Advances in Brief

Mutation, Allelotyping, and Transcription Analyses of the p73 Gene in Prostatic Carcinoma

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Abstract

A novel gene, p73, encoding a protein with significant homology to p53, was recently identified at 1p36. To investigate penetrance of p73 in prostatic carcinogenesis, mutation, allelotyping, and transcription analyses of p73 were performed in prostatic carcinoma. No types of mutation causing amino acid substitutions or frameshifts were found in 106 cases examined. Loss of heterozygosity in the gene was found in 2 of 38 cases (5.3%). Various expression levels of p73 α variant were observed in tumor compared with those in normal tissue. These data suggest that the p73 gene is not playing an essential role, but expression of p73 may associate with tumor growth in prostatic carcinogenesis.

Introduction

Recently, a novel gene was identified at chromosome 1p36.2-3 with some characteristics (1). This gene, termed p73, encodes a protein possessing similar to p53 throughout its DNA-binding, transcription, and oligomerization domains. The gene produces two splicing variants, p73 α and β; the latter lacks exon 13 at the COOH terminus. Simultaneously, an analysis using the yeast two-hybrid system showed data of strong interaction between p73 β and p53 but insignificant interaction between p73 α and p53.

Genetic imbalances of the 1p36 region are found in many types of human cancers (2-5). In prostate cancer, association of chromosome 1, including 1p36, with the tumor is still controversial (6-8). Previous reports concluded that ~20-50% of advanced-stage prostatic carcinomas possessed mutations in the p53 gene (9).

In this report, we examined mutation analysis of the entire coding region and intronic splice donor and acceptor regions of the p73 gene by SSCP, allelotyping analysis by intragenic polymorphic marker, and transcription assay by RT-PCR to investigate the penetrance of p73 in prostatic carcinogenesis.

Materials and Methods

Tissue Acquisition and Preparations of DNA and RNA. Clinical prostatic carcinoma tissues from 106 patients were collected at total prostatectomy or transrectal prostate biopsy for DNA analysis. All samples were from Japanese men. Informed consent was obtained from each patient before tissue acquisition. The samples consist of 54 cases of fresh frozen tissue and 52 cases of formalin-fixed, paraffin-embedded tissue. Simultaneously, 65 cases of corresponding normal tissue samples were prepared. For those tissues, tumor and normal areas were designated on H&E-stained sections and microdissected separately, and genomic DNA samples were prepared by standard proteinase K digestion, phenol/chloroform extraction, and ethanol precipitation protocols.

A pair of prostatic carcinoma and corresponding normal tissue, two carcinomas, and one normal prostate tissue were prepared for RNA analysis. Total RNA samples were isolated using RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The RNA quality was assessed by gel electrophoresis. Subsequently, the RNA samples were stored at -80°C until used.

Mutation and Allelotyping Analyses. Chromosome 1p, especially the 1p36 locus, is known to be frequently deleted in many types of cancers (2-5). In prostate cancers, cytogenetic data showed that chromosome 1 is one of the most frequently affected part in whole chromosomes (6). On the other hand, a report concluded that no LOH of chromosome 1, including 1p36, in prostatic adenocarcinoma was found (7). Kaghad et al. (1) identified p73 gene in the 1p36 locus, which showed lack of coding region mutations of the gene in multiple cancer cell lines (1). We screened the entire 14 exons and intronic splice donor and acceptor regions of p73 gene for mutations by SSCP analysis on 106 prostatic carcinoma. Several SSCP shifts were detected in a few exons, but all of those were polymorphisms or silent mutations, also existing in normal DNA or showing no amino acid substitutions (data not shown). Not any type of mutation causing amino acid transitions or frameshifts were found in the 106 cases examined in this study. Allelotyping analysis, which detects LOH in the p73 gene and used 65 pairs of tumor/normal DNA, showed somatic LOH only in 2 cases of 38 informative cases (5.6%; Fig. 1). Thirty cases in 106 cases of the tumor set were examined previously for p53 mutation, and four cases (13.3%) have shown mutations in p53 gene (12). These data (summarized on Table 1) suggest that the p73 gene, unlike p53, is preserved in a majority of prostatic carcinomas, and the gene does not play an essential role in prostatic carcinogenesis.

RT-PCR Analysis. Five μg of total RNA were reverse transcribed to generate cDNA, using SUPERSCRIPT II reverse transcriptase (Life Technologies, Inc., Rockville, MD) and random hexamers (Takara, Otsu, Japan) according to manufacturers' protocols. cDNA was diluted to 1:10 and amplified by PCR using a primer set covering exons 12-14 to detect expressions of both α and β variants.

Results and Discussion

Mutation and Allelotyping Analyses. Chromosome 1p, especially the 1p36 locus, is known to be frequently deleted in many types of cancers (2-5). In prostate cancers, cytogenetic data showed that chromosome 1 is one of the most frequently affected part in whole chromosomes (6). On the other hand, a report concluded that no LOH of chromosome 1, including 1p36, in prostatic adenocarcinoma was found (7). Kaghad et al. (1) identified p73 gene in the 1p36 locus, which showed lack of coding region mutations of the gene in multiple cancer cell lines (1). We screened the entire 14 exons and intronic splice donor and acceptor regions of p73 gene for mutations by SSCP analysis on 106 prostatic carcinoma. Several SSCP shifts were detected in a few exons, but all of those were polymorphisms or silent mutations, also existing in normal DNA or showing no amino acid substitutions (data not shown). Not any type of mutation causing amino acid transitions or frameshifts were found in the 106 cases examined in this study. Allelotyping analysis, which detects LOH in the p73 gene and used 65 pairs of tumor/normal DNA, showed somatic LOH only in 2 cases of 38 informative cases (5.6%; Fig. 1). Thirty cases in 106 cases of the tumor set were examined previously for p53 mutation, and four cases (13.3%) have shown mutations in p53 gene (12). These data (summarized on Table 1) suggest that the p73 gene, unlike p53, is preserved in a majority of prostatic carcinomas, and the gene does not play an essential role in prostatic carcinogenesis.
Gene Transcription Analysis. RNA samples from one normal/tumor pair, two tumors, and one normal tissue were used for RT-PCR analysis to detect expression levels of p73 α and β. The data showed relatively low level expressions of p73, compared to control (glycer-aldehyde-3-phosphate dehydrogenase), which ensured the data by Kaghad et al. (1). More intense expressions of α variant than β were observed in all samples examined. We compared the expressions in tumor cells to those in normal cells, and various expressions of p73 α transcripts were detected in tumor samples. One tumor showed strong expression (Fig. 2, Lane 4). In a pair of normal/tumor RNAs (Fig. 2, Lanes 2 and 3), p73 α was more intensely expressed in tumor than in normal tissue. As a correlation of p73 with p53, the previous report indicated relatively strong interaction of p73 β and p53 (1). Subsequent data suggested that p73 can act similar to p53 and inhibit cell growth, although different pathways of induction between p73 and p53 were envisioned by the investigators (12). At present, mechanisms of p73 induction, activation, and correlation with other genes are not clarified. Although our data in this report are too small to make a conclusion, the preliminary data show different expression levels of p73 in tumors, compared with those in normal prostate, suggesting that p73 expression may associate with tumor growth in prostatic carcinogenesis. Additional studies to explain p73 functions, regulation mechanisms, and correlation with other genes, including p53 are necessary to fully understand the roles of p73 in tumor biology.

Acknowledgments

We are grateful to Akemi Sudo and Michiko Takagi for technical assistance.

References


Table 1 Summary of clinicopathological and molecular biological data

<table>
<thead>
<tr>
<th>Age</th>
<th>Gleason's score</th>
<th>p73 mutation</th>
<th>p73 mutation</th>
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<tr>
<td>56-91</td>
<td>2-4</td>
<td>5</td>
<td>18</td>
<td>5</td>
</tr>
<tr>
<td>70-80</td>
<td>5</td>
<td>18</td>
<td>15</td>
<td>22</td>
</tr>
</tbody>
</table>

*p = 60.

Data by Watanabe et al. (13).

Average.

Percentage.

Fig. 2. RT-PCR analysis of p73 showing different expression levels between PCR products from tumor and normal RNA samples; Lane 1, size marker. Lanes 2 and 6, 5.6–91 5 113bp 207bp expressions in normal prostate. Lanes 2–5, expressions in prostatic carcinomas. Lanes 3 and 4 correlate as tumor and corresponding normal tissue, respectively. p73 α and β variants are identified as 207- and 113-bp PCR products, respectively.

Fig. 1. Example of LOH analysis of prostatic carcinoma using a polymorphic dinucleotide repeat marker on the p73 gene. The autoradiogram of gel electrophoresis comparing PCR products with tumor (T) and corresponding normal (N) DNA samples reveals LOH in Lanes 2 and 5.
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