Distinct Mutational Spectrum of the p53 Gene in Lung Cancers from Chinese Women in Hong Kong

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ABSTRACT

Accumulating evidence suggests that the p53 gene is a major target for molecular epidemiological studies to search for risk factors in carcinogenic events. The lung cancer incidence for females in Hong Kong is unusually high, ranking among the highest in the world despite a low percentage with a history of smoking. To gain insights into possible etiological risk factors responsible for this high incidence, we examined p53 mutations in 35 lung cancer specimens from Chinese females living in Hong Kong and compared them with 35 matched cases from Japanese women as well as previously reported p53 mutations in the world literature. p53 mutations in exons 5–8 were present in 20 and 31% of the Hong Kong and Japanese cases, respectively. Notably, single-base deletions within runs of identical bases were observed in 3 (43%) of the 7 mutations in the Hong Kong cases, in contrast to the absence of such mutations in the controls and the extreme scarcity in the literature, suggesting that distinct environmental and/or genetic factors might be involved. Although the frequent occurrence of characteristic single-base deletions could be a reflection of mutator mutations leading to inefficient mismatch repair of slipped strand mispairings, none of the lung cancer specimens exhibited such microsatellite instabilities.

INTRODUCTION

The p53 gene is mutated in a large proportion of most human cancers, including lung cancer. Increasing evidence suggests that the frequency and spectrum of p53 mutations may represent fingerprints left by specific carcinogenic exposures, thus providing information about the molecular epidemiology of human cancer risk (1). For example, dietary aflatoxin B1 exposure is correlated with G:C to T:A transversions in human tumors. The p53 gene is mutated in a large proportion of most human cancers, including lung cancer. Increasing evidence suggests that the frequency and spectrum of p53 mutations may represent fingerprints left by specific carcinogenic exposures, thus providing information about the molecular epidemiology of human cancer risk (1). For example, dietary aflatoxin B1 exposure is correlated with G:C to T:A transversions in human tumors.

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2 To whom reprint requests should be addressed.

To this high incidence, we investigated whether or not there are any distinctive p53 mutations in lung cancers of Chinese females living in Hong Kong when compared with those of matched Japanese females as well as previously reported p53 mutations in the world literature. We report here characteristically frequent occurrence of single-base deletions among the Hong Kong cases, in contrast to the absence of such mutations in matched Japanese female cases and the extreme scarcity in the literature, suggesting that distinct environmental factor(s) might be involved in the development of lung cancers in females in Hong Kong. Although frequent occurrence of single-base deletions within runs of identical bases could be regarded as a reflection of inefficient mismatch repair, none of the cases showed replication errors at microsatellite markers.

MATERIALS AND METHODS

Tumors. Lung cancer samples were obtained from 35 Chinese females, including 28 who had never smoked, at the time of surgery at Kowloon Hospital in Hong Kong and stored at −80°C until analysis at the Aichi Cancer Center (Nagoya, Japan). Thirty-four lung cancer patients were interviewed in person (in the thirty-fifth case, the patient’s son-in-law was interviewed) about their smoking, diet, and demographic backgrounds at the hospital during postoperative care. The lung tumors were histologically typed according to the WHO’s histological classification. Japanese female control lung cancer samples, which were matched for age, histological type, disease stage, and smoking history, were obtained at the time of surgery at the Aichi Cancer Center (Table 1).

Analysis of p53 Mutations. PCR-SSCP analysis was performed to detect p53 mutations in the region between exons 5 and 8 using genomic DNA as described previously (12). The oligonucleotide primers and PCR conditions used to amplify genomic DNA were identical to those detailed in our previous report (12). Genomic DNAs demonstrating an altered mobility shift in this PCR-SSCP analysis were further analyzed by sequencing, and the identified mutations were confirmed by separate PCR and subsequent sequence analysis as described previously (7, 13).

Analysis of Microsatellite Instabilities. Genomic DNAs of lung cancers and corresponding normal lungs were analyzed for microsatellite instabilities of dinucleotide repeat sequences using PCR in the presence of [32P]dCTP. The microsatellite markers examined were: D3S663 (3p), D3S1228 (3p), D5S107 (5q), and p53 (17p). The PCR products were separated by electrophoresis in 6% denaturing polyacrylamide gels followed by autoradiography.

RESULTS

Distinct Mutational Spectrum of p53 in Lung Cancers of Hong Kong Females. PCR-SSCP analysis yielded that mutations in exons 5 through 8 of the p53 gene were present in 7 (20%) of 35 female cases from Hong Kong, while p53 mutations were detected in 11 (31%) of 35 cases from the matched Japanese female patients (Table 2). These relatively low incidences probably reflect the fact that the majority of the cohorts studied here were adenocarcinoma cases without a smoking history. Sequencing analysis showed a marked
DISTINCT p53 MUTATIONS IN LUNG CANCER IN HONG KONG

Table 1 Patient characteristics of female lung cancers in Hong Kong and in Japan

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Hong Kong (n = 35)</th>
<th>Japan (n = 35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>Median 60</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Range 21–82</td>
<td>24–76</td>
</tr>
<tr>
<td>Histology</td>
<td>Adenocarcinoma 29</td>
<td>27</td>
</tr>
<tr>
<td>Squamous cell carcinoma 6</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Large cell carcinoma 0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Adenosquamous carcinoma 0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td>I 14</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>II 9</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>III/IVB 10</td>
<td>11</td>
</tr>
<tr>
<td>Smoking</td>
<td>Ever-smoked 7</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Never-smoked 28</td>
<td>28</td>
</tr>
</tbody>
</table>

There was a significant difference in the mutational spectra between Chinese patients in Hong Kong and Japanese patients in Japan (Table 3). Three (43%) of the 7 mutations identified in the Chinese cases were single-base deletions within runs of identical bases at different codons, whereas 0 (0%) of 11 mutations in Japanese cases were of this type of genetic alteration (P = 0.043 by Fisher's exact probability test). A search of a database revealed that 398 mutations, including those earlier reported by our group, were found to involve single-base deletions (P = 0.0019 by Fisher's exact probability test). As to the mutational spectrum in relation to smoking status, G:C to T:A transversions (three cases) were observed exclusively in smokers, whereas G:C to A:T transitions were present only in never-smokers (three of three cases). In addition, G:C to C:G transversions appeared to be frequent in the Japanese cases of never-smokers.

Absence of Microsatellite Instabilities in Both Hong Kong and Japanese Cases. We also examined four independent loci containing dinucleotide repeats to investigate whether microsatellite instabilities are significantly associated with lung cancers in Chinese women, since the single-base deletions were all found within runs of identical bases such as Gs and As, suggesting possible involvement of inefficient mismatch repair (17, 18). However, microsatellite instabilities were not observed in any lung cancer specimens of both Chinese and Japanese female cases (Fig. 1). Two colon cancer cases, which were included as positive controls for the detection of microsatellite instabilities, showed clear differences in banding patterns between normal and tumor DNAs (data not shown for one of the two positive controls), indicating that the absence in lung cancer specimens were not due to inappropriate assay conditions.

DISCUSSION

Numerous epidemiological studies have indicated that a majority of the high incidence of lung cancer in women is related to factors other than cigarette smoking (10, 19–21). The predominance of single-base deletions found here suggests that there might be a distinct etiological cause leading to the unusually high incidence of lung cancer in this population. In addition, this might also explain the lower frequency of p53 mutations in Hong Kong than in Japan, because frame-shift mutations resulting from a single-base deletion do not necessarily occur within the hot spot exons examined in the present study. In fact, among a total of 21 single-base deletions in lung cancers reported in the literature thus far, 7 (33%) such mutations were identified to be outside exons 5 through 8 (16).

Table 2 p53 mutations in lung cancers from Chinese women in Hong Kong and from Japanese women in Japan

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (yr)</th>
<th>Histology</th>
<th>Stage</th>
<th>Codon</th>
<th>Base change</th>
<th>Microsatellite instability</th>
<th>Smoking</th>
</tr>
</thead>
<tbody>
<tr>
<td>C6</td>
<td>60</td>
<td>AD</td>
<td>II</td>
<td>270</td>
<td>TTT to TGT</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>C11</td>
<td>77</td>
<td>SQ</td>
<td>III</td>
<td>245</td>
<td>GCC to GCA</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>C19</td>
<td>34</td>
<td>AD</td>
<td>I</td>
<td>213</td>
<td>CGA to TGA</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>C20</td>
<td>65</td>
<td>AD</td>
<td>I</td>
<td>273</td>
<td>CGT to CTG</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>C22</td>
<td>65</td>
<td>AD</td>
<td>II</td>
<td>188/189</td>
<td>CTGGCC to CTG-CG</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>C24</td>
<td>68</td>
<td>SQ</td>
<td>III</td>
<td>288</td>
<td>AAT to A^5A</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>C35</td>
<td>74</td>
<td>AD</td>
<td>I</td>
<td>175</td>
<td>CGC to CAC</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

The other 28 cases had neither mutations in exons 5–8 of the p53 gene nor microsatellite instabilities.

Table 3 Spectrum of p53 mutations in lung cancers of Hong Kong females in comparison to those of Japanese females and the world literature

<table>
<thead>
<tr>
<th>Mutation</th>
<th>% Hong Kong (n = 7)</th>
<th>% Japan (n = 11)</th>
<th>% Worldwide* (n = 398)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-base deletion</td>
<td>43</td>
<td>0</td>
<td>3.5</td>
</tr>
<tr>
<td>Base substitution</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G:C to T:A</td>
<td>14</td>
<td>18</td>
<td>38</td>
</tr>
<tr>
<td>G:C to A:T</td>
<td>28</td>
<td>18</td>
<td>26</td>
</tr>
<tr>
<td>Others</td>
<td>14</td>
<td>64</td>
<td>33</td>
</tr>
</tbody>
</table>

* p53 mutations occurring in exons 5–8 in lung cancers were retrieved from the data base of p53 somatic mutations in human tumors and cell lines (released January 1995; Ref 16).

a Hong Kong vs. Japan, P = 0.043; Hong Kong vs. worldwide, P = 0.0019 (by Fisher’s exact probability test).

Previously reported in Ref. 7.

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certain agents are known to be associated with specific mutational spectra. For example, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, which is present in fried meats and fish, can cause specific single-base deletions (23), while 1-nitroso-8-dinitropyrene, which is an environmental contaminant detectable in diesel exhausts and urban air particulates, has been shown to preferentially induce single-base deletions, possibly by a mechanism of mutational bypass of replication-blocking lesions of the DNA adducts (24).

It is also possible that the high frequency of single-base deletions within runs of identical bases such as Gs and As may reflect an inefficient repair of slipped strand mispairings in Chinese women, since alterations in the mismatch repair genes such as hMLH2 and hMLH1 have been shown to render human cells prone to replication errors, as manifested by the presence of frequent somatic instability at (CA) repeats and shortening of a poly(A) tail at Alu sequences (17, 25–31). However, the absence of microsatellite instability suggests that defects in the mismatch repair system may not be significantly associated with lung cancers in Chinese women, although further investigations using tri- or tetrانucleotide repeat markers are necessary to draw any definite conclusion. The dinucleotide repeats examined in the present study have been shown to be less susceptible to microsatellite instability than tri- or tetrанucleotide repeats (32).

To identify the environmental and/or genetic risk factor(s) responsible for the high incidence of lung cancers in Chinese women in Hong Kong, additional comparative studies are warranted using lung cancer specimens from Chinese immigrants in other regions such as Hawaii and San Francisco. In addition, analysis of p53 mutations among female lung cancer cases of other ethnicities and localities such as the United States and the United Kingdom would be of interest, because increased incidence rates of lung cancer, especially adenocarcinoma, appear to be worldwide (10).

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REFERENCES


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