a large sequence, it is feasible for the first time to compare the mutational spectra of a variety of tumors, of different proposed etiology, at the same locus. The most salient finding from this analysis of p53 mutations is that the spectra differ depending on the tumor type. This is consistent with the hypothesis that the origins of mutations are distinct in different tumor types.

Differences in metabolic and DNA repair capacities among different tissues and cell types are some of the factors expected to underlie these differences (*57*). In addition, both exogenous carcinogens and endogenous biological processes are known to cause mutations. Important sources of the spontaneous generation of point mutations in human cells include DNA polymerase infidelity (*58*), depurination (*59*), oxidative damage from free radicals generated by biological processes (*60, 61*), and deamination of 5-methylcytosine (*62*).

Superimposed over endogenous sources of mutation are exposures to environmental carcinogens. Many identified human carcinogens elicit base substitutions in bacteria, mammalian cells in vitro, and experimental animals (*63*). This activity is the basis of short-term tests to identify candidate carcinogens. Electrophilic attack of DNA bases by carcinogen metabolites, followed by fixation of the damage at the site of the adducted base during DNA replication has been the principal mechanistic model. There are ample biochemical

Fig. 1. Localization of p53 base substitutions in human cancer. Symbols at the top refer to cancer types: B, bladder; C, colon; E, esophagus; H, liver; K, leukemias and lymphomas; L, lung; M, breast; N, brain; O, ovary; and S, sarcoma. A period directly above the tumor symbol indicates that the mutation is a transition at a CpG dinucleotide. Single-letter amino acid abbreviations below p53 sequences designate substituted residues in the tumors. Abbreviations for the amino acid residues at the bottom are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. X refers to mutations resulting in stop codons. Underlined residues in domain iv of the human p53 sequence show positions of germline mutations detected in families with Li-Fraumeni syndrome (*44, 45*). Amino acid sequences of p53 (residues 110 to 310, human protein) are taken from the review by Soussi *et al.* (*19*). In many instances, analysis of the p53 gene in tumors was limited to exons 5 through 8, corresponding to residues 126 through 306. Five of the 280 mutations were found outside the 200-codon stretch presented here, four of which resulted in chain-terminating codons.

data characterizing the adducts and specific base substitutions expected from electrophilic metabolites of various important classes of carcinogens, including N-nitrosamines, polycyclic aromatic hydrocarbons, and fungal toxins in particular, and these are consistent with the results of animal cancer experiments on the specificity of tumor mutations in *ras* (*64, 65*).

Other classes of carcinogens have no specific interaction with DNA. The contribution of nongenotoxic as well as genotoxic chemicals to the carcinogenic process has been attributed in part to effects on cell proliferation (*66, 67*). Tissue regeneration after toxicity would increase the frequency of mutation from replication errors. Other biological responses, such as inflammation and oxygen radical DNA damage accompanying tissue injury, chronic active hepatitis caused by hepatitis B virus, or a flux of macrophage cells induced by tobacco smoke may also contribute to the mutation frequency (*67, 68*).

Specific mutations. The three most notable features of the p53 mutation spectra in human cancers are as follows: (i) transitions at CpG dinucleotides contribute heavily to the mutation frequency in many cancers; (ii) a mutation at codon 249 predominates in HCCs in individuals from high-incidence regions; and (iii) there is a high frequency of nonclustered G to T transversions in lung cancer. We comment here on their possible origins, the first of which is characteristic of spontaneously arising mutations in mammalian cells whereas the second and third suggest a significant influence of the external environment on mutation events.

The unusual mutability of CpG dinucleotides is well documented and is attributed to the presence of 5-methylcytosine residues found at these dinucleotides in the mammalian genome (*69*). Nearly one-third of the 280 human p53 tumor mutations are transitions at hot spot codons with CpG sites (codons 175, 196, 213, 248, 273, and 282). It is not known why the fraction of tumor mutations that are transitions at CpG varies so greatly from one cancer type to another (0 to 68%). Rideout *et al.* (*70*) determined the methylation status in sperm and white blood cells of three (codons 175, 273, and 282) of the p53 CpG hot spots and found all three methylated in both cell types. Methylation of CpG sites and the level of spontaneous deamination may differ in various cell types even within the same tissue. Other possibilities to consider are tissue differences in DNA repair capacity such as G:T mismatch repair. A DNA glycosylase activity in HeLa cell extracts that cleaves thymines at G:T mismatches has recently been described (*71*). Deamination at nonmethylated cytosine in cells with lowered uracil glycosylase activity could also affect the frequency of transitions at CpG (*72*).

Aflatoxin B_1 and hepatitis B virus are risk factors for liver cancer in the two areas, Qidong (People's Republic of China) and southern Africa, where the eleven tumors with G to T mutations at codon 249 originated (*39, 43*). Aflatoxin B_1 is a potent liver carcinogen with widespread human exposure in high-risk areas (*73*). In mutagenesis experiments with this compound, induction of base substitutions, principally G to T transversions, and preferential interaction at specific DNA sequences have been demonstrated (*74–77*). Analysis of tumors from other areas will allow investigators to explore further the association between aflatoxin exposure and codon 249 G to T transversions. The absence of this hot spot mutation among six base substitutions in HCCs in patients from Japan, and among a series of ten tumors in patients from Taiwan and mainland China (region unspecified) is noteworthy (*49, 78*). The particular pathobiological effects of a substitution of an arginine for serine at residue 249 could have specific effects on hepatocytes leading to selective clonal expansion (with or without hepatitis B or C virus infection), although the mutation presumably affects growth of other cell types because the identical substitution has been rarely detected in cancers of the esophagus, lung, and breast.

G to T transversions also predominate in non-SCLC tumors, but the 17 mutations are distributed over ten codons. Most lung cancers occur in smokers, and some carcinogens in tobacco, for example, benzo[a]pyrene, induce this substitution (*79–81*); however, there is also an array of chemicals, DNA adducts, and endogenous processes known to elicit G to T transversions. Studies comparing the p53 mutational spectra in cancers of smokers and nonsmokers will be of interest. 8-Hydroxy-2'-deoxyguanosine, which is found in considerable amounts in human DNA (*67, 82*), also generates G to T transversions (*83, 84*). The presence of oxidized DNA bases is attributed to free radical damage from normal biochemical reactions in living cells (*85*). It has been proposed that these and other natural processes giving rise to mutations can outweigh the effects of additional environmental burden from genotoxic carcinogens (*66, 67*). As there are only six kinds of base pair changes generating missense mutations, and an abundance of chemical and biological factors able to generate one or another characteristic substitution, information on mutation position in a defined DNA sequence (in addition to the nature of the base change) is crucial for inferences on the origins of mutations in tumors. Preferential mutation sites based on sequence context can be highly specific for different mutagens and mutagenic processes (*16–18, 86*).

The balance between mutations from endogenous factors and exogenous carcinogens may vary for different cancers from the same population and for different populations with the same cancer. Where the contributions from endogenous factors vastly outweigh those from the exogenous ones, one might anticipate a mutational spectrum to reflect what is known about preferred base substitutions from DNA polymerase infidelity, base pairing opposite oxidized residues, base insertions opposite apurinic sites, and so on, and to match to some degree the pattern of spontaneous mutation spectra in untreated mammalian cells (*72*). In other instances, for example where the level of exposure of human populations to mutagenic carcinogens in the environment approaches the doses used experimentally to generate tumors in laboratory animals, the spectrum in human tumors may coincide with experimental mutagenesis data on the compounds.

The differences in p53 mutational spectra for different tumor types reviewed here indicate that a different composite of promutagenic factors may be at work in different tissues. Each microenvironment is defined by different exogenous and endogenous mutagen exposures, metabolic capacities, DNA repair enzyme activities, and degrees of DNA methylation. Interpretation of mutational differences among diverse tumor types may be confounded by inherent biochemical differences related to the generation of mutations in different cell types. For the many cancers with multiple and complex etiologies, elucidation through the study of gene damage will be particularly challenging. However, p53 gene mutations have already provided some tantalizing clues about the nature of the genetic alterations underlying several types of human tumors, with significant implications for understanding etiology.

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96. We are indebted to E. Chang, R. Dalla-Favera, D. Lane, J. Minna, Y. Murakami, J. Prosser, T. Sekiya, C. Theillet, J. Yokota, S. Hirohista and their co-workers for communicating data before publication. We are grateful to R. Tarone for helpful discussions and review of calculations. We thank R. Julia for editorial assistance. This work was supported in part by NIH training grant CA 09071 to D.S. and grant CA 43460 to B.V.

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