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p53 REVIEW ARTICLE

The *TP53* Gene, Tobacco Exposure, and Lung Cancer

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Of the various genetic alterations in lung cancer, the abnormalities of the *TP53* gene (*p53*) are among the most frequent and important events. Because of its importance, many aspects of *TP53* have been studied, including preneoplastic lesions and *TP53* as a marker for early detection and prognosis and as a therapeutic option. We summarize recent knowledge of *TP53* in lung cancer with a special emphasis on the relationship between smoking exposure (e.g, cigarette, etc.) and specific mutational pattern of *TP53* by analyzing the latest version of the International Agency for Research on Cancer (IARC) database on *TP53* mutations in human cancer. Our analysis confirmed several other studies showing significant differences in the frequencies of G:C to T:A transversions between ever-smokers and never-smokers. Furthermore, when comparing the mutational spectrum by gender, important differences were noted between male and female never-smokers. We concluded that the previously noted G:C to T:A transversions were mainly due to female smokers having a high frequency of these changes compared to female never-smokers. There was no relationship between adenocarcinomas and squamous cell carcinomas independent of gender. We also examined the seven codons which have been previously identified as hot spots, that is, the sites of frequent G:C to T:A transversions in smoking-related lung cancers. However, there was no specific codon which was strongly related to smoke exposure despite a moderate relationship. We considered the term "warmspot" may be more appropriate. While mutations of *TP53* are frequent in lung cancers, further investigation is necessary to understand their role for lung carcinogenesis, especially as they relate to gender differences, and to translate our laboratory knowledge to clinical applications. Hum Mutat 21:229–239, 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

Lung cancer is the leading cause of cancer deaths in the world with over one million cases diagnosed every year [Parkin et al., 2001], and the vast majority of cases are smoking related. Abnormality of the *TP53* gene is one of the most significant events in lung cancers playing an important role in tumorigenesis of lung epithelial cells. Human lung cancers are classified into two major types, small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC), the latter consisting of several types [Travis et al., 1995]. Adenocarcinoma is the most common of the NSCLC subtypes, and its frequency is rising in the United States and in other parts of the world [Travis et al., 1995]. Adenocarcinoma is the most frequent type of lung cancer in women and never-smokers.

Mutations of the *TP53* tumor suppressor gene (MIM# 191170) occur in about 50% of NSCLC and more than 70% of SCLC [Bodner et al., 1992; Mao, 2001; Takahashi et al., 1989]. *TP53* mutations are the most extensively studied mutations in lung cancer

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and, as discussed below, a database of all published mutations is maintained mainly by the International Agency for Research on Cancer (IARC), Lyon, France (www.iarc.fr/p53) [Olivier et al., 2002]. Another database is curated by Dr. Therry Soussi, Curie Institute, Paris, France (http://p53.curie.fr/) [Caron de Fromentel and Soussi, 1992; Soussi and Beroud, 2001; Beroud and Soussi, 2003]. These databases are valuable resources to study the role of the gene in lung tumorigenesis. Previous reports demonstrated that the *TP53* mutational spectra of lung cancer showed some specific hotspots that are rarely observed in other types of human tumors, suggesting different carcinogen-specific mutations. While exposure to tobacco smoke is the best known and studied lung-cancer mutagen [Hecht, 2002], there are other carcinogens for lung cancers derived from occupational and environmental factors [Vahakangas, 2003]. Because of its importance in lung tumorigenesis, there are many studies which are related to *TP53* alteration in lung cancers. In this review we discuss important aspects of the multifactorial relationship between *TP53* and lung cancer pathogenesis. We have relied heavily on the IARC database for some of the critical information published herein. However, during our analysis of the database, we noted important new information of the influence of gender on the spectrum of *TP53* mutations in lung cancer and the influence of smoke exposure.

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (IARC) *TP53* DATABASE

In 1991, a database of published *TP53* mutations was established by Dr. Curt Harris and his collaborators in order to facilitate the retrieval and analysis of *TP53* mutations [Hollstein et al., 1997]. Since 1994 this database has been maintained at the International Agency for Research on Cancer (IARC) [Olivier et al., 2002] and is made freely available as a service to the scientific community (www.iarc.fr/p53). The current release is version R6 (January, 2002). It contains 16,285 somatic mutations and 213 germline mutations. In the R6 version, 1,860 cases of lung tumors are registered (we excluded six mesotheliomas).

Smoke Exposure and *TP53* Mutations

Tobacco smoking is the most important risk factor for the development of lung cancer. Several investigators have noted that the *TP53* mutational spectrum of lung cancers was different from those of other cancers. In particular, an excess of G:C to T:A transversions was characteristic of lung cancers related to smoke exposure [Hainaut et al., 2001; Hainaut and Pfeifer, 2001; Harris, 1996; Hussain et al., 2001b; Vahakangas et al., 2001]. Mutations frequently occur at bases known to be the sites of formation of adducts of polycyclic aromatic hydrocarbons, especially benzo[a]pyrene, in the coding sequence of *TP53* [Hainaut et al., 2001; Smith et al., 2000]. Three prominent hotspots at codons 157, 248, and 273, which are the strongest binding sites for these adducts, account for a disproportionate number of G:C to T:A mutations in lung cancers arising in smokers. Other hotspots include codons 158, 175, 245, and 249 [Hainaut and Pfeifer, 2001; Vahakangas et al., 2001]. Thus, tobacco exposure is associated with both a higher frequency of *TP53* mutations as well as a specific transversion at defined codon hotspots. This *TP53* mutational spectrum was observed in cancers arising in active smoker as well as in former smokers [Vahakangas et al., 2001]. Human bronchial epithelial cell cultures offer an in vitro system to study the effects of tobacco carcinogens on inducing mutations at specific codons in *TP53* [Hussain et al., 2001a].

While these findings are regarded as gospel by many authorities, some dissenting voices have questioned this dogma. Rodin and Rodin's examination of the IARC data concluded that physiological stresses (not necessarily genotoxic) aggravated by smoking are the leading risk factor in the *TP53*-associated etiology of lung cancer [Rodin and Rodin, 2000]. Paschke analyzed the R3 version of the IARC *TP53* mutation database and concluded that there was no difference in the frequencies of G:C to T:A transversions between smokers and never-smokers and that there were no specific hotspots for smokers [Paschke, 2000]. These findings have been challenged and counter-challenged [Hainaut et al., 2001; Paschke, 2001].

Databases such as the IARC represent the interpretation of published literature from many investigators and are subject to wrong interpretation. Different techniques of analysis result in differences in the quality and reliability of the published work. In addition, some investigators may have published the same data in several publications, and the duplications may not have been clearly identified. These limitations also result in wrongful interpretations. However, if these limitations are understood, much useful information may be gleaned from these databases.

Our analysis utilized the R6 version of the IARC database, updated in January 2002. This version represents an effort by the organizers to identify and eliminate published duplicate cases, to check for errors, and to include additional information. To our knowledge, no analysis of lung cancers has been published using this updated and improved version. As have other reviews, we eliminated one study suspected of having an artificially high rate of mutations [Gao et al., 1997]. In addition, we excluded 132 cases with known occupational exposures including radon gas, coal mines, asbestos, and mustard gas. After these eliminations, we were left with a total of

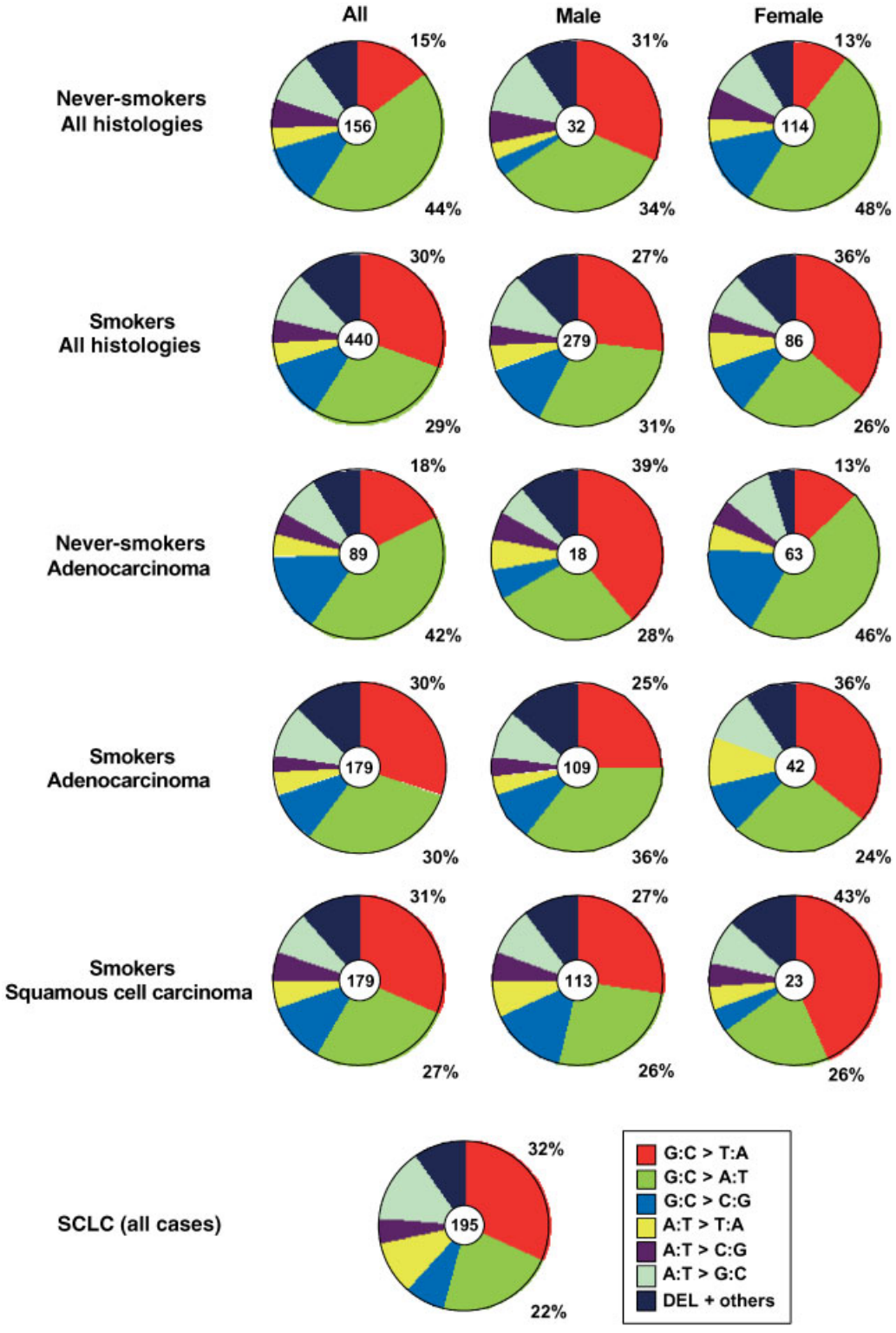

FIGURE 1. **The *TP53* mutational spectra of lung cancers. The numbers in each circle indicate the number of cancers analyzed. DEL = deletion.**

1,647 lung cancer cases for analysis. Smoke exposure data were available for 596 cases. We classified samples based on smoke exposure, gender, and histological types (Fig. 1).

Statistical Analysis

Differences of frequencies of specific mutation in two groups (smokers and never-smokers) were

compared using odds ratio and statistical significance were determined by chi-square or Fisher exact tests.

The odds ratios were obtained to estimate whether G:C to T:A transversions were related to smoking exposure. As controls, we employed G:C to A:G mutational type (control 1) and the total number of G:C to C:G, A:T to T:A, A:T to C:G, A:T to G:C, and Del+others mutational types (control 2) that are not assumed to be related to smoking exposure [Hainaut and Pfeifer, 2001; Paschke, 2000; Vahakangas et al., 2001]. One of the most important selection biases in the present study is known as "referent (control) selection bias," which is induced in case-control studies when the smoke exposure information of the control is not the same as that of the study base [Norell, 1955; Rothman and Greenland, 1998]. This referent selection bias underestimates the odds ratio if it exists. Therefore, even if we judge the cause effect by G:C to T:A transversion from the present observations, the decisions are not changed by the potential data.

Furthermore, the G:C to T:A and G:C: to A:G mutational types were combined for comparison with other mutational types (control 2). Then, the odds ratio was estimated to examine the difference on the G:C to T:A and G:C to A:G combined proportion between smoker and never-smoker.

THE SPECTRUM OF *TP53* MUTATIONS IN LUNG CANCER

Differences Between Smokers and Never-smokers

Because previous studies suggested a reciprocal relationship between G:C to T:A transversions and G:C to A:T transitions in smokers and never-smokers, respectively, we calculated the odd ratios of the combined rate of G:C to T:A and G:C to A:T for control 2. All odds ratios were around the null value, and did not show any significance (data are not shown). This indicates the combined proportion of G:C to T:A transversions and G:C to A:T transitions is invariable between smoking status.

As with several other studies, we noted that there were significant differences in the frequencies of G:C to T:A transversions between smokers (30%) and never-smokers (15%) for all lung cancer cases (Fig. 1) and the odds ratios were 3.20 (95% CI, 1.88–5.43, $P<0.0001$) for control 1 and 2.07 (95% CI, 1.22–3.51, $P=0.006$) for control 2. In never-smokers, there was a reciprocal increase in G:C to A:T transitions, while the proportions of the other mutation types were relatively unchanged (means and range; 11% and 3–17% for G:C to C:G, 4% and 3–10% for A:T to T:A, 5% and 0–6% for A:T to C:G, 10% and 6–14% for A:T to G:C, and 11% and 5–13% for DEL+others mutational types). We refer to the ratio of G:C to T:A transversions to G:C to A:T transitions as the GT:GA ratio. In smokers, the GT:GA ratio was near unity, while in never-smokers the GT:GA ratio was 0.34 (Fig. 1). In smokers, the GT:GA ratios of squamous cell and adenocarcinomas were similar and near unity (Fig. 1). Because the vast majority of cancers arising in never-smokers are adenocarcinomas, histology differences could not be analyzed in this group.

Gender-Related Differences

When we compared the mutational spectrum by gender, important differences were noted between male and female never-smokers. However, the differences between male and female smokers were much more subtle (Fig. 1). The previously noted G:C to T:A transversions were mainly due to female smokers having a high frequency (36%) compared to female never-smokers (11%) (Fig. 1) and the odds ratios were 6.77 (95% CI, 2.94–15.6, $P<0.0001$) for control 1 and 3.57 (95% CI, 1.61–7.94, $P=0.001$) for control 2. Similarly the GT:GA ratios between female smokers (1.5) and never-smokers (0.23) were highly different. These frequencies and ratios for male never-smokers and smokers were very different from those of females. In fact, there were no major differences in the mutational spectra of male never-smokers and smokers. Thus the previously noted differences in the mutational spectra of never-smokers and smokers (which we confirmed in our present analysis) are almost entirely due to differences between female never-smokers and smokers.

As previously mentioned, almost all lung cancers arising in never-smokers are adenocarcinomas. The number of female never-smokers with lung cancer ($n=114$) is considerably higher than for male never-smokers ($n=32$). Thus, we also examined the mutational spectrum of adenocarcinomas arising in female never-smokers and smokers (Fig. 1). This analysis eliminated any possible confounding effects of gender and histological type. The frequency of G:C to T:A transversions in adenocarcinomas arising in female smokers (36%) was significantly different from the frequency in never-smokers (13%) (Fig. 1) and the odds ratios were 4.94 (95% CI, 1.64–14.9, $P=0.003$) for control 1 and 3.05 (95% CI, 1.06–8.8, $P=0.04$) for control 2. Similarly the GT:GA ratios between female never-smokers (0.28) and smokers (1.4) were very different.

Our findings about gender-related mutational differences are of particular interest. Zang and Wynder [1996] have reported that the odds ratios for major lung cancer types are consistently higher in women than in men at every level of exposure to cigarette smoke and that these gender differences cannot be explained by differences in baseline exposure, smoking history, or body size, but are likely due to the higher susceptibility to tobacco carcinogens in women. A recent review suggested that the risk of lung cancer may be different for men and women in response to

a complex interaction between biological factors such as hormonal difference and gendered factors such as smoking behavior [Payne, 2001]. A report from Taiwan found that DNA adduct levels of BPDE (a tobacco carcinogen) in females with lung cancer were markedly greater than those arising in males, suggesting gender differences in susceptibility to DNA damage derived from environmental carcinogen exposure [Cheng et al., 2001].

People who stop smoking, even well into middle age, avoid most (but not all) of their subsequent risk of lung cancer [Peto et al., 2000]. While very limited numbers of cases have been analyzed, the mutational frequency and spectrum of *TP53* mutations in cancers arising in former smokers appears to resemble those of current smokers [Vahakangas et al., 2001]. In the IARC database there are only 26 cases of lung cancer arising in former smokers without exposure to asbestos or radiation. For analysis we included these cases with other smokers.

Histological Differences in *TP53* Mutational Spectra

The vast majority of lung cancer cases (462 of 539; 86%) in the IARC database identified by smoking status and histological type were either adenocarcinomas or squamous cell carcinomas (Table 1). Thus, we investigated differences in the *TP53* mutational spectra of these two dominant types. Gender and smoke exposure status significantly influenced the adenocarcinoma to squamous cell carcinoma (AD:SQ) ratio. In males the AD:SQ ratio was unity, whereas in females it was higher (1.8) ($P = 0.03$). The gender differences were particularly striking in never-smokers, where the AD:SQ ratio in males was 2.0 while in females it was 10.5 ($P = 0.003$). Thus the vast majority of cancers in never-smokers, especially females, were adenocarcinomas. Therefore, analyses comparing smoke exposure were limited to all cases and the adenocarcinoma subtype. Similarly, analyses comparing histological types (adenocarcinoma vs. squamous cell carcinoma) were restricted to those who had a history of smoking.

TABLE 1. Demographic Data of Lung Cancer Cases in the IARC Database for Smoker (A) and Never-smoker (B) Associated Cases

A. Smokers (n = 440)	Male (n = 279)	Female (n = 86)	Gender unknown
Adenocarcinoma (n = 179)	109	42	28
Squamous cell (n = 178)	113	23	42
Large cell (n = 36)	23	11	2
Adenosquamous (n = 3)	2	1	0
SCLC (n = 24)	20	2	2
Others/not classified (n = 20)	12	7	1

B. Never-smokers (n = 156)	Male (n = 32)	Female (n = 114)	Gender unknown
Adenocarcinoma (n = 89)	18	63	8
Squamous cell (n = 16)	9	6	1
Large cell (n = 4)	2	2	0
Adenosquamous (n = 8)	1	6	1
Not classified (n = 39)	2	37	0

For smokers, there were no important differences in the mutational spectra of the two major histological subtypes of NSCLC (Fig. 1). For adenocarcinomas arising in women, the mutational spectra have been discussed previously. The mutational spectra of SCLC and other neuroendocrine tumors is discussed in the next section.

The mutational spectrum for SCLC (Fig. 1) closely resembled the spectrum of other smoking-associated tumors, with a frequency of G:C to T:A transversions of 32% and a GT:GA ratio of 1.5. Of 195 cases of SCLC in the IARC database, the smoking status of only 24 cases is recorded (all of these cases arose in smokers). Thus an analysis by smoking status is not possible. However, SCLC is exceedingly rare in never-smokers, and we can presume that almost all (if not all) of the cases in the database arose in smokers.

While alcohol is not a known mutagen, the combination of alcohol and tobacco smoke has been implicated in the pathogenesis of head and neck cancers [reviewed in Blons and Laurent-Puig, 2003]. Recently, Ahrendt et al. [2000] found that both alcohol consumption and tobacco use are associated with *TP53* mutations in NSCLC, suggesting the possibility that alcohol may enhance the mutagenic effects of cigarette smoke in the lung.

Mutational Hotspots and Warmspots

As previously discussed and referenced, seven codons have been identified as the sites of frequent G:C to T:A transversions in smoking-related tumors (so called hotspots). In Table 2, we state the frequencies of G:C to T:A transversions at the seven hotspots. For NSCLC, the differences between smokers and never-smokers were significant only for codon 245 ($P = 0.03$), although for six of the hotspots the frequencies were higher in smokers. In never-smokers, the frequency was higher in codon 249. Of interest, this is not a strong binding site for BPDE [Hainaut and Pfeifer, 2001]. A possible reason for the lack of significant differences is the relatively low frequencies of G:C to T:A transversions at these codons in smoke-associated tumors (0.9–3.6%). Perhaps the term "warmspot" may be more appropriate! However, the total frequency of G:C to T:A transversions at these sites in smokers (16%) was significantly higher than that of never-smokers (5.8%) ($P = 0.001$). For the SCLC cases, smoking information in the database is scant, although as discussed previously, we presume that most if not all cases arose in smokers. The total frequency for G:C to T:A transversions at the seven warmspots in SCLC

TABLE 2. Difference of Hotspot for *TP53* Mutation in Smoker and Nonsmoker

	All NSCLC (n = 572)			
Codon	All smoker (n = 416)	Never-smoker (n = 156)	P value	All SCLC (n = 195)
157	9 (2.3%)	0	> 0.05	4 (2.6%)
158	13 (3.2%)	1 (0.6%)	> 0.05	3 (1.5%)
175	4 (0.9%)	0	> 0.05	0
245	16 (3.6%)	1 (0.6%)	0.03[a]	2 (1.0%)
248	4 (0.9%)	0	> 0.05	7 (3.6%)
249	9 (2.3%)	5 (3.2%)	> 0.05	4 (2.6%)
273	13 (3.2%)	2 (1.3%)	> 0.05	4 (2.6%)
Total	68 (16%)	9 (5.8%)	0.001[b]	24 (12.3%)

P value with [a]Fisher exact test or [b]chi-square test indicated the significant difference between all smokers and never-smokers.

(12.3%) were not significantly different for the total in NSCLC smoker cases (16%) but was significantly different ($P = 0.04$) from the total frequency in NSCLC never-smoker cases (5.8%). It should be noted that the totals for G:C to T:A transversions at all seven codons are low (5.8–16%), and that the vast majority of *TP53* mutations in lung cancers of all types, whether smoke-related or not, are of other types or occur at different codons.

Neuroendocrine Tumors

SCLC have features of classic neuroendocrine (NE) tumors which distinguish them from most other lung cancers. However, other less common forms of pulmonary NE tumors occur, including bronchial carcinoids (consisting of typical carcinoids and the more aggressive atypical carcinoids) and large-cell NE carcinomas [Travis et al., 1991]. Carcinoids are not smoking-associated, indicating that their pathogenesis is different from the NE carcinomas. A study of *TP53* mutations in pulmonary NE tumors indicated that the incidence of *TP53* gene abnormalities progressively increased with increasing severity of tumor type and that the patterns of *TP53* gene mutations were different between atypical carcinoids and NE carcinomas [Onuki et al., 1999]. Although NE lung tumors have varied etiologies, the results of this study support the clinico-pathologic concept that they represent a spectrum ranging from low-grade typical carcinoids to the highly malignant NE carcinomas.

Carcinogens Other Than Tobacco Smoke

Several other carcinogens are known to be associated with lung cancer. However, many of these cancers arise in smoker subjects, confounding analysis of the effects of the carcinogen. For a few of these carcinogens, *TP53* mutation data are available from very limited numbers of cases.

Radon

Radon-222, a decay product of uranium-238 and a source of high linear-energy transfer (LET) alpha-particles, has been implicated in the increased risk of lung cancer in uranium miners as well as other exposed individuals. The studies of *TP53* mutation spectra of radon-associated lung cancers are somewhat contradictory [Hollstein et al., 1997; Hussain et al., 1997; McDonald et al., 1995; Vahakangas et al., 2001; Wiethege et al., 1999; Yang et al., 2000], perhaps because many of the cases are also smoking-associated. Thus of 50 cases in the IARC database (version R6), only five occurred in never-smokers.

Asbestos

Asbestos exposure (particularly in combination with smoking) is a carcinogen for lung cancer, and some reports have identified increased *TP53* gene mutations in asbestos-associated lung cancers [Guinee et al., 1995; Liu et al., 1998; Wang et al., 1995]. However, the number of cases analyzed to date are too few to draw conclusions about the mutational spectrum (there are 26 asbestos-associated cases in the IARC database, of which 24 arose in smoker subjects). Of interest, while mesotheliomas are not strictly lung cancers, they are also strongly asbestos-related. However, *TP53* gene mutations are rare in mesotheliomas [Liu et al., 1998; Mayall et al., 1999; Mor et al., 1997]. One reason may be the presence of SV40 virus in approximately 50% of human mesotheliomas [Carbone et al., 2002; Shivapurkar et al., 2000]. The largest antigen of SV40 binds to and inactivates the TP53 gene product, eliminating the necessity for inactivating mutations.

CLINICAL APPLICATIONS OF *TP53* MUTATIONS

Angiogenesis

Angiogenesis is one of the hallmarks of cancer [Hanahan and Weinberg, 2000] and vascular endothelial growth factor (VEGF) is a multifunctional cytokine that increases and stimulates angiogenesis. Angiogenesis is also regulated by several oncogenes and tumor suppressor genes including *TP53* [Bouck, 1996; Giatromanolaki and Koukourakis, 1998; Niklinska et al., 2001a]. A recent report found a strong,

statistically significant association between the presence of *TP53* gene mutations and expression of VEGF [Niklinska et al., 2001a; Yuan et al., 2002], and the wild-type *TP53* gene is antiangiogenic [Nishizaki et al., 1999].

TP53 Alternations During Multistage Pathogenesis

Tumors arise after a series of progressive histological and molecular changes. In the bronchial epithelium, a continuing series of steps has been identified in the formation of squamous cell carcinomas [Wistuba et al., 1999]. For SCLC these steps are largely unknown [Wistuba et al., 2000]. Peripherally arising adenocarcinomas are believed to arise from lesions known as atypical adenomatous hyperplasias (AAH) [Kitamura et al., 1999]. Multiple studies have demonstrated that *TP53* alterations including allelic losses, immunostaining, and occasional mutations commence during bronchial preneoplasia [Bennett et al., 1993a; Bennett et al., 1993b; Mitsudomi et al., 1993; Sozzi et al., 1992; Wistuba et al., 1997] and can be detected in AAH lesions [Kerr et al., 1994; Kitaguchi et al., 1998; Kitamura et al., 1995; Slebos et al., 1998]. Detection of *TP53* alterations in exfoliated cells present in sputum and bronchiolo-alveolar lavage fluids may help identify smokers at increased risk or aid in the diagnosis of early detection of lung cancers [Ahrendt et al., 1999; Mao et al., 1994].

Individuals with one aerodigestive tract malignancy have a high incidence of second primary aerodigestive tumors, a phenomenon known as field carcinogenesis. Franklin et al. [1997] studied an individual with widespread dysplastic changes in the respiratory epithelium but no overt carcinoma. The entire tracheobronchial tree obtained at autopsy was embedded in paraffin, and bronchial epithelial cells were isolated by microdissection. DNA extracted from the microdissected cells was analyzed for point mutations in the *TP53* gene. A single, identical point mutation consisting of a G:C to T:A transversion in codon 245 was identified in dysplastic bronchial epithelium from 7 of 10 sites in both lungs. The widespread presence of a single somatic *TP53* point mutation in the bronchi of a smoker suggests that a single progenitor bronchial epithelial clone may expand to populate broad areas of the bronchial mucosa; a novel mechanism for field carcinogenesis in the respiratory epithelium that may be of importance in assessing individuals for risk of a second primary tumor as well as in devising effective strategies for chemoprevention of lung cancer.

Clinical Significance of *TP53* Mutations

Multiple reports have addressed the question as to whether *TP53* mutations in NSCLC tumors are a negative prognostic factor [reviewed in Mitsudomi et al., 2000]. These results of these reports are controversial, with some suggesting a negative prognostic effect, some a positive prognostic effect, and others showing no effect! Most of the mutations identified in lung cancers are missense mutations. The half-life of the wild-type TP53 protein is short and usually not detected by most immunohistochemical (IHC) methods. By contrast, missense mutations in exons 5 to 8 are frequently associated with a prolonged half-life of the mutant protein and its detection by IHC in lung cancers [Bodner et al., 1992] and other tumors. Because of the simplicity of IHC, it has been widely used as a surrogate for *TP53* mutation status. However, the correlation is not exact and many cases of discrepancy have been reported. Differences in methodology and sensitivity may also result in discrepant results. Most studies that rely on molecular analyses only examine exons 5 to 8, which are the locations of most of the *TP53* mutations. For these multiple reasons, discrepancies between the various studies are not surprising. For these reasons, Mitsudomi and coworkers [Mitsudomi et al., 2000] performed a meta-analysis of 43 published reports and concluded that *TP53* mutations (as determined by IHC and mutational analysis) were a significant marker of poor prognosis in patients with pulmonary adenocarcinoma. Recent reports have confirmed and extended these observations [Laudanski et al., 2001; Niklinska et al., 2001b].

Several studies indicate that *TP53* mutations confer chemoresistance to lung cancer cells in vivo and in vitro [Fujiwara et al., 1994; Higashiyama et al., 1998; Rusch et al., 1995; Vogt et al., 2002], thus providing one possible explanation for its action as a negative prognostic marker. Recently, Baptiste et al. [2002] reported that the proline-rich domain of TP53 is essential for the ability of TP53 to respond to DNA-damaging agents to cause cell death.

TP53 GeneTherapy

Clinical trails utilizing *TP53* gene therapy offer an innovative approach for lung cancer therapy [Roth et al., 1996]. Advances in biotechnology made it possible to deliver the *TP53* gene into lung cancer cells by viral vectors [Fujiwara et al., 1993; Zhang et al., 1994] or liposomes [Ramesh et al., 2001] and demonstrated antitumor effect including a bystander effect induced by the wt-*TP53* gene transfer on adjacent tumor cells [Nishizaki et al., 1999]. However a recent phase II study of local adenovirus-mediated *TP53* gene therapy to systematically assess the clinical efficacy of this novel therapeutic approach in patients undergoing an effective first-line chemotherapy showed no convincing evidence for an additional local benefit [Schuler et al., 2001]. One of the problems with such approaches is that they were directed at pulmonary or intrabronchial tumors, while deaths from lung cancer are usually from widespread or metastatic disease.

CONCLUSIONS

The TP53 protein plays important roles in multiple cellular functions such as cell cycle control, DNA synthesis and repair, cell differentiation, gene transcription, and programmed cell death. Of the multiple genetic alterations described in lung cancers, mutations and other alterations of the *TP53* gene are among the most frequent and important events, commencing during multistage pathogenesis. The vast majority of lung cancers, both of the SCLC and NSCLC types, have abnormalities of the *TP53* gene. Despite several hundred reports that have studied the status or effects of *TP53* mutations in lung cancer, major gaps in our knowledge exist, and some of the reported findings are controversial.

Tobacco smoke is the major cause of lung cancer. Carcinogens present in tobacco smoke are associated with specific mutations at a relatively small number of *TP53* codons resulting in differences in cancers arising in smokers and never-smokers. However, these findings remain controversial, prompting us to re-evaluate them. Because no single study can analyze more than a modest number of mutations, we analyzed the latest (R6) version of the IARC database. While we confirmed several reports that indicated that tobacco carcinogens targeted G:C to T:A transversions at five or six specific codons, only the minority of mutations in cancers arising in smokers were of this category. Our analysis has resulted in an important new finding: there are major gender differences associated with the mutational spectrum, with cancers arising in women smokers demonstrating significantly more tobacco-related mutations. These findings may help to explain the reported higher susceptibility of women to tobacco carcinogens. SCLC and squamous cell carcinomas are centrally arising lung cancers (i.e., those arising from the major bronchi) and more frequently arise in smokers than the peripherally arising adenocarcinomas. To our surprise, there were no important differences in the *TP53*mutational spectra between the major histological types of lung cancer. However, carcinoid tumors, a relatively rare group of low-grade neuroendocrine lung tumors that are not smoking-associated, had a low frequency of *TP53* mutations.

A study of *TP53* gene alterations may, potentially, have major clinical applications, impacting on risk assessment, early diagnosis, prognosis, response to chemotherapy, and the development of novel therapeutic approaches. However, most of these applications remain theoretical, and have not been universally accepted or applied. Thus, despite great promise and intense study by investigators from all parts of the world, major gaps exist in our knowledge. Until we have a fuller understanding of *TP53* gene alterations in lung cancer, the full clinical potential of these critical events will not be realized.

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