p53: At the Crossroads of Molecular Carcinogenesis and Risk Assessment

Curtis C. Harris

Mutations in the tumor suppressor gene *p53* are common in human cancer and, in fact, can be found in about half of all cases. The location and characteristics of these *p53* mutations can reveal clues about the etiology and molecular pathogenesis of cancer (*1*).

Mutations in *p53* are quite different from those in most other tumor suppressors. The tumor suppressor genes defective in retinoblastoma (*Rb*) and adenomatous polyposis coli (*APC*) are commonly inactivated by nonsense mutations that cause truncation or instability of the protein. But in *p53*, more than 90 percent of the mutations are missense mutations that change the identity of an amino acid. Changing amino acids in this way can alter the conformation and increase the stability of the p53 protein; these mutations also can indirectly alter the sequence-specific DNA binding and transcription factor activity of p53 (*2*). Indeed the frequency of *p53* mutations in human cancer may be so high because this missense class of mutation can cause both a loss of tumor suppressor function and a gain of oncogenic function by alteration of the repertoire of genes controlled by p53 (*3*).

The spectrum of mutations in *p53* induced in human cancer can help identify particular carcinogens and define the biochemical mechanisms responsible for the genetic lesions in DNA that cause human cancer. 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 (*4*). 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 (Fig. 1). 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, and 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 (*5*); dietary aflatoxin B_1 exposure is correlated with G:C to T:A transversions that lead to a serine substitution at residue 249 of p53 in hepatocellular carcinoma (*6*); and exposure to cigarette smoke is correlated with G:C to T:A transversions in lung and head and neck carcinomas (*1*, *7*).

How these mutations arise can be further tested in the laboratory. For example, the predominant base changes in *p53* 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 (*8*). 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) phosphoribosyl transferase gene, and ultraviolet light–induced cross-linked dipyrimidines in the nontranscribed DNA strand of the *p53* gene also are more slowly repaired than in the transcribed strand (*9*). Because DNA repair rates can be sequence-dependent (*10*), 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 *ERCC-3* genes, have recently been identified and provide a mechanistic underpinning for strand-specific repair (*11*). Another example comes from areas of China and Mozambique with 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 B_1 or to a selective growth advantage of hepatocyte 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 B_1 is preferentially mutated (*12*). Other *p53* codons show a lower frequency 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 (*1*) is consistent with mutagenesis by endogenous deamination mechanisms. A transition of C to T would be generated by spontaneous deamination of 5-methylcytosine (*13*) or by enzymatic deamination

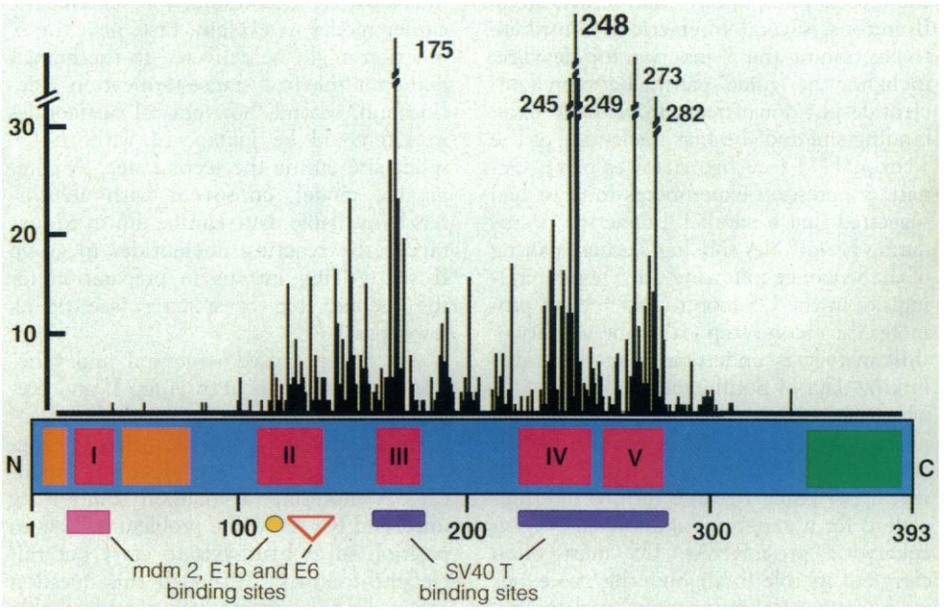

Fig. 1. Mutations in the p53 protein. Functional (transactivation, orange; oligomerization, green) and evolutionarily conserved (red) domains of p53. Other cellular (Mdm2) and oncoviral proteins (SV40 T antigen, E1b from adenovirus, and E6 from human papilloma virus) bind to p53. The bar graph indicates base substitution mutations (n = 1361) and mutational hot spots at codons 175 (n = 77), 245 (n = 55), 248 (n = 112), 249 (n = 55), 273 (n = 89), and 282 (n = 42).

The author is in the Laboratory of Human Carcinogenesis, Division of Cancer Etiology, National Cancer Institute, Bethesda, MD 20892.

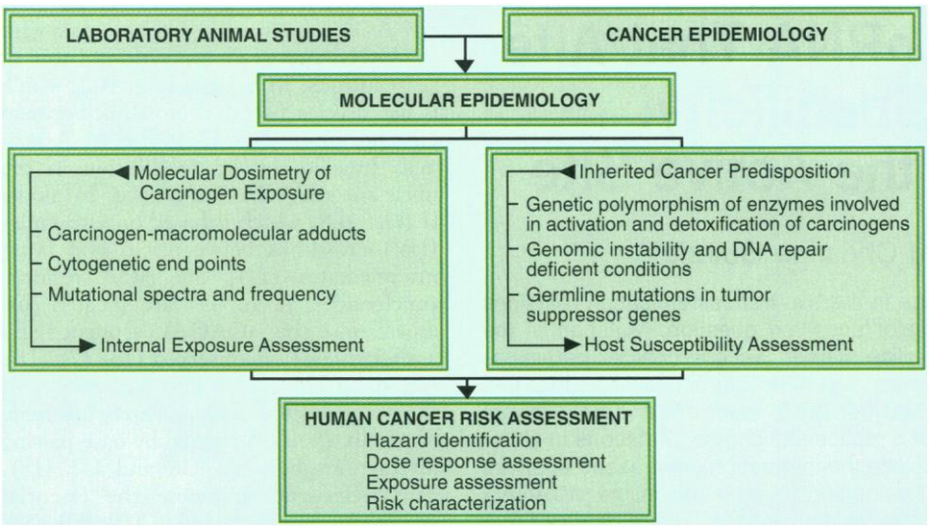

Fig. 2. Human cancer risk assessment.

of cytosine by DNA (cytosine-5)-methyl transferase when *S*-adenosylmethionine is in limiting concentration (or by both mechanisms) (*14*). Because oxygen radicals enhance the rate of deamination of deoxynucleotides (*15*), chronic inflammation and nitric oxide generated by nitric oxide synthases may contribute to the mutagenesis of the *p53* gene. This mechanism 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 germline mutation, a concept first proposed for the *Rb* tumor suppressor gene (*16*). Germline *p53* mutations are missense and occur frequently in the cancer-prone individuals with Li-Fraumeni syndrome (*17*). Laboratory animals with either a mutant *p53* transgene or a deleted *p53* gene, that is, homozygous or heterozygous "gene knockouts," also are particularly susceptible to cancer (*18*). These mutations in *p53* are associated with instability in the rest of the genome (*19*). 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. Furthermore, loss of the wild-type alleles of the *p53* gene abrogates DNA damage–induced delay of the cell cycle at G1 (*20*). 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 the p53 protein is an integral component in one pathway of programmed cell death (apoptosis) induced by DNA-damaging chemotherapeutic drugs or ionizing radiation (*21*), 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 (Fig. 2). Cancer risk assessment is a highly visible discipline in public health and has historically relied on classical epidemiology, results 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 (*22*). 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 (*23*).

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, the apparent interspecies differences could 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* protooncogenes in rodent, as compared to human cancer, a reflection of a pathway that is parallel and equivalent to the p53 pathway in humans 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" individuals may allow the identification of those at increased cancer risk and their consequent protection by preventive measures.

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