The TP53 Gene, Tobacco Exposure, and Lung Cancer

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For the p53 Special Issue

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.

KEY WORDS: p53; TP53; cancer; lung cancer; tobacco; exposure; carcinogen; risk factor; mutagen

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
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
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; Vahakan-gas 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 odds 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 GTGA ratio. In smokers, the GTGA ratio was near unity, while in never-smokers the GTGA ratio was 0.34 (Fig. 1). In smokers, the GTGA 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 GTGA 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 GTGA 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 smoking status significantly influenced the adenocarcinoma to squamous cell carcinoma (AD:SQ) ratio.

<table>
<thead>
<tr>
<th>Cases</th>
</tr>
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<tbody>
<tr>
<td><strong>TP53 Mutational Spectra</strong></td>
</tr>
<tr>
<td><strong>Adenocarcinoma</strong></td>
</tr>
<tr>
<td>(n = 440)</td>
</tr>
<tr>
<td>Adenocarcinoma ( n = 179 )</td>
</tr>
<tr>
<td>Squamous cell ( n = 178 )</td>
</tr>
<tr>
<td>Large cell ( n = 36 )</td>
</tr>
<tr>
<td>Adenosquamous ( n = 3 )</td>
</tr>
<tr>
<td>SCLC ( n = 24 )</td>
</tr>
<tr>
<td>Others/not classified ( n = 20 )</td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
</tbody>
</table>

**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...
(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,

<table>
<thead>
<tr>
<th>Codon</th>
<th>All smoker ((n = 416))</th>
<th>Never-smoker ((n = 156))</th>
<th>P value</th>
<th>All SCLC ((n = 195))</th>
</tr>
</thead>
<tbody>
<tr>
<td>157</td>
<td>9 (2.3%)</td>
<td>0</td>
<td>&gt;0.05</td>
<td>4 (2.6%)</td>
</tr>
<tr>
<td>158</td>
<td>13 (3.2%)</td>
<td>0 (0.6%)</td>
<td>&gt;0.05</td>
<td>3 (1.5%)</td>
</tr>
<tr>
<td>175</td>
<td>4 (0.9%)</td>
<td>0</td>
<td>&gt;0.05</td>
<td>0</td>
</tr>
<tr>
<td>245</td>
<td>16 (3.6%)</td>
<td>1 (0.6%)</td>
<td>0.03*</td>
<td>2 (1.0%)</td>
</tr>
<tr>
<td>248</td>
<td>4 (0.9%)</td>
<td>0</td>
<td>&gt;0.05</td>
<td>7 (3.6%)</td>
</tr>
<tr>
<td>249</td>
<td>9 (2.3%)</td>
<td>5 (3.2%)</td>
<td>&gt;0.05</td>
<td>4 (2.6%)</td>
</tr>
<tr>
<td>273</td>
<td>13 (3.2%)</td>
<td>2 (1.3%)</td>
<td>&gt;0.05</td>
<td>4 (2.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>68 (16%)</td>
<td>9 (5.8%)</td>
<td>0.001b</td>
<td>24 (12.3%)</td>
</tr>
</tbody>
</table>

\(P\) value with *Fisher exact test or \(^b\)chi-square test indicated the significant difference between all smokers and never-smokers.
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 bronchioalveolar 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 that may 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 Gene Therapy**

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 intrabronchical 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. Among the most frequent and important events, TP53 with specific mutations at a relatively small number of 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 frequency 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, adenocarcinomas, 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.

REFERENCES

Giromonolaki A, Koukourakis MI. 1998. p53 and angio-
Guiney DG Jr, Travis WD, Trivers GE, De Benedetti VM,
Cawley H, Welsh JA, Bennett WP, Jett J, Colby TV, Tazelaar
analysis of p53 mutations, anti-p53 serum antibodies and C-
Hainaut P, Olivier M, Pfeifer GP. 2001. TP53 mutation
spectrum in lung cancers and mutagenic signature of
components of tobacco smoke: lessons from the IARC
Hainaut P, Pfeifer GP. 2001. Patterns of p53 G→T transver-
sions in lung cancers reflect the primary mutagenic signature
of DNA-damage by tobacco smoke. Carcinogenesis 22:
367–374.
100:57–70.
Harris CC. 1996. p53 tumor suppressor gene: from the basic
research laboratory to the clinic: an abridged historical
Hecht SS. 2002. Cigarette smoking and lung cancer: chemical
mechanisms and approaches to prevention. Lancet Oncol
3:461–469.
Higashiyama M, Kodama K, Yokouchi H, Takami K, Doi O,
Kobayashi H, Tanisaka K, Minamigawa K. 1998. Immuno-
histochemical p53 protein status in non-small cell lung cancer
is a promising indicator in determining in vitro chemosensi-
Hollstein M, Bartsch H, Wesch H, Kure EH, Mustonen R,
Mühlbauer KR, Spiethoff A, Wegenker H, Wiethege T, Muller
KM. 1997. p53 gene mutation analysis in tumors of patients
Hussain SP, Kennedy CH, Amstad P, Lui H, Lechner JF, Harris
codons 249 and 250 to 238Psi alpha-particles in human
Hussain SP, Amstad P, Raja K, Sawyer M, Hofseth L, Shields
PG, Hewer A, Phillips DH, Ryberg D, Haugen A, Harris CC.
diol epoxide BPDE and the frequency of p53 mutations in
Hussain SP, Hofseth L, Harris CC. 2001b. Tumor suppressor
genes: at the crossroads of molecular carcinogenesis,
molecular epidemiology and human risk assessment. Lung
Identification of carcinomaembryonic antigen-producing cells
circulating in the blood of patients with colorectal carcinoma
by reverse transcriptase polymerase chain reaction. Gut
hyperplasia: relationship with pulmonary adenocarcinoma,
Proliferative activity, p53 expression and loss of hetero-
zygosity on 3p, 9p and 17p in atypical adenomatous
Kitamura H, Kameda Y, Nakamura N, Nakatani Y, Inayama Y,
Proliferative potential and p53 overexpression in precursor
and early stage lesions of bronchioloalveolar lung carcinoma.
Kitamura H, Kameda Y, Ito T, Hayashi H. 1999. Atypical
adenomatous hyperplasia of the lung. Implications for the
pathogenesis of peripheral lung adenocarcinoma. Am J Clin
Pathol 111:610–622.
Laudanski J, Niklinska W, Burzykowski T, Chyczewski L,
abnormalities in operable non small cell lung cancer. Eur
mutations in asbestos associated cancers. Biomed Environ
Detection of oncogene mutations in sputum precedes
Mao L, 2001. Molecular abnormalities in lung carcinogenesis
and their potential clinical implications. Lung Cancer
34(Suppl 2):S27–S34.
gene and SV40 sequences in asbestos associated
52: 291–293.
McDonald JW, Taylor JA, Watson MA, Saccomanno G,
lung adenocarcinoma. Cancer Epidemiol Biomarkers Prev
4: 791–793.
Mitsudomi T, Lam S, Shirakusa T, Gazdar AF. 1993. Detection
and sequencing of p53 gene mutations in bronchial biopsy
samples in patients with lung cancer [see comments]. Chest
Prognostic significance of p53 alterations in patients with
6:4055–4063.
Mor O, Yaron P, Huszar M, Yellin A, Jakobovitz O, Brok-Simoni
in malignant mesotheliomas. Am J Respir Cell Mol Biol
Expression of vascular endothelial growth factor VEGF
in non-small cell lung cancer NSCLC: association with
p53 gene mutation and prognosis. Lung Cancer 34(Suppl 2):
S59–S64.
Niklinska W, Chyczewski L, Laudanski J, Sawicki B, Niklinski J.
2001b. Detection of P53 abnormalities in non-small cell lung
Cancer by yeast functional assay. Folia Histochem Cytobiol
Nishizaki M, Fujiwara T, Tanida T, Hizuta A, Nishimori H,
Mitsudomi T, Lam S, Shirakusa T, Gazdar AF. 1993. Detection
and sequencing of p53 gene mutations in bronchial biopsy
samples in patients with lung cancer [see comments]. Chest
University Press.
Olivier M, Eles R, Hollstein M, Khan MA, Harris CC, Hainaut
P. 2002. The IARC TP53 database: new online
mutation analysis and recommendations to users. Hum


