p53 Tumor Suppressor Gene: At the Crossroads of Molecular Carcinogenesis, Molecular Epidemiology, and Cancer Risk Assessment

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Carcinogenesis is a multistage process involving the inappropriate activation of normal cellular genes to become oncogenes, e.g., ras, and the inactivation of other cellular genes called tumor suppressor genes. p53 is the prototypic tumor suppressor gene that is well suited as a molecular link between the causes of cancer, i.e., carcinogenic chemical and physical agents and certain viruses, and the development of clinical cancer. The p53 tumor suppressor gene is mutated in the majority of human cancers. Genetic analysis of human cancer is providing clues to the etiology of these diverse tumors and to the functions of the p53 gene. Some of the mutations in the p53 gene reflect endogenous causes of cancer, whereas others are characteristic of carcinogens found in our environment. In geographic areas where hepatitis B virus and a dietary chemical carcinogen, aflatoxin B1, are risk factors for liver cancer, a molecular signature of the chemical carcinogen is found in the mutated p53 gene. A different molecular signature in the p53 gene is found in skin cancer caused by sunlight. Because mutations in the p53 gene can occur in precancerous lesions in the lung, breast, esophagus, and colon, molecular analysis of the p53 gene in exfoliated cells found in either body fluids or tissue biopsies may identify individuals at increased cancer risk. p53 mutations in tumors generally indicate a poorer prognosis. In summary, the recent history of p53 investigations is a paradigm in cancer research, illustrating both the convergence of previously parallel lines of basic, clinical, and epidemiologic investigation and the rapid translation of research findings from the laboratory to the clinic. — Environ Health Perspect 104(Suppl 3):435–439 (1996)

Key words: p53 gene, molecular carcinogenesis, molecular epidemiology, cancer risk assessment

The crucial differences between normal and cancer cells stem from discrete changes in specific genes controlling proliferation and tissue homeostasis. Over 100 such cancer-related genes have been discovered, several of which are implicated in the natural history of human cancer because they are consistently found mutated in tumors. The p53 tumor suppressor gene is the most striking example because it is mutated in about half of almost all cancer types arising from a wide spectrum of tissues. Other tumor suppressor genes important in human oncology such as APC, WT1, p16INK4a, or NF1 may have a more limited distribution (Table 1); given the variety of hereditary cancers and allelic deletions found in human cancers, additional tumor suppressor genes should be identified in the future, some of which may also have a conspicuous role in carcinogenesis.

Tumor suppressor genes are vulnerable sites for critical DNA damage because normally they function as physiological barriers against clonal expansion or genomic mutability and are able to hinder growth and metastasis of cells driven to uncontrolled proliferation by oncogenes. Loss of tumor suppressor function can occur by damage to the genome through mutation, chromosomal rearrangement and nondisjunction, gene conversion, imprinting, or mitotic recombination. Tumor suppressor activity can also be neutralized by interaction with other cellular proteins or with viral oncoproteins. Comprehensive reviews of this rapidly advancing field of molecular carcinogenesis are available (4–6).

The p53 suppressor gene is the most prominent tumor suppressor gene because it is mutated in about half of human cancer cases (7,8). Although the retinoblastoma and APC tumor suppressor genes are most commonly inactivated by nonsense mutations that cause the protein to be truncated or unstable, about 80% of p53 mutations are missense mutations that change the identity of an amino acid. Changing amino acids in this way can alter the protein conformation and increase the stability of p53; it can also alter sequence-specific DNA binding and transcription factor activity of p53 (9). One explanation for the high frequency of p53 mutation is that the missense class of mutations can cause both a loss of tumor suppressor function and a gain of oncogenic function by changing the repertoire of genes whose expressions are controlled by this transcription factor (10,11). The central role of p53 in multistage carcinogenesis places it at the intellectual crossroads of molecular carcinogenesis, molecular epidemiology of human cancer, and cancer risk assessment.

p53 participates in many cellular functions: cell cycle control, DNA repair, differentiation, genomic plasticity, and programmed cell death (8,10,12). p53 is one component of the DNA damage response pathway in mammalian cells (Figure 1). Some of these normal cellular functions of p53 can be modulated and sometimes inhibited by interactions with either cellular proteins (e.g., mdm2) or oncoviral proteins (e.g., hepatitis B virus X protein) of certain DNA viruses. p53 is clearly a component in a biochemical pathway or pathways central to human carcinogenesis, and p53 mutations provide a selective advantage for clonal expansion of preneoplastic and neoplastic cells.

The mutation spectrum of p53 in human cancer can help identify particular carcinogens and define the biochemical mechanisms responsible for the genetic lesions in DNA that cause human cancer.
Table 1. Examples of tumor suppressor genes involved in human cancers.a

| Tumor suppressor gene | Locus | Location/functionb | Somatic mutations | Inherited mutations |
|-----------------------|-------|--------------------|-------------------|--------------------|
|                       |       |                    | Major types       | Heterozygote carrier ratec | Typical neoplasms |
| p53                   | 17p13.1 | Nuclear/transcription factor | Missense | Most human cancer types examined to date | Li-Fraumeni | ~2 | Carcinomas of breast and adrenal cortex; sarcomas; leukemia; brain tumors |
| RB1                   | 13q14 | Nuclear/transcription modifier | Deletion and nonsense | Retinoblastoma; osteosarcoma; carcinomas of the breast, prostate, bladder, and lung | Retinoblastoma | ~2 | Retinoblastoma; osteosarcoma |
| APC                   | 5q21 | Cytoplasmic/unknown | Deletion and nonsense | Carcinoma of the colon, stomach, and pancreas | Familial adenomatous polyposis | ~10 | Carcinomas of colon, thyroid and stomach |
| ATM                   | 11q22 | Unknown/kinase | Deletion | Unknown | Ataxia telangiectasia | ~2 | Leukemia; lymphoma |
| WT1                   | 11p13 | Nuclear/transcription factor | Missense | Wilms' tumor | Wilms tumor | ~0.5–1 | Wilms' tumor |
| NF1                   | 17q11 | Cytoplasmic/GTPase activating protein | Deletion | Schwannomas | Neurofibromatosis type 1 | ~30 | Neural tumors |
| NF2                   | 22q | Cytoplasmic/cytoskeletal-membrane linkage | Deletion and nonsense | Schwannomas and meningiomas | Neurofibromatosis type 2 | ~3 | Central schwannomas and meningiomas |
| p16INK4               | 9p21 | Nuclear/cyclin dependent kinase inhibitor | Deletion and nonsense | Mesothelioma; pancreas; melanoma; glioblastoma | Familial melanoma | ? | Melanoma |
| VHL                   | 3p25 | Nuclear/adaptor | Deletion | Unknown | von Hippel-Lindau | ~3 | Hemangioblastoma and renal cell carcinoma |

*a*Data from Savitsky et al. (1); reviewed in Harris et al. (2). bProposed function. c*Per 10⁵ births. dDecreased expression controlled by epigenetic mechanism (DNA methylation) (3).

Figure 1. DNA damage leads to p53 accumulation and subsequent changes in gene expression and protein–protein interactions.

The frequency and type of p53 mutations can also act as a molecular dosimeter of carcinogen exposure and thereby provide information about the molecular epidemiology of human cancer risk. The p53 gene is well-suited for this form of molecular archaeology. The majority of mutations in p53 are in the hydrophobic midregion of the protein (Figure 2) (8). The function of the p53 protein as a transcription factor is exquisitely sensitive to conformational changes in this region that result from amino acid substitutions (13); p53 binding to other cellular and oncoviral proteins can easily be disrupted by mutations in these regions. How can p53 mutation spectra lead to identification of the carcinogens that caused a particular tumor? Different carcinogens seem to cause different characteristic mutations. Exposure to one common carcinogen, ultraviolet light, is correlated with transition mutations at dipyrimidine sites (14); dietary aflatoxin B₁ exposure is correlated with G:C to T:A transversions that lead to a severe substitution at residue 249 of p53 in hepatocellular carcinoma (15,16); and exposure to cigarette smoke is correlated with G:C to T:A transversions in lung carcinomas (17).

How these mutations arise can be further tested in the laboratory. For example, the predominant base changes in p53 found in lung cancers (G:C to T:A transversions) and skin carcinomas (C:G to T:A transitions) suggest that the causal lesion likely occurred on the nontranscribed strand, a finding that is consistent with the preferential repair after damage of the transcribed strand of active genes (18). Benzo[a]pyrene, a carcinogen in tobacco smoke, forms DNA adducts that are more slowly repaired when present on the nontranscribed strand than on the transcribed strand of the hypoxanthine (guanine) phosphoribosyltransferase gene (19), and ultraviolet light-induced cross-links of dipyrimidines in the nontranscribed DNA strand of the p53 gene also are more slowly repaired than in the transcribed strand.
p53 TUMOR SUPPRESSOR GENE

Figure 2. Schematic representation of p53 molecule. Functional domains include the transactivation region (amino acids 20–24, [ ], sequence-specific DNA binding region (amino acids 100–203), nuclear localization sequence (amino acids 316–325, [ ]), and oligomerization region (amino acids 319–360, [ ]). Cellular or oncoviral proteins bind to specific areas of the p53 protein. Evolutionarily conserved domains (amino acids 17–29, 97–292, and 324–352, [ ]) were determined using the MACAW program. Seven mutational hotspot regions within the large conserved domain are identified (amino acids 130–142, 151–164, 171–181, 190–200, 213–223, 234–258, and 270–286, [ ]). Vertical lines above the schematic are missense mutations; lines below schematic are nonmissense mutations. The majority of missense mutations are in the conserved hydrophobic midregion, while nonmissense (nonsense, frameshift, splicing, and silent mutations) are distributed throughout the protein, determined primarily by sequence context.

Because DNA repair rates can be sequence dependent (21), the p53 mutation spectrum could be influenced by both the type and location of the promutagenic lesion. Transcription–repair coupling factors, the products of the mfd and XPD gene, have been recently identified and provide a mechanistic underpinning for strand-specific repair (22–24). The p53 protein binds to XPD and XPD DNA helicases in the TFIIH complex and modulates their function in nucleotide excision repair (25). Another example comes from areas of China and Mozambique where there is a high incidence of liver cancer. The high frequency of G:C to T:A transversions in human hepatocellular carcinomas in this region could be due to the high mutability of the third base of codon 249 by aflatoxin B1 or a selective growth advantage of hepatocytic clones carrying this specific p53 mutant in liver chronically infected with hepatitis B virus. Indeed, the third base of codon 249 in a human liver cell line exposed to aflatoxin B1 is preferentially mutated (26), and transfected 249<sup>mut</sup> p53 mutant enhances the growth rate of the p53 null hepatocellular carcinoma cell line, Hep3B (27). Other p53 codons show lower frequencies of G:C to T:A, G:C to A:T, and G:C to C:G mutations, which suggests that both preferential mutability and clonal selection are involved in human hepatocellular carcinogenesis.

The p53 mutational spectra also can indicate that a particular cancer did not result from an environmental carcinogen but instead was caused by endogenous mutagenesis. The high frequency of C to T transitions at CpG dinucleotides in colon carcinomas (7) is consistent with mutagenesis by endogenous deamination mechanisms. A C to T transition would be generated by spontaneous deamination of 5-methylcytosine (28) or by enzymatic deamination of cytosine by DNA (cytosine-5)-methyl transferase when S-adenosylmethionine is in limiting concentration (or by both mechanisms) (29). Because oxygen radicals enhance the rate of deamination of deoxynucleotides (30,31), chronic inflammation and nitric oxide generated by nitric oxide synthases may explain why rats that inhale particulate materials, which cause inflammation but do not act directly on DNA, have a high incidence of lung cancer.

Mutations in p53 can also reveal that an individual has an increased susceptibility to cancer owing to inheritance of a germ-line mutation, a concept first proposed for the retinoblastoma (Rb) tumor suppressor gene (32). Germline p53 mutations are missense and occur frequently in the cancer-prone individuals with Li-Fraumeni syndrome (33). Laboratory animals with either a mutant p53 transgene or a deleted p53 gene, i.e., homozygous or heterozygous gene knockouts, also are particularly susceptible to cancer (34,35). These mutations in p53 are associated with instability in the rest of the genome (36). Such instability could generate multiple genetic alterations leading to cancer. Indeed, genomic instability (including gene amplification) increases in frequency in cells that lack a normal p53 gene (37,38). Furthermore, loss of the wild-type alleles of the p53 gene abrogates DNA damage-induced delay of the cell cycle in G1 (39). DNA repair of certain promutagenic lesions can proceed prior to DNA synthesis in S phase. Less time for repair would increase the frequency of mutations. Since p53 is an integral component in one pathway of programmed cell death (apoptosis) induced by DNA-damaging chemotherapeutic drugs or ionizing radiation (40,41), inactivation of p53 could increase both the pool of proliferating cells and the probability of their neoplastic transformation by inhibition of programmed cell death.

Such progress in the fields of molecular carcinogenesis and molecular epidemiology increases our ability to accurately assess cancer risk (Figure 3). Cancer risk assessment, a highly visible discipline in public health, has historically relied on classical epidemiology, from chronic exposure of rodents to potential carcinogens, and the mathematical modeling of these findings. The field has been forced to steer a prudent course of conservative risk assessment because of limited knowledge of the complex pathobiological processes during carcinogenesis; differences in the metabolism of carcinogens, different DNA repair capacities, variable genomic stability among animal species, and variation among individuals with inherited cancer predisposition have made definitive analysis of cancer risk almost impossible (5,42). Because regulatory decisions based on cancer risk assessments have significant public health and economic consequences,
the scientific basis of risk assessment continues to be, and should continue to be, actively investigated (43).

Many questions remain. Are the pathways of molecular carcinogenesis similar in rodents and humans? Because the time to develop cancer is generally shorter in rodents than in humans, could the apparent interspecies differences be due to the number of genetic and epigenetic events required for malignant progression or to the rate of transit between the events? Is the more frequent mutation of the ras proto-oncogenes in rodent cancer a reflection of a pathway that is parallel and equivalent to the p53 pathway in human carcinogenesis? Are the selective pressures for clonal expansion of preneoplastic and neoplastic cells in human carcinogenesis similar to those in animal models?

Investigations of the p53 tumor suppressor gene are an example of the recent progress in molecular aspects of cancer research. A better understanding of molecular carcinogenesis and molecular epidemiology will eventually decrease the qualitative and quantitative uncertainties associated with the current state of cancer risk assessment and improve public health decisions concerning cancer hazards. Indeed, determination of the type and number of mutations in p53 and other cancer-related genes in tissues from healthy people may allow the identification of those at increased cancer risk and their consequent protection by preventive measures.

| Laboratory animal studies | Cancer epidemiology |
|---------------------------|---------------------|
| Molecular dosimetry of carcinogen exposure | Inherited cancer predisposition |
| - Carcinogen–macromolecular adducts | - Genetic polymorphism of enzymes involved in activation and detoxification of carcinogens |
| - Cytogenetic end points | - Genomic instability and DNA repair-deficient conditions |
| - Mutational spectra and frequency | - Germ line mutations in tumor suppressor genes |
| Internal exposure assessment | Host susceptibility assessment |

**Human cancer risk assessment**
- Hazard identification
- Dose-response assessment
- Exposure assessment
- Risk characterization

**Bioethical issues**
- Autonomy
- Privacy
- Justice
- Equity
- Quality
- Specificity
- Effectiveness
- Limit genetic testing to conditions that are correctable by successful intervention

**Intervention and risk management**
- Reduce carcinogen exposure
- Increase medical surveillance
- Therapeutic strategies including chemoprevention
- Formulation of health policy

**Figure 3.** Human cancer risk assessment and bioethical issues associated with molecular epidemiology and human cancer.

### REFERENCES

1. Savitsky K, Bar-Shira A, Gilad S, Rotman G, Ziv Y, Vanagaite L, Tagle DA, Smith S, Uziel T, Sfez S, Ashkenazi M, Pecker I, Frydman M, Harnik R, Panatjali SR, Simmons A, Clines GA, Sarriel A, Gatti RA, Chessa L, Sanai O, Lavin MF, Jaspers NG, Malcolm A, Taylor R, Arlett CF, Miki T, Weissman SM, Lovett M, Collins FS, Shiloh Y. A single ataxia-telangiectasia gene with a product similar to PI-3 kinase. Science 268:1749–1753 (1995).
2. Harris CC, Hollstein M. Clinical implications of the p53 tumor-suppressor gene. N Engl J Med 329:1318–1327 (1993).
3. Herman JG, Latif F, Weng Y, Lerman MI, Zbar B, Liu S, Samid D, Duan DS, Gnarra JR, Linehan WM, Baylin SB. Silencing of the VHL tumor-suppressor gene by DNA methylation in renal carcinoma. Proc Natl Acad Sci USA 91:9700–9704 (1994).
4. Bishop JM. Molecular themes in oncogenesis. Cell 64:235–248 (1991).
5. Harris CC. Clinical and physical carcinogenesis: advances and perspectives. Cancer Res 51:5023s–5048s (1991).
6. Weinberg RA. Tumor suppressor genes. Science 254:1138–1146 (1991).
7. Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. Science 253:49–53 (1991).
8. Greenblatt MS, Bennett WP, Hollstein M, Harris CC. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res 54:4855–4878 (1994).
9. Vogelstein B, Kinzler KW. p53 function and dysfunction. Cell 70:523–526 (1992).
10. Lane DP, Benchimol S. p53: oncogene or anti-oncogene. Genes Dev 4:1–8 (1990).
11. Dittmer D, Pati S, Zambetti G, Chu S, Teresky AK, Moore M, Finlay C, Levine AJ. Gain of function mutations in p53. Nature Genet 4:42–46 (1993).
12. Levine AJ, Momand J, Finlay CA. The p53 tumour suppressor gene. Nature 351:453–456 (1991).
13. Cho Y, Gorina S, Jeffrey P, Pavletich NP. Crystal structure of a p53 tumor suppressor–DNA complex: a framework for understanding how mutations inactivate p53. Science 265:346–355 (1994).
14. Brash DE, Rudolph JA, Simon JA, Lin A, McKenna GJ, Baden HP, Halperin AJ, Ponten J. A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma. Proc Natl Acad Sci USA 88:10124–10128 (1991).
15. Hsu IC, Metcalfe RA, Sun T, Welsh JA, Wang NJ, Harris CC. Mutational hotspot in the p53 gene in human hepatocellular carcinomas. Nature 350:427–428 (1991).
16. Bressac B, Kew M, Wands J, Ozturk M. Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa. Nature 350:429–431 (1991).

17. Takeshima Y, Seyama T, Bennett WP, Akiyama M, Tokuoka S, Inai K, Mabuchi K, Land CE, Harris CC. p53 mutations in lung cancers from non-smoking atomic-bomb survivors. Lancet 342:1520–1521 (1993).

18. Bohr VA. Gene specific DNA repair. Carcinogenesis 12:1983–1992 (1991).

19. Chen RH, Maher VM, Brouwer J, van de Putte P, McCormick JJ. Preferential repair and strand-specific repair of benzo[a]pyrene diol epoxide adducts in the HPRT gene of diploid human fibroblasts. Proc Natl Acad Sci USA 89:5413–5417 (1992).

20. Evans MK, Taffe BG, Harris CC, Bohr VA. DNA strand bias in the repair of the p53 gene in normal human and xeroderma pigmentosum group C fibroblasts. Cancer Res 53:5377–5381 (1993).

21. Tornaletti S, Pfeifer GP. Slow repair of pyrimidine dimers at p53 mutation hotspots in skin cancer. Science 263:1436–1438 (1994).

22. Schaeffer L, Roy R, Humbert S, Moncollin V, Vermeulen W, Hoeijmakers JH, Chambon P, Egly JM. DNA repair helicase: a component of TFIIF (TFIIH) basic transcription factor. Science 260:58–63 (1993).

23. Selby CP, Sancar A. Molecular mechanism of transcription-repair coupling. Science 260:53–58 (1993).

24. Buratowski S. DNA repair and transcription: the helicase connection. Science 260:37–38 (1993).

25. Wang XW, Yeh H, Schaeffer L, Roy R, Moncollin V, Egly JM, Wang Z, Friedberg EC, Evans MK, Taffe BG, Bohr VA, Hoeijmakers JH, Forrester K, Harris CC. p53 Modulation of TFIIF-associated nucleotide excision repair activity. Nature Genet 10:188–195 (1995).

26. Aguilar F, Hussain SP, Cerutti P. Aflatoxin B1 induces the transversion of G→T in codon 249 of the p53 tumor suppressor gene in human hepatocytes. Proc Natl Acad Sci USA 90:8586–8590 (1993).

27. Ponchel F, Puisieux A, Tabone E, Michot JP, Froschl G, Morel AP, Frebourg T, Fontaniere B, Oberhammer F, Ozturk M. Hepatocarcinoma-specific mutant p53-249ser induces mitotic apoptosis but has no effect on transforming growth factor β 1-mediated apoptosis. Cancer Res 54:2064–2068 (1994).

28. Rideout WM III, Coetzee GA, Oulumi AF, Jones PA. 5-Methylcytosine as an endogenous mutagen in the human LDL receptor and p53 genes. Science 249:1288–1290 (1990).

29. Shen JC, Rideout WM III, Jones PA. High frequency mutagenesis by a DNA methyltransferase. Cell 71:1073–1080 (1992).

30. Nguyen T, Brunson D, Crespi CL, Penman BW, Wishnok JS, Tannenbaum SR. DNA damage and mutation in human cells exposed to nitric oxide in vitro. Proc Natl Acad Sci USA 89:3030–3034 (1992).

31. Wink DA, Kasprzak KS, Maragos CM, Elespuru RK, Misra M, Dunams TM, Cebula TA, Koch WH, Andrews AW, Allen JS, Keefe LK. DNA deaminating ability and genotoxicity of nitric oxide and its progenitors. Science 254:1001–1003 (1991).

32. Knudson AG, Jr. Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci USA 68:820–823 (1971).

33. Malkin D, Li FP, Strong LC, Fraumeni JF, Nelson CE, Kim DH, Kassel J, Gryka MA, Bischoff FZ, Tainsky MA, Friend SH. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 250:1233–1238 (1990).

34. Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery CA Jr, Bulet JS, Bradley A. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature 356:215–221 (1992).

35. Lavigneaur A, Maltby V, Mock D, Rossant J, Pawson T, Bernstein A. High incidence of lung, bone, and lymphoid tumors in transgenic mice overexpressing mutant alleles of the p53 oncogene. Mol Cell Biol 9:3982–3991 (1989).

36. Hartwell L. Defects in a cell cycle checkpoint may be responsible for the genomic instability of cancer cells. Cell 71:543–546 (1992).

37. Livingston LR, White A, Sprouse J, Livanos E, Jacks T, Tsuj TD. Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. Cell 70:923–935 (1992).

38. Yin Y, Tainsky MA, Bischoff FZ, Strong LC, Wahl GM. Wild-type p53 restores cell cycle control and inhibits gene amplification in cells with mutant p53 alleles. Cell 70:937–948 (1992).

39. Kastan MB, Zhou Q, el-Deiry WS, Carrier F, Jacks T, Walsh WV, Plunkett BS, Vogelstein B, Fornace AJ Jr. A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. Cell 71:587–597 (1992).

40. Clarke AR, Purdie CA, Harrison DJ, Morris RG, Bird CC, Hooper ML, Wyllie AH. Thymocyte apoptosis induced by p53-dependent and independent pathways. Nature 362:849–852 (1993).

41. Lowe SW, Schmitt EM, Smith SW, Osborne BA, Jacks T. p53 is required for radiation-induced apoptosis in mouse thymocytes. Nature 362:847–849 (1993).

42. Barrett JC, Wiseman RW. Molecular carcinogenesis in humans and rodents. Prog Clin Biol Res 376:1–30 (1992).

43. National Research Council. Science and Judgement in Risk Assessment. Assessment of Toxicology (National Academy of Sciences, eds). Washington:National Academy Press, 1994;56–67.