

Molecular Epidemiology in Environmental Health: The Potential of Tumor Suppressor Gene *p53* as a Biomarker

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One of the challenges in environmental health is to attribute a certain health effect to a specific environmental exposure and to establish a cause-effect relationship. Molecular epidemiology offers a new approach to addressing these challenges. Mutations in the tumor suppressor gene *p53* can shed light on past environmental exposure, and carcinogenic agents and doses can be distinguished on the basis of mutational spectra and frequency. Mutations in *p53* have successfully been used to establish links between dietary aflatoxin exposure and liver cancer, exposure to ultraviolet light and skin cancer, smoking and cancers of the lung and bladder, and vinyl chloride exposure and liver cancer. In lung cancer, carcinogens from tobacco smoke have been shown to form adducts with DNA. The location of these adducts correlates with those positions in the *p53* gene that are mutated in lung cancer, confirming a direct etiologic link between exposure and disease. Recent investigations have also explored the use of *p53* as a susceptibility marker for cancer. Furthermore, studies in genetic toxicology have taken advantage of animals transgenic for *p53* to screen for carcinogens *in vivo*. In this review, we summarize recent developments in *p53* biomarker research and illustrate applications to environmental health. — Environ Health Perspect 105(Suppl 1):155–163 (1997)

Key words: aflatoxin B₁, biomarker, cancer, environmental health, exposure, genetic toxicology, mutations, *p53*, polymorphism, risk assessment, smoking, susceptibility, ultraviolet light, vinyl chloride

Introduction

Because environmental exposures to chemical agents or radiation can occur at low levels over long periods of time, it is often difficult to determine associations between exposure and disease, let alone prove causality. Therefore, investigations concerning the health consequences of environmental exposure face a multitude of challenges. First, the environmental fate of xenobiotic agents must be evaluated before they come into contact with human populations. Factors such as transformation, bioaccumulation, and bioavailability in the environment will differentially affect the exposure an individual receives (1) and the routes by which an individual is exposed. In addition,

population heterogeneity and interactions between biological variables such as inherited metabolic characteristics and synergistic effects of multiple environmental exposures may play an important role as well.

Once the complex task of establishing exposure levels has been addressed, health risks resulting from such exposure can be determined. Toxicological dose-response relationships and threshold effects are usually extrapolated from animal data. Molecular epidemiology offers a new approach to establish associations between exposure and disease by using types and frequencies of mutations as molecular dosimeters (2–4).

By combining environmental epidemiology with modern molecular techniques, molecular epidemiology attempts to find answers to questions regarding exposure and risk (5). For example, is the lung cancer of a miner due to environmental radon exposure or smoking? Do dietary carcinogens play a role in the liver cancer of a patient, or is the disease a result of endogenous processes? Molecular epidemiology offers a new approach to risk assessment in cases such as these.

The first interaction between xenobiotic compounds and organisms occurs on a molecular level prior to any clinical manifestation. Thus, if a target molecule of a toxic agent is known, changes in that molecule may be used as a biomarker and the biological dose or effect of an environmental exposure may be measured directly in biological samples. The power of molecular epidemiology in environmental exposure assessment lies in the use of such biomarkers, which attempt to infer exposure and identify exposure-related health risks.

When biomarkers are used to directly measure the molecular impact of exposure, subclinical responses can be detected. Therefore, biomarkers allow exposure to be determined prior to any clinical manifestation of health effects, allowing the minimization of adverse outcomes through the prevention of continued exposure.

The tumor suppressor gene (TSG) *p53* is an ideal biomarker of effect for addressing such questions of exposure and risk. The *p53* gene is expressed in every cell of the human body and the *p53* protein has been shown to play a crucial role in the regulation of cell division, making it a central molecule in the life of a cell (6). Inactivation of the *p53* gene can result in uncontrolled cell division and ultimately lead to cancer (7). Such inactivation frequently occurs as a result of mutations caused by exposure to environmental mutagens (3,4). However, inactivation of *p53* can also occur sporadically through endogenous processes, and in rare cases, mutations in *p53* can be inherited. The ability to distinguish between different types of mutations in *p53* as well as to link exposure to specific patterns and types of mutations in the gene makes *p53* an ideal tool in molecular epidemiology.

p53 and Cancer

Cancer is a complex process, a result of the cell cycle gone awry. Tight control over cellular proliferation is essential for the life

Manuscript received 29 May 1996; manuscript accepted 23 September 1996.

The authors thank M.T. Smith for his invaluable support of this review. We would also like to acknowledge L. Balluz, H. Falk, D. Flanders, A. Henderson, M. Khoury, M. McGeehin, C. Rubin, T. Sinks, and A. Wasley for their helpful comments on the manuscript.

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Abbreviations used: AFB₁, aflatoxin B₁; Arg, arginine; ASL, angiosarcoma of the liver; B[a]P, benzo[a]pyrene; BCC, basal cell carcinoma; CDK, cyclin dependent kinase; DMN, dimethylnitrosamine; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; PAH, polycyclic aromatic hydrocarbon; Pro, proline; SCC, squamous cell carcinoma; TSG, tumor suppressor gene; UV, ultraviolet.

of an organism, in which growth is stimulated by protooncogenes and counterbalanced by growth-inhibiting TSGs such as *p53*. Mutations that activate protooncogenes can turn them into oncogenes and result in tumor growth. On the other hand, mutations that inactivate TSGs release growth inhibition, also leading to tumorigenesis (8).

While mutations in protooncogenes result in a gain of function and are therefore dominant, overriding the presence of the wild-type copy of the gene, recessive TSG mutations result in loss of function and a single mutant copy will thus be masked by the presence of the remaining wild-type copy (8). Therefore, in most cases, both copies of a TSG must be mutated in order to see an effect. In many instances, one copy of *p53* is inactivated by a point mutation while the other allele has been deleted (7). The initiation of cancer often results when mutations in both protooncogenes and TSGs occur simultaneously.

The *p53* gene is one of the most prominent members of the TSG family. Its protein product is a nuclear transcription factor (7) that regulates the activity of several genes, including that of *p21*, which is directly involved in cell cycle control (9–11). *p21* exerts its effect by binding to and inactivating cyclin-dependent kinases (CDK), preventing the cell cycle from progressing (Figure 1). Mutated *p53* as found in human cancers is unable to activate the *p21* gene, leading to a release of the normal cell cycle block and thus to uncontrolled cellular growth (12).

Control of the cell cycle checkpoint becomes increasingly important when a cell has suffered from extensive DNA damage. *p53* plays a crucial role in preventing such cells from multiplying by initiating programmed cell death (apoptosis) (13–15). When *p53* is mutated and can no longer activate apoptosis, such damaged cells are able to continue proliferating, potentially resulting in cancer.

p53 as a Biomarker

The usefulness of *p53* as a biomarker of effect is 5-fold:

a) *p53* is altered in tumors of more than 50% of all cancer patients (16–18). This finding reflects the central role played by *p53* in cell cycle control and cancer. This high prevalence of *p53* mutations means that the probability of finding mutations in this gene in a given cancer patient population is likely, allowing for the establishment of statistically significant associations between mutations and exposures.

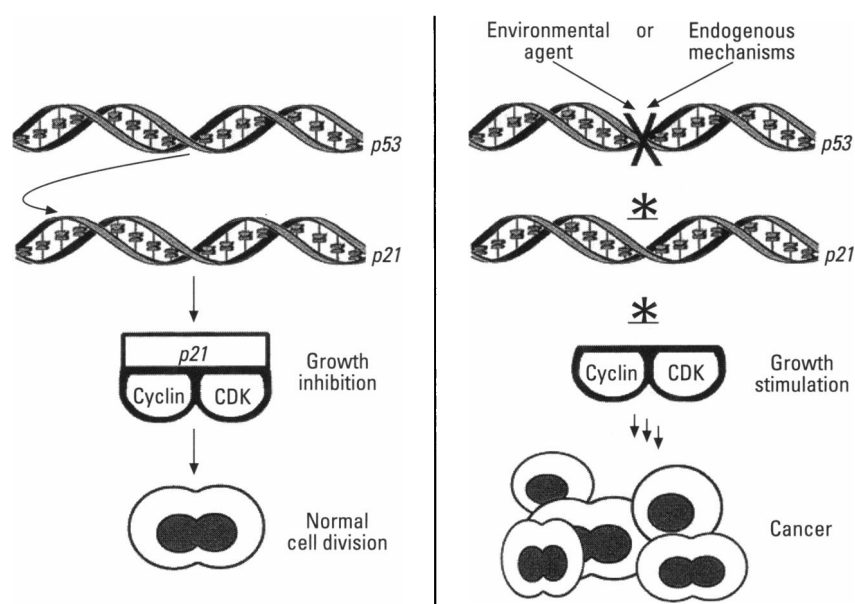


Figure 1. Transcriptional activation of the *p21* gene by *p53* and cyclin-mediated cell cycle control.

b) Mutations in the *p53* gene are found in nearly all types of cancer studied (19). Because mutations in *p53* are widespread, having been identified in such diverse types of cancer as lung, breast, colon, esophagus, lymphomas, and leukemias, (16) *p53* is a useful tool for assessing associations between environmental exposure and a multiplicity of types of cancer. Thus, *p53* lends itself to the study of the carcinogenic process in relation to carcinogens even in the case of rare tumor types.

c) Specific types of cancer are associated with characteristic mutation patterns in *p53*. Particular mutation patterns in *p53* have been observed to correlate with specific types of cancer (16). The so-called mutational spectrum, the pattern of location and type of mutations within the *p53* gene, has been shown to differ between cancers of the lung, colon, liver, breast, brain, esophagus, reticuloendothelial tissue, and hemopoietic tissues, allowing investigation of the etiology of the carcinogenic process in different tissues.

d) Specific mutational spectra in *p53* can be linked to specific exposures (18). Most importantly for environmental health, certain mutagens have been shown to leave mutational fingerprints in the *p53* gene—characteristic patterns of mutations that can be indicators of specific exposures. On the basis of these fingerprints, endogenous mutagenic events can often be distinguished from those resulting from exposure to exogenous agents, thus shedding light

on the etiology and molecular pathogenesis of cancer. Furthermore, it has been possible to identify carcinogens on the basis of such mutational spectra and to determine the dose by using the frequency of mutations as a molecular dosimeter (3,18).

e) The location of adducts between carcinogens and DNA can be mapped along the sequence of *p53* (20). Preferential location of such adducts at particular positions in the *p53* gene correlates with the sites of mutational hot spots found in certain cancer types. Thus, the *p53* gene can be used to identify direct causal links between chemical exposures and disease.

p53 and Exposure Assessment

Because of the widespread involvement of *p53* in many types of cancer, significant information concerning links between specific exposures, mutations in *p53*, and cancer type is available. The *p53* gene has been isolated and sequenced in a wide variety of cancers and the presence of signature mutations in several exposure-induced types of cancer has been described (Table 1). These findings indicate that the *p53* gene can be used as an indicator to reconstruct exposure and to identify the carcinogen responsible for the mutation. Mutations resulting from endogenous mechanisms are thought to differ from exposure-induced events. For example, more than two-thirds of the mutations found in colon cancer are transitions at CpG sites (16). It has been

Table 1. Exposure to environmental agents, mutation in *p53*, and cancer outcome.

Exposure	Cancer type	Mutation in <i>p53</i>	Reference
Dietary aflatoxin	Hepatocellular carcinoma (liver)	G→T or G→C transversions, particularly at codon 249	(21–23)
UV irradiation	Squamous and basal cell carcinomas (skin)	C→T substitutions at dipyrimidine sites	(24–26)
Tobacco smoke	Lung cancer	G:C to T:A transversions, particularly at codons 157, 248, 273	(16,27–30)
	Bladder cancer	G:C to C:G transversions, higher double mutation frequency	(31,32)
Vinyl chloride	Angiosarcoma (liver)	A:T to T:A transversions, anti- <i>p53</i> antibodies in sera	(33,34)

speculated that CpG sites are susceptible to spontaneous methylation followed by deamination and that the resulting mutation is due to endogenous rather than exogenous processes (3).

The usefulness of *p53* in exposure assessment is illustrated in the following sections.

Dietary Aflatoxin B₁

One of the best documented examples of the value of *p53* as a biomarker of effect is the association that has been established between exposure to dietary aflatoxin B₁ (AFB₁), hepatocellular carcinoma (HCC) of the liver, and mutation in the *p53* gene. AFB₁, a fungal mycotoxin produced by *Aspergillus flavus*, is a common contaminant in grains, nuts, and oil seeds in certain geographical regions. In areas where exposure is endemic, such as sub-Saharan Africa and southern China, dietary AFB₁ is considered a major risk factor for HCC (35,36), which occurs at high levels in the population. Hepatitis B infection is often also common in these regions and is considered to have a substantial and perhaps synergistic causative role in the development of HCC (37,38).

Examination of the *p53* gene in HCC tumors from patients residing in Qidong, China, an area of both high AFB₁ exposure and endemic Hepatitis B infection, revealed a prevalence of point mutations at the third base position of codon 249. Many of the point mutations observed were G→T or G→C transversion events (21). In contrast, nonmalignant liver tissue from patients with *p53* mutations in the tumor showed no mutation in *p53*. Thus, there is a striking correlation between the presence of *p53* mutation and malignancy. Studies of HCC tumors from patients residing in sub-Saharan Africa, also a region of both high AFB₁ exposure and high rates of hepatitis B virus (HBV) infection, revealed strikingly similar patterns of mutation in

the *p53* gene; many of the mutations observed were G→T transversions, with a hot spot at codon 249 (22).

An association between dietary AFB₁ exposure and G→T transversions in HCC is supported by *in vitro* studies that have demonstrated that metabolites of AFB₁ are capable of binding to DNA and inducing G→T transversions (23). In studies of DNA-repair deficient human cells, AFB₁ metabolite exposure resulted in a high frequency of base substitutions at G:C pairs and transition mutations at GG sites. Furthermore, experiments in which human hepatocytes were exposed to AFB₁ metabolites *in vitro* revealed a high frequency of G→T transversion events at codon 249, a result consistent with observations in tumors from populations in areas with high AFB₁ exposure (39).

Whereas these studies implicate AFB₁ exposure in specific patterns of mutation in *p53*, HBV infection has also been established as a risk factor for HCC and therefore is likely to also play a role in events leading to carcinogenesis. However, a study of HCC in Hong Kong, a high prevalence area for HBV infection but a low-exposure area for AFB₁, showed a lower frequency of mutation in *p53* than was observed in tumors from China and sub-Saharan Africa, with no clustering of mutations at codon 249 (40). In addition, when tumors from Qidong, China, were assessed for the presence of HBV infection, no correlation was found between the state of HBV DNA and mutations in *p53* (41). Experimental evidence suggests that HBV infection is likely to contribute to HCC by inhibiting the activity of wild-type *p53*, rather than through mutagenesis of the *p53* gene. A protein encoded by HBV, protein X, has been shown to bind to the wild-type *p53* gene product and inhibit its DNA binding and transcriptional activity *in vitro* (42). Thus, it is likely that HBV infection increases the probability of developing

HCC as a result of a synergistic effect with mutations in *p53* stemming from AFB₁ exposure.

The specificity of the link between G→T transversion events, particularly at codon 249, and AFB₁ exposure is further supported by studies of HCC cases from regions where neither AFB₁ exposure nor HBV infection is common. One of these studies revealed a high frequency of *p53* overexpression in HCC tumor cells linked to mutations in the *p53* gene (43). However, the codon 249 mutation that was frequently observed in AFB₁-exposed populations was not a predominant mutation event in these HCCs (43). Studies with additional cases showed no consistent pattern of mutation in *p53* and the frequency of mutation at codon 249 was low (44,45). Additional support for the causality of AFB₁ in the development of HCC is provided by the presence of mutations at codon 249 in nonmalignant liver tissue from HCC patients in areas with high AFB₁ exposure (46). These studies corroborate the evidence linking exposure to AFB₁ with mutations at codon 249 of the *p53* gene in cases of HCC tumors.

Ultraviolet Irradiation

Exposure to ultraviolet (UV) irradiation has been implicated in the etiology of many types of skin cancers (47). Because UV exposure is often preventable by the use of sunscreens or the reduction of time spent in the sun, public health implications of the relationship between UV exposure and skin cancer are of great practical importance. Because UV exposure is well established as the most consistent and significant cause of skin cancer in humans, the ability to locate specific patterns of mutation in *p53* on the causal pathway between exposure and disease provides an excellent example of the power of biomarkers in assessing environmental exposure.

Exposure to UV light has been shown to increase the risk of squamous cell (SCC) and basal cell (BCC) carcinomas, as well as the risk of melanoma (47,48). At the molecular level, it has been demonstrated that exposure to UV light leads to CC→TT double base substitutions and C→T substitutions at dipyrimidine sites. Such dipyrimidine mutations are a signature of UV exposure; rarely do these types of mutations result from exposure to other mutagens (49,50).

In cases of skin cancer, particularly SCC (24) and BCC (25,26), mutations in *p53* have been detected predominantly at

dipyrimidine sites and are frequently C→T substitutions, suggesting a relationship between UV exposure, *p53*, and carcinogenesis. Further implicating an association between UV exposure and the presence of *p53* mutations in these cases is the fact that all of the tumors in which *p53* mutations were observed came from sun-exposed sites on the body, whereas tumors occurring at nonexposed sites on the body showed no correlation with mutations in *p53* (25,51). When normal skin cells were subjected to varying degrees of UV irradiation *in vitro* and assessed for CC→TT transitions at codons 245 and 247/248 of *p53*, such mutations were observed to occur according to a dose–response relationship. The presence of pyrimidine dimer mutations at certain hot spots in the *p53* gene is thought to be a result of slow repair of dimers at specific sites (52).

Smoking

Tobacco smoking has been associated with an elevated risk for multiple types of human cancers (53,54). Moreover, several of the approximately 3800 chemicals identified in tobacco smoke have been shown to cause cancer in laboratory animals (55). Two components of tobacco smoke, benzo[*a*]pyrene (B[*a*]P), a polycyclic aromatic hydrocarbon (PAH), and 4-aminobiphenyl, an arylamine, form adducts with DNA, suggesting that these components may be direct mutagens. DNA adducts containing B[*a*]P have been found in lung tumors from smokers (56–59) and 4-aminobiphenyl has been identified in DNA adducts in bladder tumors from smokers (60). The presence of tobacco smoke components in DNA adducts from smoking-associated tumors strongly suggests that direct DNA mutagenesis plays a role in the increased cancer risk associated with tobacco smoking.

Several studies have determined that G:C to T:A transversions are the most commonly observed mutation events following experimental exposure to polycyclic aromatic hydrocarbons such as B[*a*]P (27–30,61), and other components of tobacco smoke have been shown to lead to G:C to T:A transversions as well (16,62).

Studies investigating *p53* mutations in lung cancers have shown that G:C to T:A transversions on the nontranscribed strand of the *p53* gene occur at a significantly higher frequency in lung cancers than in other types of cancer (4,63,64), suggesting an association between exposure to tobacco smoke components, such as PAHs, and

these mutations. Conversely, the frequency of transition mutations at CpG residues, presumed to arise from endogenous causes, is also significantly lower overall in lung cancer than in most other types of cancer. This would suggest a stronger than usual role of direct mutagenesis in lung cancers as compared to other types of cancer. However, among the small subgroup of lung cancers occurring in nonsmokers (tobacco smoking is thought to account for up to 90% of all pulmonary malignancies), the pattern is reversed. Fewer incidences of G:C to T:A transversion events in *p53* have been observed; most of the *p53* mutations in these cancers are G:C to A:T transition mutations (65,66). Thus, the specific and distinctive mutation pattern of G:C to T:A transversion events in the *p53* gene predominates in cases of lung cancer associated with smoking, whereas lung cancer cases not associated with smoking tend to display the more widespread and endogenous G:C to A:T transition mutations. Therefore, the presence of G:C to T:A transversions in the *p53* gene, particularly on the nontranscribed strand, serves as a specific biomarker of effect of tobacco smoke and is likely to be induced by one or more components found in tobacco smoke.

Further supporting the association between such mutations in *p53*, lung

cancer, and smoking are the results of several studies (64–66). These studies show that in lung cancers not only is there a direct association between smoking and the frequency of mutation or state of the *p53* protein, but the relative frequency of such events correlates with lifetime cigarette consumption. Additionally, several hot spots at guanine residues have been identified in lung cancers at codons 157, 248, and 273, which lie within the DNA binding domain of *p53* (20). Of these, the mutational hot spot at codon 157 is specific for lung cancer and has not been identified as a hot spot in other cancer types.

Recently, a direct etiologic link between tobacco smoke and lung cancer has been established in a study examining B[*a*]P adduct formation in the *p53* gene (Figure 2). When bronchial epithelial cells were exposed to benzo[*a*]pyrene diol epoxide, the metabolically active form of B[*a*]P, strong and selective adduct formation was observed at codons 157, 248, and 273 (20). Since these codons are identical to those mutated in lung cancer, this finding formally establishes the link between exposure and disease in tobacco smoke exposure.

Tobacco smoking has also been associated with increased risk for bladder cancer (67–72). Mutations in *p53* were one of the first genetic alterations to be associated

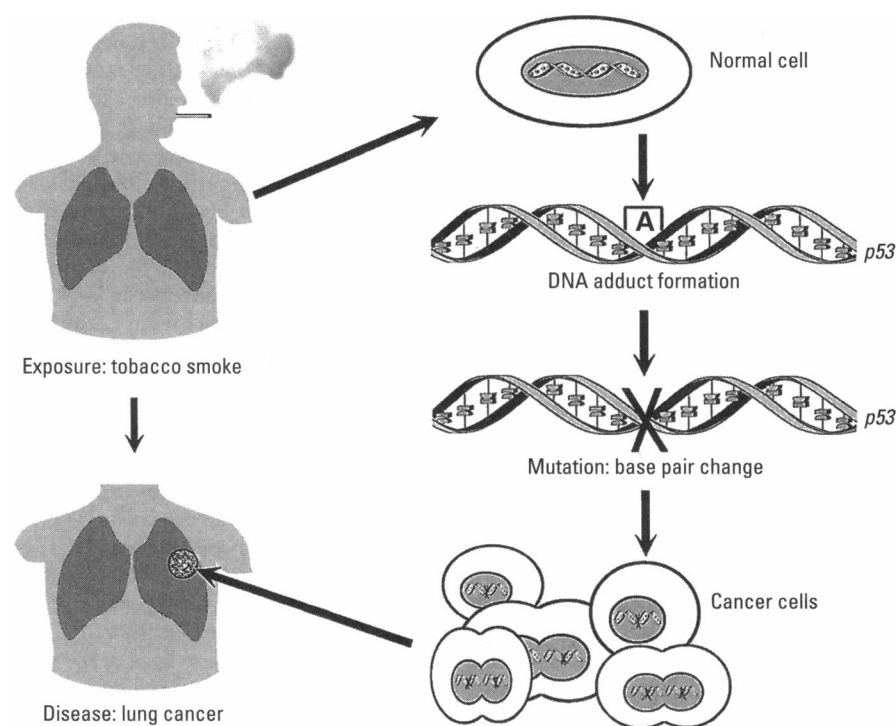


Figure 2. Causal link between exposure and disease: tobacco smoke and lung cancer.

with primary invasive bladder cancers (73). In immunohistochemical studies of patients with early stage bladder cancer, a significant association between the number of cigarettes smoked per day and the frequency of nuclear staining for *p53* was observed (74). Studies comparing cases of bladder cancer from smoking and non-smoking patients showed that an increased frequency of G:C to C:G transversion events in the *p53* gene was associated with both groups (31,32). Although no difference in the spectrum of mutation types was found, smokers were observed to have a higher frequency of double mutation events in the *p53* gene. One study of Japanese patients with a variety of urothelial cancers, including bladder cancer, showed that although the frequency of *p53* mutation did not substantially differ between smokers and nonsmokers, tumors from smokers exhibited A:T to G:C transitions in the *p53* gene at a significantly higher frequency than tumors from nonsmokers (31).

Vinyl Chloride

Occupational exposure to vinyl chloride during the manufacture of plastics has been tied to the occurrence of angiosarcoma of the liver (ASL), a rare form of cancer (75,76). Most exposure to vinyl chloride occurred between 1950 and 1980, prior to the discovery of its carcinogenic effect. Because such exposure has been remarkably well documented, studies of workers exposed to vinyl chloride provide an excellent opportunity for establishing associations between environmental exposure, mutation spectra, and ASL.

Serum from workers exposed to vinyl chloride tested positive for anti-*p53* antibodies; many of those who tested positive either had ASL at the time of testing or went on to develop ASL at a later date (33). In contrast, serum from lung cancer patients tested positive at a significantly lower frequency, whereas serum taken from individuals with neither vinyl chloride exposure nor cancer showed no reactivity at all (33). Thus, the presence of anti-*p53* antibodies in serum from workers exposed to vinyl chloride may be a useful indicator for risk or presence of ASL.

When ASL tumors from workers exposed to vinyl chloride were analyzed for mutations in *p53*, a significant frequency of A:T to T:A transversions, an uncommon mutation event in the majority of human types of cancer, was observed (34). Tumor tissues from those vinyl chloride-exposed workers who tested positive for anti-*p53*

antibodies also showed these unusual A:T to T:A transition events in *p53* (33). Usually, mutations in *p53* are rare events in sporadic ASL (77) and when they do occur, they are usually G:C to A:T transitions consistent with endogenous mechanisms. However, A:T to T:A transversions are characteristic of chloroethylene oxide, a carcinogenic metabolite of vinyl chlorides (76). Therefore, such mutations can be used as biomarkers of effect resulting from exposure to vinyl chloride.

p53 as a Susceptibility Marker

Mutations

p53 mutations can be inherited as well as acquired. Individuals with the rare Li-Fraumeni syndrome are at increased risk of acquiring breast carcinomas, soft tissue sarcomas, brain tumors, osteosarcomas, leukemia, and adrenocortical carcinomas (78). This syndrome results from germline mutations in *p53* (79).

Although individuals carrying germline mutations in *p53* can usually be identified on the basis of a family history of cancer (i.e., Li-Fraumeni syndrome), the identification of individuals at risk who lack such a family history poses technical problems for traditional techniques. In the absence of a family history of cancer, a molecular assay developed in yeast can be used to screen for individuals with germline mutations (80). This functional assay distinguishes between mutations that affect the function of the protein (and are therefore likely to lead to increased susceptibility to cancer) and polymorphisms not likely to alter wild-type *p53* function.

Polymorphisms

Polymorphisms differ from mutations in that they occur at a higher frequency in the population (>1%) and do not necessarily affect the function of the protein. In many cases, polymorphisms do not alter the amino acid sequence, although they sometimes alter the expression of the gene. Therefore, polymorphisms in *p53* cannot be identified by the functional yeast assay because they do not affect protein function (although they can still result in a predisposition for certain cancers). To date, several polymorphisms in *p53* have been described, two of which have been implicated in cancer susceptibility and are described below.

Codon 72 (Pro) Polymorphism

The wild-type form of the *p53* protein occurs in two variants at codon 72, arginine

(Arg) and proline (Pro), which appear to be functionally equivalent. An initial study investigating smoking-associated lung cancer in Japan identified a possible association between the homozygous Pro/Pro polymorphism at codon 72 of the *p53* gene and cancer risk (81). Additional studies found a similarly increased frequency of the Pro/Pro polymorphism at codon 72 in smoking-related cancers, although the statistical significance of these results was borderline (82,83). The importance of polymorphisms in codon 72 as a marker for cancer susceptibility remains to be determined.

Polymorphism in Intron 3

A recent study with a Swedish population of lung cancer patients determined that the Pro/Pro polymorphism at codon 72 itself showed no association with cancer; in combination with the lack of a 16 bp duplication in intron 3 of the *p53* gene, however, the Pro/Pro polymorphism was associated with risk for lung cancer (84). The same disequilibrium between the Pro/Pro polymorphism at codon 72 and the lack of the 16 bp duplication has been associated with increased risk for colorectal cancer (85). Conversely, the presence of the same 16 bp duplication in intron 3 has recently been associated with sporadic ovarian cancer in a population of Caucasian Germans (86). However, an increased allele frequency for the 16 bp insertion was not detected in an American population of ovarian cancer patients, possibly as a result of geographic differences and allele frequencies (87). Because there is no consistent causal relationship between either the absence or the presence of the 16 bp insertion in intron 3 and cancer, both this polymorphism and the Pro/Pro polymorphism at codon 72 in the *p53* gene are probably linkage markers for an as-yet unidentified region that confers susceptibility to cancer (84).

The use of susceptibility markers to identify individuals with an inherited predisposition to cancer can improve the efficiency of cancer screening and cancer prevention efforts. A simple blood test has been used to identify the 16 bp insertion polymorphism in patient serum and could be applied to circumvent the traditionally difficult diagnosis of early ovarian cancer by identifying individuals at risk. If cancer-prone individuals can be identified by the use of susceptibility markers, they can be assisted in minimizing environmental exposures (i.e., tobacco smoke) that put them at increased risk.

p53 in Genetic Toxicology

Cancerogenesis is thought to be a multistage process involving initiation, promotion, and progression. Understanding how carcinogens function requires the elucidation of the roles they play in each of these stages of cancer development. Because *p53* is a central molecule in cell cycle regulation and growth control, its interaction with various carcinogens can shed light on the ways in which they contribute to the cancerous state.

The development of transgenic animals possessing null alleles of *p53* provides an *in vivo* model for analysis of carcinogen function. Although animals homozygous for null alleles of *p53* have been shown to be developmentally normal, they have shown an increased susceptibility to spontaneous tumors, thus making them inappropriate for toxicological studies (88). However, animals heterozygous for null alleles of *p53* have a reduced rate of spontaneous tumors relative to homozygotes; these animals have proven to be useful tools for studying the carcinogenic process and for identifying carcinogens and teratogens.

Transgenic mice possessing one copy of a null allele of *p53* have proven to be valuable for rapid and sensitive assessment of the effects of xenobiotic exposures in relation to the timing of tumorigenesis. A recent study of skin cancer in such animals found that in one instance of chemically induced tumorigenesis, *p53* was involved in malignant progression but not in initiation or promotion (89).

Heterozygous null *p53* animals can also be useful in identifying chemicals that are potential environmental carcinogens. Exposure of these animals to dimethylnitrosamine (DMN), a potent liver carcinogen, showed a higher frequency of spontaneous tumors than in exposed wild-type animals, providing a sensitive assay with which to assess the effects of carcinogens (90). Furthermore, in heterozygous null animals, the mutational spectra for specific chemicals can be evaluated for different types of cancer and compared with human data. Certain mutant forms of *p53* can be reintroduced into heterozygous null and homozygous null mice in order to study the selective predisposition of certain mutant forms of *p53* to specific tumor type (91,92). Such mice can also aid in the determination of signature mutations in *p53* in cases of radiation-induced tumors, since *p53*-deficient mice are highly susceptible to even minor doses of radiation (93,94).

Transgenic mice deficient for *p53* have also been used to screen for environmental teratogens and have been found to be a sensitive tool with which to study embryonic development. In one such study, the embryotoxicity and teratogenicity of benzo[*a*]pyrene was found to be 2- to 4-fold higher in *p53*-deficient mice than in wild-type controls (95). *In utero* death was 2.6- and 3.6-fold higher, respectively, in heterozygous and homozygous *p53*-deficient embryos.

However, neither standardized lifetime bioassays in wild-type rodents exposed to daily, near-toxic doses, nor short-term tests to assess the mutagenicity of chemicals in wild-type animals, have resulted in *p53* mutational spectra comparable to that seen in human cancers. Thus, *p53* seems not to play the same central role in animal tumorigenesis as it does in human tumorigenesis (96). These newly developed *p53*-deficient mice will aid genetic toxicology in identifying natural and synthetic compounds that pose potential risk to human health (97). In controlled animal experiments, the effect of a certain chemical on *p53* can be evaluated and compared with results from exposed human populations.

Methods for Identifying Mutations in p53

Because specific types or patterns of mutation in the *p53* gene can serve as indicators or predictors of cancers resulting from exposure, the detection of such mutations is important not only for establishing a cause-effect relationship between exposure and disease, but also for screening of exposed populations prior to the onset of cancer. Therefore, accurate, timely, and cost-efficient methods of identifying such mutations are needed. Three general diagnostic approaches to the detection of *p53* mutations can currently be used to screen for mutations in *p53*.

The first and most direct approach is to evaluate the *p53* gene sequence from tumor tissue and analyze it for mutations. However, since this is often a time-consuming and costly procedure, especially when conducted on a widespread basis, other preliminary screening approaches are often used to detect cases in which *p53* mutation is likely. The most common of these is immunohistochemistry, a method that relies on the increased half-life of many mutant *p53* proteins. Because of their longer half-life, mutant *p53* proteins can be detected in cells, whereas wild-type *p53* eludes detection because of its short half-life (98).

Recent studies also indicate that antibodies to *p53* are present in the serum of individuals with *p53* mutations. This can be applied as a screening method; studies of both breast and lung cancer have shown elevated levels of anti-*p53* antibodies in the serum of individuals with mutations in *p53* (99,100).

Analysis of the *p53* protein is not always a good indicator of mutations in the *p53* gene, since the protein can be inactivated, for example by interactions with viral proteins. Furthermore, analysis of the protein by immunohistochemistry cannot determine the type and location of mutations in *p53*, which is essential information when the *p53* gene is used as a biomarker of exposure or dose. Therefore, even when analysis of the *p53* protein suggests that a mutation may be present, it is still necessary to screen for mutations at the gene level.

There are several ways that the *p53* gene can be analyzed at the genetic level and screened for mutations in its sequence. If the site of the mutation is not known, prescreening with denaturing gradient gel electrophoresis or single-strand conformation polymorphism can be used (101,102). Either of these two methods will allow rapid screening of mutations without labor-intensive sequencing of the entire gene. When the location of the mutation has been narrowed to a particular exon, polymerase chain reaction in combination with restriction fragment length polymorphism can effectively identify the specific nature of individual mutations (103,104). If no restriction site is associated with the mutation in question, then direct sequencing must be used to determine the nature of the mutation.

Limitations

Population-based studies are needed to evaluate critical epidemiologic features of *p53*, such as the predictive value positive, sensitivity, and specificity. Studies presented in this review are predominantly case reports and thus the prevalence of *p53* mutations in the general population cannot be estimated. Therefore, it is difficult to estimate how predictive a certain mutation is for a specific exposure with the data available in the current literature. It would be desirable to use *p53* as a biomarker of effect in different epidemiologic study designs such as cohort and case control. Rigorous epidemiologic evaluation of such data would be required to translate and implement these findings in public health practice.

Furthermore, there are substantial ethical considerations which must be taken into account concerning the use of *p53* as a biomarker in public health. The use of such biomarkers is susceptible to considerable abuse, i.e. cancellation of insurance policies and violation of equal opportunity laws. Therefore, the practical implication of *p53* as a biomarker may be limited due to social restraints.

Conclusions

In this brief review we have restricted our discussion to *p53* research as it pertains to

environmental health. Because of the primary role *p53* plays in the life of a cell, including its involvement in apoptosis, DNA repair, and cell proliferation and division, a vast body of knowledge exists regarding its function (6). This knowledge can be applied to the development of effective environmental health tools for using *p53* as a biomarker of effect. Several examples of mutational spectra associated with specific exposures and doses have already been elucidated; further research is likely to extend the range of environmental exposures that can be associated with disease through the use of *p53*.

In particular, *p53* shows promise for the assessment of cancer susceptibility as well as for *in vivo* identification of carcinogens. More work is needed to identify specific markers for susceptibility, and the development of animals transgenic for *p53* is likely to facilitate an understanding of how carcinogens act on *p53* and lead to cancer. The use of biomarkers, particularly genes such as *p53*, is a rapidly expanding field in environmental health and has vast potential for increasing our knowledge and awareness of the impact of the environment on human health.

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