

p53 REVIEW ARTICLE

TP53 Mutations in Workers Exposed to Occupational Carcinogens

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In some cases, evidence exists that exogenous carcinogenic exposures contribute to the mutation spectrum of the TP53 gene (p53) in human cancers. Although the clearest examples come from dietary and environmental sources, only a restricted number of papers have concentrated specifically on TP53 mutations in tumors from workers exposed to occupational carcinogens. In populations exposed to dietary aflatoxin B1 with liver cancer (AFB1) and ultraviolet (UV)-radiation with skin cancer, a single specific-looking TP53 mutation has been described in some of the tumors. Whether these fingerprints in the TP53 gene can be used to reveal an occupational etiology remains to be shown. In other cases, although differences in the TP53 mutation spectrum exist, they are more diffuse and difficult to interpret at this point. For instance, cigarette smoking seems to induce long-lasting molecular footprints in TP53. However, their use to rule out other occupational exposures as etiological factors in occupational cancers is still very questionable, especially due to the putative synergistic effects of cigarette smoke with other carcinogens. Although interesting implications of possible typical mutation spectra among cancers with other occupational etiologies exist, the data are scanty and await further development of TP53 mutation databases. *Hum Mutat* 21:240–251, 2003. © 2003 Wiley-Liss, Inc.

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DATABASES:

TP53 – OMIM: 191170; GenBank: NM_000546 (mRNA)

<http://p53.curie.fr/> (p53 Web Site at Institut Curie)

www.iarc.fr/p53 (IARC p53 Mutation Database)

INTRODUCTION

Shortly after our paper on p53 (TP53; MIM# 191170) gene mutations in uranium miners was published [Vähäkangas et al., 1992], there was an inquiry as to whether we could use the TP53 mutations as evidence in a court case for occupational exposure causing larynx cancer in a miner. At that point the hypothesis of TP53 mutations as an indicator of exposure was new [Hollstein et al., 1991; Harris, 1991], and it was self-evident that no firm scientific conclusions, let alone use as evidence in court, could be drawn. We had to disappoint the attorney, who, optimistically however, after a long correspondence of the pros and cons of mutation analysis of TP53, asked whether he could now send the tumor sample for me to analyze ... end of correspondence! How far have we come from those days until now – or is it still even a viable hypothesis any more to try to identify exposure by the TP53 mutation spectrum?

TP53 GENE AND P53 PROTEIN

The status of TP53 mutations as among the most important genetic changes in human tumors has definitely not changed. This is illustrated both by the increasing number of publications on p53 and the excellent reviews published at short intervals [for recent general reviews on p53 see Vogelstein et al., 2000; Colman et al., 2000; Soussi, 2000a; Fisher, 2001; Bargonetti and Manfredi, 2002]. To consider TP53 mutations in cancers from workers exposed to occupational carcinogens is relevant for two reasons: First, chemical carcinogens at large are genotoxic agents and p53 protein is one of the protective

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molecules in the cells against genotoxic damage. Second, the TP53 mutation spectrum (location and type of mutations) differs in different cancer types associated with different exposures. When talking about a TP53 mutation spectrum, it is necessary to realize that we operate at the population level: one tumor contains usually only one TP53 mutation, if any [Vähäkangas, 2002].

The 393 amino-acid-containing p53 protein of humans is a signal integrator from cellular stress with a special role in the protection against genomic damage [Lane, 1992]. As a transcription factor, p53 increases the expression of genes that can block the cell cycle to give time for repair or trigger apoptosis to destroy the damaged cell [Bennett et al., 1999]. In addition, p53 protein binds to DNA repair proteins and has also a more direct role in different forms of DNA repair [for an extensive review, see Bernstein et al., 2002]. It is involved in homologous recombination repair, nucleotide excision repair, as well as base excision repair.

The TP53 gene in chromosome 17p13.1 is different from other tumor suppressor genes in that the mutations found in TP53 are mainly missense base changes, while those in other tumor suppressor genes are mainly deletions [Bennett et al., 1999]. Mutations in TP53 may have different consequences depending on the location and type of mutation. Mutations in the central region of the gene coding for the specific DNA binding region may destroy the conformation and inhibit DNA binding [Hainaut and Vähäkangas, 1997]. Hot spots for mutations (sites of frequent mutations), indeed, seem to occur at codons encoding amino acids in direct contact with DNA. Other critical sites for conformation are also targeted in human tumors, such as codons responsible for zinc-binding amino acids. Thus, one determinant in the type of the mutations found in tumors is the selection

for growth advantage they give the cell while destroying the normal function of p53 protein.

Another determinant seems to be the etiology of the tumor. Tumors in different organs have different TP53 mutation spectra [Hollstein et al., 1991; Greenblatt et al., 1994]. In some cases, evidence now exists that exogenous carcinogenic exposures contribute to this difference [Greenblatt et al., 1994; Hainaut and Vähäkangas, 1997; Bennett et al., 1999; Soussi, 2000a; Hainaut and Pfeifer, 2001]. Although the clearest examples come from dietary and environmental sources, same data exist about carcinogens that also occur as occupational exposures (Table 1). To date, only a restricted number of papers have specifically concentrated on TP53 mutations in tumors from workers exposed to occupational carcinogens.

**TP53 MUTATIONS AND CARCINOGENS WITH MAIN EXPOSURE FROM THE ENVIRONMENT
Aflatoxin B1**

Aflatoxin B1 (AFB1) is typically a contaminant of food, such as grain and peanuts, stored in poor conditions. Occupational exposure to AFB1 could occur to some extent in the food industry. A specific TP53 mutation has been described in liver cancer from hepatitis B virus positive patients living in certain areas of China and Africa [Hsu et al., 1991; Bressac et al., 1991; Scorsone et al., 1992]. In human liver this mutation in TP53 codon 249 in exon 7 (AGG to AGT, arg to ser) is clearly associated with aflatoxin B1 exposure [Ozturk, 1991; Aguilar et al., 1994; Teramoto et al., 1994; Kirby et al., 1996]. Some experimental studies support the targeting of the last nucleotide of p53 codon 249 by AFB1 [Puisieux et al., 1991; Aguilar et al., 1993] while others contradict this

TABLE 1. Association of Environmental and Occupational Chemicals With Possibly Specific p53 Mutations

Carcinogen	Cancer tissue	TP53 mutations	Reference
Aflatoxin B1	Liver	Codon 249 AGG to AGT	Ozturk [1991]
Cigarette smoke, benzo(a)pyrene and other PAH- compounds	Lung	G:C to T:A in codons 157, 158, 247-249 and 273	Chiba et al. [1990]; Bennett et al. [1999]; Hainaut & Pfeifer [2001]; Vähäkangas et al. [2001]
Cigarette smoke and alcohol	Head and neck	Increased incidence of p53 mutations	Somers et al. [1992]
UV-radiation	Esophagus, head and neck	Codons 205, 245, 248	Brennan et al. [1995]
	Skin	G:C to T:A CC to TT	Brash et al. [1991]; Dumaz et al. [1994]; Daya-Grosjean et al. [1995]
Radon	Lung	Codons 146-151, 195-209, deletions	Vähäkangas et al. [1992]
Vinyl chloride	Liver	A:T to T:A	Hollstein et al. [1994]; Marion [1998]
Mustard gas	Lung	Double G:C to A:T	Takeshima et al. [1994]
Chromate exposure, nickel exposure, metal industry	Lung	G:C to T:A on non-coding strand	Harty et al. [1996]
Petrochemical industry	Lung	G:C to A:T at non-CpG sites	Harty et al. [1996]

notion [Fujimoto et al., 1992; Dennissenko et al., 1998].

Cigarette Smoke

Smoking is the most important risk factor for lung cancer, but passive smoking increases the risk as well [IARC, 1986; Hackshaw et al., 1997]. For example, restaurant employees and office workers can be occupationally exposed to cigarette smoke. Both qualitative and quantitative data link TP53 G to T mutations at CpG sites in lung cancer with smoking [Bennett et al., 1999; Hainaut and Pfeifer, 2001; Pfeifer et al., 2002]. The few studies on lung cancer in non-smokers published so far can be interpreted as supporting the hypothesis that smoking induces TP53 mutations: The incidence of TP53 mutations is 10–26% in non-smokers and about 60% in smokers with overrepresentation of G to T mutations in smokers (30% vs. 12%).

Similar effects have been noted by former or passive smoking on TP53 mutations. Husgafvel-Pursiainen and co-workers [Husgafvel-Pursiainen et al., 2000] showed that lung cancers of passive smokers have an increased risk for p53 mutations. We found that the few cases of former smokers had a significantly higher frequency of TP53 mutations than the ones with no smoking history [Vähäkangas et al., 2001]. Also, the sites of mutations in non-smokers seemed to differ from the typical smoking-related cases. Thus, the existing molecular epidemiology studies on different aspects of smoking-related lung cancer support the TP53 gene as a target of carcinogens in cigarette smoke and a tobacco-specific TP53 mutation spectrum at a population level. Workers exposed to second-hand smoke, provided they are genetically susceptible [see. e.g., Nair and Bartsch, 2001; Vähäkangas, 2002], are at an increased risk to develop lung cancer [Hackshaw et al., 1997] and would be expected to harbor tobacco-specific mutations in their tumors. However, so far no studies exist on TP53 mutations specifically in lung cancers putatively due to occupational exposure to passive smoking.

Polycyclic Aromatic Hydrocarbons (PAH)

PAH-compounds are the products of incomplete combustion of organic material and thus ubiquitous in the environment [IARC, 1983]. Occupational exposure to PAH-compounds increasing the risk of lung and putatively also other cancers, is highest in coke oven workers, other jobs in the steel industry, asphalt and bitumen workers, and those exposed to exhaust and working with gasoline. However, the highest exposure for those who smoke comes from smoking [IARC, 1986]. Nobody is ever exposed to a single PAH-compound, and rarely to the group of PAH-compounds only. Theoretically, it would thus be impossible to find a specific mutation spectrum in

human cancers caused by a single PAH-compound, like benzo(a)pyrene (BP). Unlikely as it seems, experimental studies have implicated that polycyclic aromatic hydrocarbons in cigarette smoke are related to the TP53 mutations in lung cancer. The best known carcinogen in cigarette smoke, BP, induces G:C-T:A transversions experimentally [Hainaut and Vähäkangas, 1997], which are the main mutation type in smoking-related lung cancer [Hainaut and Pfeifer, 2001]. Pfeifer and coworkers [Denissenko et al., 1996; Pfeifer and Denissenko, 1998; Smith et al., 2000] have shown that the codons 157, 248, and 273 in the TP53 gene that are most mutated in lung cancer are also targets for DNA-adduct formation by BP and more prone to mutations by it. Other PAH-compounds have a similar preference for adduct formation in TP53 codons 157, 158, 245, 248, and 273 [Smith et al., 2000].

Cooking is still a major part of the occupation of housewives. In China smoky coal is used for cooking and this is associated with a high incidence of lung cancer among the non-smoking Chinese women [Lan et al., 2000 and references within]. The indoor air levels of benzo(a)pyrene can reach levels comparable to the ones experienced by workers in old type coking plants [Band et al., 1990]. Experimentally, smoky coal, which contains a large percentage of polycyclic aromatic hydrocarbons [Chuang et al., 1992], is both mutagenic [Nakanishi et al., 1997] and carcinogenic [Mumford et al., 1990]. DeMarini and coworkers [DeMarini et al., 2001] found that the TP53 mutation spectrum in lung cancers from smoky coal exposed non-smoking Chinese women is in many respects similar with the smoking-associated mutation spectrum. A clear difference was a hotspot at the TP53 codon 154, which is a hotspot for PAH adducts [Denissenko et al., 1996; Smith et al., 2000], but not found in lung cancers from smokers [Hainaut and Pfeifer, 2001].

UV-Radiation

Another example of a clear finger-print mutation in the TP53 gene, in addition to the AFB1-hepatitis-related TP53 mutation, is the CC to TT double mutation in skin cancer [Brash et al., 1991; Greenblatt et al., 1994; Dumaz et al., 1994]. Such mutations are typically induced by UV-radiation in experimental systems [see Hainaut and Vähäkangas, 1997]. C to T single mutations are also clearly associated with UV-radiation. Because of such a clear association, Weihrauch and coworkers [Weihrauch et al., 2002] studied recently whether it would be possible to use TP53 mutations as an indication of UV etiology in 12 work-related basal cell carcinomas (BCC) definitively exposed to UV. As controls they had 20 BCCs from unexposed skin areas. Of the six point mutations in the workers, 4 were CC to TT double mutations and

2 C to T mutations, while in controls there were no CC to TT double mutations and no preference for C to T transitions. These studies support CC to TT double mutations in the TP53 as a specific marker of UV-radiation in the causation of BCC, although the number of work-related cases is still small. Such mutations seem not to be restricted to TP53 because Bodak and coworkers [Bodak et al., 1999] found a similar mutation spectrum in the hptc gene, the human homolog of *Drosophila* segment polarity gene patched, that has been implicated in human skin carcinoma syndrome.

Arsenic

People can be exposed to arsenic through medication, artesian well water, or occupationally in the electronic industry and inorganic arsenic compounds are lung and skin carcinogens in humans [IARC, 1980]. Arsenic has been suggested to act synergistically with UV in skin cancer induction [Gloster and Brodland, 1996]. Dimethylarsenic acid is a major metabolite of arsenic both in animals and in humans, and in rats it induces cancer, but no TP53 mutations in bladder [Wei et al., 2002]. In human cells and tissues arsenic compounds are genotoxic and induce p53 protein expression [see Yih and Lee, 2000]. Only a few studies with a small number of cases exist on TP53 mutations and arsenic exposure in humans, and none of the studies include occupational exposure. No TP53 mutations were found in 16 arsenic-related carcinomas in situ of the skin by Hsieh and coworkers [Hsieh et al., 1994]. Using PCR-SSCP, we found an indication of a TP53 mutation in 3 out of 29 lesions in 7 patients with multiple BCCs who were exposed in the past to arsenic as medication which supposedly had etiological importance in their disease [Castren et al., 1998]. They had no excessive UV exposure, and in most of their tumors p53 protein was detectable by immunohistochemistry. On the other hand, a much higher frequency of TP53 mutations has been described in various skin (up to 55%) [Hsu et al., 1999] and bladder (62%) [Shibata et al., 1994] cancers from Taiwan, from the endemic area for arsenic-related blackfoot disease. None of the skin mutations found were typical UV-induced CC to TT double mutations and only one was a C to T transition.

TP53 MUTATIONS AND CARCINOGENS IN OCCUPATIONAL SETTING

Gasoline

Gasoline and diesel fuel contain benzene among other hydrocarbons including PAH-compounds. The exposure to gasoline is usually expressed as cumulative exposure in benzene equivalents. Roth and coworkers [Roth et al., 1997] found a low level (13%) of TP53

mutations in renal cell cancers from 23 gasoline-exposed workers and accumulation of the mutations in smokers among all the 53 studied cases including controls not exposed to gasoline. This suggests that in renal cell cancer TP53 mutations occur due to smoking, which is a known risk factor for this cancer type.

Radon

Radon is a decay product of uranium and emits α -radiation which is carcinogenic in animals although experimental data of radon in animals is partly discrepant: Risk of lung cancer increases significantly in rats and dogs but hamsters are more resistant [IARC, 1988]. Because radon originates from the foundation rock, miners are exposed to high levels of radon. From the rock radon is liberated to the air where it is diluted, except in tightly built houses where the base is in contact with the rock. Epidemiology in humans shows an increased risk of lung and laryngeal cancer in uranium miners [Jostes, 1996], and environmental radon may have a similar effect [Lubin and Boice, 1997]. Interestingly, experimental studies in vitro and in vivo suggest chemicals as controlling factors in radiation carcinogenesis [IARC, 1988; Cross, 1994]. However, so far epidemiological studies are inconsistent on the importance of such a synergism in humans [e.g., Pershagen et al., 1994; Alavanja et al., 1994; Auvinen et al., 1996].

Biological effects of α -radiation include different types of DNA and chromosome damage leading to mutations, micronuclei formation, chromosomal aberrations, chromosomal instability, and cell transformation [Jostes, 1996; Brooks et al., 1997; Little, 1998]. Molecular data suggests harmful effects of very low levels of α -radiation: traversal of a single α -particle through a cell is only slightly cytotoxic (about 80% of cells survive), but it is highly mutagenic to cells [Hei et al., 1997] and α -radiation effects neighbouring cells not in direct contact with α -particles [Lehnert and Goodwin, 1997a, 1997b and references within]. Among other things, this leads to the induction of p53 protein in neighboring cells [Hickman et al., 1994].

The first paper on TP53 mutations in radon-associated lung cancer in miners included only 19 cases (Table 2) [Vähäkangas et al., 1992]. The mutation spectrum seemed to differ from the typical one seen in smokers. Seven mutations were found, of which none were GC to TA transversions on the coding strand (the most common mutation in lung cancers from smokers) and none were located on the typical hot spot codons of lung cancer. However, the next paper published on uranium miners [Taylor et al., 1994] reported the intriguing finding of a TP53 hotspot in codon 249; 16 of the 52 tumors contained the same AGG-ATG transversion leading to an

TABLE 2. TP53 Mutations in Populations Exposed to Radon

Population	Exposure (mean WLM)	Number of cases	Number (%) of TP53 mutations	Codon 249 AGG-ATG	Reference
<i>Uranium miners</i>					
New Mexico, USA	111	19	7 mutations in 5 cases (26%)	0	Vähäkangas et al. [1992]
Colorado, USA	1382	52	30 mutations in 29 miners (56%)	16 (31%)	Taylor et al. [1994]
Germany	1011	50	NS	0	Bartsch et al. [1995]; Hollstein et al. [1997]
Colorado, USA	1270	23	NS	0	McDonald et al. [1995]
Germany	NK	29	NS	2 (7%)	Yang et al. [2000]
<i>Environmental exposure</i>					
England	10			0	Lo et al. [1995]
Sweden	2	139	29 (20.9%)	0	Yngveson et al. [1999]
	0.5	90	23 (25.6%)		
	0.25	14	6 (42.9%)		
Missouri, USA	< 0.46 in 83%	Non 120	22 (18%)	0	Vähäkangas et al. [2001]
Women		Ex 11	6 (54%)	0	

WLM, working level months (1WLM corresponds to 170 hours in a place where air contains 130 000 MeV of α -radiation in 1 liter of air; 1Bq/m³ is equivalent to 0.005 WLM); NS, not studied; NK, not known; Non, non-smokers; Ex, ex-smokers.

amino acid change (arg to met). The majority of the mutations (12) were found in squamous cell carcinomas (SCC) and a few in large cell carcinomas. The found mutation was different from the aflatoxin- and hepatitis B-associated mutation in liver cancers (AGG-AGT, arg to ser) [Hsu et al., 1991; Bressac et al., 1991]. Since the study by Taylor and coworkers [Taylor et al., 1994] included no adenocarcinomas, 23 such lung cancers from the same population of miners were later analyzed for the specific AGG to ATG mutation in TP53 codon 249. However, no such mutations were found in this sample set implicating tumor histology as one determinant for the specific mutation [McDonald et al., 1995]. In following studies in lung cancers among uranium miners from Germany [Bartsch et al., 1995; Hollstein et al., 1997; Yang et al., 2000] the specific mutation was described in 2 out of 79 lung cancers, both of them in SCC. This was, however, not significantly different from the frequency of codon 249 mutations in lung cancer in the IARC-based p53 mutation database [Yang et al., 2000]. Mycotoxins in the mines have been offered as a possible explanation for the discrepancy between the findings by Taylor and other studies [Venitt and Biggs, 1994].

Among those exposed environmentally to radon (Table 2) [Lo et al., 1995; Yngveson et al., 1999; Vähäkangas et al., 2001] no codon 249 AGG-ATG mutations have been found in lung cancers. Experimental exposure to α -particles of normal human bronchial epithelial cells induced also a different mutation (AGG-AAG transitions) in codon 249 in addition to CCC-ACC transversions in codon 250 [Hussain et al., 1997]. It can be thus concluded that although the radon-induced mutation spectrum in lung cancer may be different from the mutation

spectrum in cigarette smoke-induced lung cancer from people not exposed to radon, there is probably no single mutation typical of radon exposure.

Vinyl Chloride

Vinyl chloride is used extensively in the plastics industry as a refrigerant and as an intermediate in organic synthesis, as in the manufacture of vinyl chloride polymers [see e.g., Weihrauch et al., 2000]. It is a human carcinogen and is associated with angiosarcoma of the liver (ASL) and probably also hepatocellular carcinoma (HCC) [see Weihrauch et al., 2000]. Exposure to vinyl chloride causes a 45-fold excess risk to ASL with 197 cases listed worldwide by 1998 [Kielhorn et al., 2000]. TP53 mutations are common in these tumors and a typical fingerprint, AT to TA transversion, has been found in vinyl chloride-associated ASL [Hollstein et al., 1994; Marion, 1998], but not in ASL with other etiologies [Soini et al., 1995; Barbin, 2000]. This is supported by rat data where similar mutations have been found in p53 gene in vinyl chloride-induced ASL [Barbin et al., 1997] and in Ha-ras gene in vinyl chloride induced HCC [Kielhorn et al., 2000].

A dose-dependent increase of mutated p53 protein and p53 antibodies is found in the serum of workers exposed to vinyl chloride [Trivers et al., 1995; Smith et al., 1998; Marion, 1998; Luo et al., 1999]. Support for the potential importance of these parameters as markers of TP53 mutations comes from the positive general correlation between the frequency of TP53 mutations and the number of positive cases for p53 serum antibodies in different tumor types [Soussi, 2000b]. Furthermore, recent results by Wong and

coworkers [Wong et al., 2002] show modulation of the level of serum p53 protein and antibodies by polymorphisms of DNA-repair enzymes and enzymes metabolizing vinyl chloride.

There is also a small excessive risk for hepatocellular carcinoma (HCC) in vinyl chloride workers, but on the basis of a 1:1 relationship between transitions and transversions, and the fact that five mutations occurred at CpG sites, Weihrauch and coworkers [Weihrauch et al., 2000] suggested that the mutations might have appeared through an endogenous mechanism. In vinyl chloride-associated HCC the frequency of p53 mutations (11/18, 61%) [Weihrauch et al., 2000] does not differ from that in AFB1-associated HCC (>50%) [Hsu et al., 1991; Bressac et al., 1991; Scorsone et al., 1992], while the frequency is much less in HCC without these exposures [Ng et al., 1994].

Asbestos

Occupations with exposure to asbestos, like working in construction or shipyards, pose a documented increased risk to mesothelioma and lung cancer with a synergistic effect with smoking [see Husgafvel-Pursiainen et al., 1999]. Probably depending on the reactivity of the used antibody [see e.g., Turpeinen et al., 2002] a very high (70% [Kafiri et al., 1992], 67% [Segers et al., 1995], 77% [Kumar-Singh et al., 1997]), or lower (25% [Ramael et al., 1992]) proportion of malignant mesotheliomas have been immunohistochemically positive for p53. However, it seems that asbestos does not target the TP53 gene in malignant mesothelioma. In 20 mesothelioma cell lines from 17 patients Metcalf and coworkers [Metcalf et al., 1992] found two point mutations and one cell line was null while the two mutated plus an additional 12 cell lines were positive for p53 in immunohistochemistry. In six separate studies including altogether 63 mesothelioma cases, only two (3%) TP53 mutations were found [Mor et al., 1997; Husgafvel-Pursiainen et al., 1997; Kitamura et al., 1998; Mayal et al., 1999; Ni et al., 2000; Kitamura et al., 2002]. Asbestosis exposure of one patient with a TP53 mutation was uncertain [Mayal et al., 1999] and in the second case the person was also a heavy smoker [Kitamura et al., 2002]. The largest material contained 17 malignant mesotheliomas and TP53 exons 5–8 were sequenced for mutations [Ni et al., 2000]. Thus, it is possible that mutations in other locations of TP53 may have skipped detection. On the other hand, other p53-related mechanisms, like viral infection, may well play a role here as in some other tumors. One such candidate appears to be Simian virus 40 (SV40), which has been detected in several human mesotheliomas. Carbone and coworkers [Carbone et al., 2002] suggest asbestos and SV40 as co-carcinogens in malignant mesothelioma, because

SV40 large tumor antigen binds p53 protein, and SV40 transforms mesothelial cells readily in vitro, an effect increased by asbestos.

A fair percentage of asbestos-related lung cancer tissues are p53 immunopositive (20/30 or 67% [Nuorva et al., 1994] 4/10 or 40%, [Husgafvel-Pursiainen et al., 1997]). We also found a positive association between the asbestos exposure and asbestos content of lung tissue with p53 immunostaining [Nuorva et al., 1994]. Again, there is a discrepancy between the immunohistochemistry and mutation analysis results implicating that if p53 is involved, the aberration is more probably at the protein than gene level. Husgafvel-Pursiainen and coworkers [Husgafvel-Pursiainen et al., 1999] found a TP53 mutation in 39% (13/33) of asbestos-exposed patients with lung cancer while the percentage was 54% in patients not exposed to asbestos. The facts that the prevalence of TP53 mutations decreased with increasing asbestos fiber content and that the mutations were less prevalent in adenocarcinoma associated with asbestos exposure than in other histological types speak against asbestos targeting the TP53 gene in lung tissue.

Chromate

Occupational chromate exposure increases the risk of lung cancer and chromium is a well-known genotoxic carcinogen associated with frequent microsatellite instability in lung cancers of exposed workers [Hirose et al., 2002]. Among 20 lung cancers from 19 chromate workers, Kondo and coworkers [Kondo et al., 1997] found six TP53 gene mutations in four workers (21%), a frequency much lower than in smokers. Although chromate thus seemed not to specifically target the TP53 gene, the mutations were not typical cigarette smoke-associated mutations. In addition to the fact that two tumors had double missense mutations, not common in lung cancers, three of the mutations were at AT base-pairs and there were no G to T transversions. Also, three of the mutations occurred in two non-smoking workers. Hanaoka and coworkers [Hanaoka et al., 1997] found that 19% of chromium workers with a long work history had higher levels of p53 serum antibodies than controls.

Mustard Gas

Increased risk to lung cancer is associated with exposure to mustard gas during production of mustard gas and among army veterans who were exposed in battle fields during the World War I [see Takeshima et al., 1994]. Among a small population of 12 mustard gas workers, Takeshima and coworkers [Takeshima et al., 1994] found eight point mutations with two G:C to A:T double mutations while 12 non-exposed lung cancers did not contain double mutations.

Aromatic Amines

These carcinogens pose an increased risk to bladder cancer in the dye/ink industry [Sorlie et al., 1998]. Another important known risk factor for bladder cancer is smoking. The frequency of TP53 mutations in occupational bladder cancer is not higher than in bladder cancers from people not occupationally exposed (Table 3). Yasunaga and coworkers [Yasunaga et al., 1997] described a peculiar TP53 mutation spectrum in bladder tumors from exposed workers with predominantly C to T transitions and a hot spot at codons 151–152, features not found in tumors from people not exposed to aromatic amines. However, in other studies the type (majority G:C to A:T) or distribution of TP53 mutations in occupational bladder cancers has not differed from other bladder cancers suggesting that the etiology is common in both groups or that TP53 mutation is a late event and not related to etiology [Esteve et al., 1995; Taylor et al., 1996; Sorlie et al., 1998]. Increased odds ratios for p53 nuclear over-expression were found by Zhang and coworkers [Zhang et al., 1994] in bladder cancers from workers in dye- and cooking-associated occupations, but the association with cigarette smoking was much stronger.

P53 PROTEIN EXPRESSION IN CELLS AND OCCUPATION

Numerous studies have been published on immunohistochemistry of p53 protein in human tumors. In antibody-based methods the specificity of the antibody and the details of the method itself determine the result [Bonsing et al., 1997; Nickels et al., 1997; Turpeinen et al., 2002]. Furthermore, the status of the protein detected needs consideration. In the case of p53 protein, a lot of misunderstanding in the literature of the meaning of positive immunohistochemistry has

resulted from too simplistic an interpretation of the result [Hainaut and Vähäkangas, 1997]. It is true that normal protein is generally invisible in immunohistochemistry due to the very short half-life (min to tens of min) and that the half-life of p53 protein is increased in many cases due to a mutation [Kastan et al., 1991; Ogretmen and Safa, 1997].

However, discrepancy between immunohistochemical positivity and mutation analysis is well documented [e.g., Mineta et al., 1998; Kropveld et al., 1999; Castren et al., 1998; see also Hainaut and Vähäkangas, 1997] and other well-known mechanisms for the increased staining of p53 are known in addition to the mutation. Cellular and viral proteins may bind to p53 and effect its half-life [Mantovani and Banks, 2001; Wang et al., 2002]. Furthermore, p53 protein is inducible by DNA-damaging carcinogens as has been shown by benzo(a)pyrene in animal tissues [Bjelogrlic et al., 1994; Serpi et al., 1999] and human cell lines [Rämet et al., 1995], by acrylonitrile in human lung fibroblasts [Rössner et al., 2002], and by UV in normal human skin [Davenport et al., 1999; Ponten et al., 2001]. Thus, theoretically, a positive result in p53 immunohistochemistry in an exposed worker may be a response to DNA-damage as well as an indication of a TP53 gene mutation. However, such effects seem to be dependent on the cell type as well as on the chemical. For instance styrene oxide, known to damage DNA and to cause neurobehavioral changes in workers occupationally exposed to styrene oxide vapors, up-regulated p53 mRNA in human lymphocytes [Laffon et al., 2001], but did not induce p53 in human neuroblastoma SK-N-MC cell line [Dare et al., 2002].

Occupational exposure to organochlorines has been suggested as an etiological factor in pancreatic cancer and p53 aberrations are frequently found. Slebos and coworkers [Slebos et al., 2000] found no difference, however, in p53 immunohistochemistry between the

TABLE 3. TP53 Mutation Frequency in Exposed Workers

Occupational exposure	Cancer		Cancers with TP53 mutations	Smokers among mutated cases	Reference
Gasoline	Renal cell ca	Exp.	3/23 (13%)	6/7	Roth et al. [1997]
		Non-exp	4/30 (13%)		
Aromatic amines	Bladder	Exp.	34 (47%)	Most of the cases whose smoking status was known	Taylor et al. [1996]
		Non-exp.	30 (54%)		
Aromatic amines	Bladder	Exp.	8/26 (31%)	Most of the cases whose smoking status was known	Yasunaga et al. [1997]
		Non-exp	12/33 (36%)		
Asbestos	Lung ca	Exp.	13/33 (39%)	Most cases	Husgafvel-Pursiainen et al. [1999]
		Non-exp	29/54 (54%)		
Mustard gas	Lung ca	Exp.	6/12 ^a (50%)	Most cases	Takeshima et al. [1994]
		Non-exp	6/12 (50%)		
Chromate UV-radiation	Lung ca BCC	Exp.	4/19 (21%)	2/4	Kondo et al. [1997]
		Exp.	7/12 (58%)		
		Non-exp	11/20 (55%)		

^aA double mutation in two cases.

BCC, basal cell carcinoma of the skin; NR, not relevant (cigarette smoking is not a known risk factor for skin cancer).

tumors from exposed and unexposed workers. Whether the frequency of TP53 gene mutations differs between those exposed to organochlorines and those unexposed remains to be studied.

CONCLUSIONS

It seems that, as to radon and most other occupational carcinogens, we still have to disappoint those who seek for a specific molecular marker of occupational etiology in the TP53 mutation spectrum. Although in some cases a mutation spectrum specific for an environmental exposure has been suggested (Table 1), for now such implications can be used to create hypothesis, but generally not as a proof of a specific mutation spectrum for an exposure. Only AFB1 in connection with hepatitis B infection, and UV seem to induce what looks like a specific TP53 mutation, but even in these cases such a mutation does not occur in all tumors.

TP53 mutation frequency is increased in smoking-associated lung cancers, and in AFB1- and vinyl chloride-exposed HCCs, compared to tumors from non-exposed patients with similar tumors. Many other carcinogens, however, do not show the same effect (Table 3). There is good evidence by now that cigarette smoke also induces a mutation spectrum with typical features. In occupational lung cancers, chromate-associated lung cancer being a possible exception, smoking seems to be a significant determinant of the TP53 mutation frequency and type, speaking for TP53 gene as a specific target of tobacco carcinogens, especially benzo(a)pyrene. The effect may be strong enough to overcome the effect of other carcinogens. This means that workers in any occupation when they are smokers or exposed to tobacco smoke will be prone to TP53 mutations and at risk to cancer initiation through the inactivation of p53. However, current TP53 mutation data does not provide a basis for using TP53 mutation(s) in individual cases as a proof of smoking etiology.

There are not sufficient numbers of cancer patients with information of occupational exposure for final conclusions, and existing studies utilize different methods and approaches which may not be totally comparable with each other. Further studies with the developing TP53 databases (www.iarc.fr/p53 [see Hainaut and Hollstein, 2000; Olivier et al., 2002] and <http://p53.curie.fr/> [see Soussi et al., 2000; Beroud and Soussi, 2003]) and data managing systems [Soussi et al., 2000] will hopefully help to overcome these difficulties in the future.

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