Differences in the p53 gene mutational spectra of prostate cancers between Japan and Western countries

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Mutations of the p53 gene are related to development of human cancers and their frequencies and spectra, the latter representing fingerprints left by carcinogens, provide information about the molecular epidemiology of the disease. Prostate cancer is the most common neoplasm in American males and although its incidence is still relatively low in Japanese people, it has recently been increasing with the westernization of life style. To assess the frequency and spectrum of p53 gene mutations in Japanese prostate cancers, we examined a series of 90 lesions using polymerase chain reaction (PCR)-single-strand conformation polymorphism (SSCP) analysis. The patients’ mean age was 69.3 years (range 57–87). Of the total, six were well-, 34 moderately- and 50 poorly-differentiated adenocarcinomas, and the median Gleason score was 7.9. Eleven of the 90 cases (12%) had mutations in exons 2–11 of the p53 gene: none of the five clinical-stage A, one of 25 stage B (4%), three of 35 stage C (9%) and seven of 25 stage D (28%) cancers. The correlation with an advanced stage was statistically significant. One insertion and 10 base pair substitutions were encountered, comprising six transversions (55%) and four transitions (36%). Two of the latter involved methylated cytosine-guanine (CpG). These 11 mutations were combined with 18 other mutations in previous reports concerning Japanese prostate cancers to facilitate comparison of the p53 gene mutational spectrum with those reported for American and European prostate cancers. In the latter, 61% were transitions and 33% were transversions. The greater proportion of transversions in the Japanese population suggests that there are different factors responsible for carcinogenesis of the prostate glands in the various countries.

Introduction

Mutations of the p53 gene are the most common genetic alteration in human cancers with a role in neoplasms now firmly established (1). Genetic alterations including point, deletion and insertion mutations can inactivate the p53 tumor suppressor function (2). Such DNA damage may be induced by numerous factors including endogenous metabolites and exogenous agents. In the case of chemical carcinogens, specific DNA base changes may result, so it is possible that the mutation patterns produced may be characteristic (3). Gene mutational analysis has suggested that the spectrum of p53 changes can be regarded as a fingerprint left by specific carcinogen exposure, thus providing information about the molecular epidemiology of human cancers (4). Clear examples of this include the C:C → T:T transitions observed in UV-induced skin cancers and the G:C → T:A transversions which are characteristic of aflatoxin B1-induced hepatocellular carcinomas (5–7).

Prostate cancer is one of the most common malignant diseases among American men, and while it has been infrequent in Japan, its incidence has recently been increasing (8–10). The available findings for the wide international variation in the incidence and mortality rates of patients with prostate cancer suggest that both genetic and environmental factors play important roles (11–14). The p53 gene mutational spectra for prostate cancers may therefore provide information on the underlying causes. We previously reported identification of p53 gene mutations in Japanese prostate cancers, suggesting that they play a role in the progression of a subset of these malignancies (15). To pursue this question further, the spectra and frequency of p53 gene mutations in Japanese prostate cancers were examined and compared with data from Western countries.

Materials and methods

Tissue samples

Samples of 90 prostate tumors were collected from the same number of patients who presented at Meie University Hospital, Chiba University Hospital or The Osaka Center for Adult Disease between 1991 and 1995. The ages of the patients ranged from 57 to 87 years with a mean age of 69.3 years. All 90 samples were primary prostate tumors. They were obtained by radical prostectomy (63 cases), radical cystoprostatectomy for bladder tumors (two cases), later diagnosed as invasive prostate cancers, and at autopsy (25 cases).

DNA extraction

From frozen tissues, genomic DNA was purified by standard methods, with proteinase K digestion, serial phenol and chloroform extractions, and ethanol precipitation.

Polymerase chain reaction (PCR*)-SSCP analysis

Eleven sets of primers were prepared to amplify DNA fragments covering exons 2–11 of the p53 gene as described previously (15). Primers were end-labeled with [γ-32P]ATP using T4 polynucleotide kinase (Takara, Kyoto, Japan). Samples of 50 ng of genomic DNA were amplified by PCR in a total of 5 μl of reaction mixture, which consisted of 80 nM end-labeled 5′ and 3′ primers, 100 μM each of deoxynucleotide triphosphate (dNTP), 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2, 0.001% gelatin and 0.1 U/ml Taq

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DNA polymerase (Perkin Elmer Cetus, Norwalk, CT). The reaction conditions were 94°C (0.5 min), 55°C (0.5 min) and 72°C (1 min) for 35 cycles. The reaction was initiated with one 3-min incubation at 94°C and ended with an incubation of 7 min at 72°C. Aliquots of 5 μl of PCR products were added to 45 μl of loading buffer (95% formamide, 20 mM EDTA, 0.05% Bromophenol Blue, and 0.05% xylene cyanol), heat denatured, and 1-μl portions were loaded per lane onto 4.9% polyacrylamide gels with or without 5% glycerol. Electrophoresis was carried out at 40 W at 18°C with a water jacket. Gels were then dried and exposed to XAR-5 (Kodak, Rochester, NY) at −80°C for 0.5–1 h. When abnormal bands were detected by PCR-SSCP analysis, we confirmed them by repeated experiments.

DNA sequencing
DNA was extracted from shifted-bands obtained by PCR-SSCP analysis and fragments were directly subcloned into the pCR vector using a TA cloning system kit (Invitrogen, San Diego, CA). They were then sequenced by the Sanger di-deoxynucleotide method with a Sequenase Ver. 2.0 kit (United States Biochemical, Cleveland, OH).

Statistical analysis
Associations between p53 mutations and other factors were evaluated using χ². The criterion for significance was P < 0.05.

Results
Analysis of p53 gene mutations in 90 prostate cancers
The PCR-SSCP analysis of exons 2–11 of the p53 gene was performed for all 90 samples. PCR products that showed band shifts on PCR-SSCP analysis were subcloned and sequenced. For each case, the sequences of 10 subclones were determined. For example, no. 11 showed a band shift in exon 9 due to a TAT to TAG transversion of codon 90 (Figure 1). Mutations of the p53 gene were detected in a total of 11 of 90 samples (11%). Table II summarizes details of these positive cases. The mean age of the affected patients was 70.5 years and not appreciably different from the mean of 69.3 years for the entire study population. The clinical stages were one stage B, three stage C and seven stage D cancers to give incidences of 4%, 9% and 28%, respectively. This correlation with clinical stage was found to be statistically significant (χ² = 6.3, P < 0.05). The involved samples were all moderately or poorly differentiated adenocarcinomas, and 10 of 11 samples were high grade in terms of the Gleason score (7–10). However, the relationship between p53 gene mutations and histological grade or the Gleason score was not significant statistically.

We detected nine missense mutations, one nonsense mutation and one frameshift mutation, the latter being a G insertion in exon 9. Of the base substitution mutations, six (55%) were transversions and four (36%) were transitions. Two of the transitions were G:C → A:T at CpG, one a G:C → A:T not at CpG and one was an A:T → G:C. Of the six transversions, two were A:T → T:A, two G:C → C:G, one A:T → C:G and one G:C → T:A. The overall distribution of these mutations was relatively random across the p53 gene exons 2–9, with four mutations detected in exons 5, two in exon 7, two in exon 9 and one in exons 2, 4 and 8, respectively.

Discussion
The present investigation of the frequency and spectrum of p53 gene mutations in a series of 90 primary prostate cancers in Japanese people, using PCR-SSCP analysis, revealed 11 (12%) to be positive, with preferential detection in late stage disease. Thus seven of 25 stage D cancers (28%) had mutations as compared with only one of 25 stage B (4%) lesions. In addition, 10 of the 11 samples were also high grade in terms of the Gleason score 7–9 (average 8.1) as compared with 7.9 for the entire study population. This study indicates that p53 gene mutations mainly involve late-stage and high-grade prostate cancers in Japanese men, which is in line with previous reports from America (18,19).

Mutations of the p53 gene have been detected in most human tumors, the majority being of the missense type, in conserved regions of the nucleotide sequence, which leads to amino acid substitutions in the protein (2,20). It has been established that DNA damage can be induced by various factors including endogenous and exogenous carcinogens and that particular agents induce specific DNA base changes (21). Therefore, p53 gene mutational spectra in tumors may represent fingerprints left by specific carcinogen exposure (3,4,22). A correspondence between mutational type detected in human tumors and that in laboratory studies of the putative carcinogen has been reported. For example, specific mutations found in patients with hepatocellular carcinomas from Africa and China were G → T transversions (6,7). In the laboratory, aflatoxin B1 binds preferentially to G residues in GC-rich regions and induces G → T transversions almost exclusively (23).

Although only a relatively limited number of tumors were analyzed in the present study, the data present clear evidence of variation in the p53 gene mutational spectra for prostate cancers between Japanese and Western cases (Table III) (15,18,19,24–33). In American and European prostate cancers, transitions predominate (69%) with G:C → A:T transitions at
Table II. Summary of details for the p53 gene mutation cases

<table>
<thead>
<tr>
<th>Sample</th>
<th>Age</th>
<th>Stage</th>
<th>Gleason score</th>
<th>p53 gene mutation</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Exon codon</td>
</tr>
<tr>
<td>13</td>
<td>73</td>
<td>B</td>
<td>6</td>
<td>7–239</td>
</tr>
<tr>
<td>5</td>
<td>70</td>
<td>C</td>
<td>7</td>
<td>5–181</td>
</tr>
<tr>
<td>9</td>
<td>67</td>
<td>C</td>
<td>8</td>
<td>9–313</td>
</tr>
<tr>
<td>11</td>
<td>69</td>
<td>C</td>
<td>9</td>
<td>9–327</td>
</tr>
<tr>
<td>25*</td>
<td>77</td>
<td>D</td>
<td>7</td>
<td>5–176</td>
</tr>
<tr>
<td>28*</td>
<td>65</td>
<td>D</td>
<td>8</td>
<td>8–288</td>
</tr>
<tr>
<td>30*</td>
<td>71</td>
<td>D</td>
<td>7</td>
<td>4–67</td>
</tr>
<tr>
<td>34*</td>
<td>66</td>
<td>D</td>
<td>9</td>
<td>2–11</td>
</tr>
<tr>
<td>50</td>
<td>72</td>
<td>D</td>
<td>8</td>
<td>7–239</td>
</tr>
<tr>
<td>61</td>
<td>70</td>
<td>D</td>
<td>9</td>
<td>5–156</td>
</tr>
<tr>
<td>65</td>
<td>76</td>
<td>D</td>
<td>9</td>
<td>5–169</td>
</tr>
<tr>
<td>Mean</td>
<td>70.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TV, transversion; TS, transition; *, previously reported samples (ref. 15).

CpG accounting for 32%. These latter transitions are due to frequent methylation of cytosine to 5-methylcytosine and subsequent spontaneous deamination to thymine (34–36). This mutation is thought to result from spontaneous or endogenous processes rather than being due to exogenous agents. G:C → A:T transitions are frequently detected in colon tumors, and 70% of the mutations occur at CpG dinucleotides (4,37). In this study, transversions were evident in six of 11 cases (55%), while transitions were found in only four (36%). In addition, G:C → A:T mutations at CpG were observed in only two of 11 cases (18%). This is in line with previous studies demonstrating a predominance of transversions in the p53 gene mutations in Japanese prostate cancers (χ² = 6.6, P < 0.05). In line with Carrielo’s paper, we used a computer program for comparison of two mutational spectra obtained from ‘UNCVX1.OIT.UNC.EDU’ using the Internet (38). The observed P value of 0.0002 indicates that the spectra of Japan and Western countries are significantly different. It has been reported that G:C → T:A transversions are commonly observed in lung, laryngeal and pharyngeal cancers (39–41). This mutation is representative of the specific types of damage caused by polycyclic aromatic hydrocarbons present in tobacco smoke (42). However, no specific exogenous carcinogens have been linked with development of either human prostate or breast cancers (43). The reason for the detected differences in mutational spectrum between Japanese prostate cancers and American prostate cancers is unclear, but they indicate differences in both exogenous and endogenous responsible agents.

The frequent G:C → C:G transversions detected in Japanese prostate cancers, viewed in the light of their lack of occurrence in the Western group, may be of particular significance.

It is difficult to draw firm conclusions on the basis of the small number of mutations so far detected (11 cases in our study and 18 cases in other Japanese studies). According to Greenblatt’s report, it is possible that p53 may be inactivated by epigenetic events in cancers characterized by low incidences of p53 gene mutations (4). Further studies are thus needed to enlarge the data base and confirm our present findings. Research into exogenous factors in human populations should also be combined with animal experiments to facilitate interpretation.

In conclusion, the present study demonstrates that p53 gene mutations are predominantly detected in advanced prostate cancers in Japanese men with a clear difference in the mutational spectrum of the p53 gene as compared with American and European cases. Thus the involvement of different causative agents is indicated.

Acknowledgement

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References


