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Advances in Brief

Mutation, Allelotyping, and Transcription Analyses of the *p73* Gene in Prostatic Carcinoma[1]

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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 possess similar to *p53* throughout its DNA-binding, transactivation, 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,[3] 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 carcinoma tissues, and one normal prostate tissue were prepared for RNA analysis. Total RNA samples were isolated using RNAzol B (Tel-Test, Inc., Friendswood, TX).

SSCP Analysis. Intron sequences surrounding exons were determined by two of the authors (Y. N. and A. N.), and 14 sets of intron-based primers were designed to amplify individual exons 1–14 of the *p73* gene.[4] PCR reactions and SSCP analysis were performed according to our standard protocol described elsewhere (10).

Allelotyping Analysis. Dinucleotide repeats in intron 9 of *p73* were used to assess LOH. Sixty-five pairs of tumor and corresponding normal DNA were examined. A primer set was designed surrounding the repeats, and PCR-based allelotyping analysis was performed as described before (11).

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.

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[3] The abbreviations used are: SSCP, single strand conformation polymorphism; RT-PCR, reverse transcription-PCR; LOH, loss of heterozygosity.

[4] Y. Nimura, M. Mihara, S. Ichimiya, N. Seki, M. Ohira, W. Adachi, J. Amano, S. Sakiyama, and A. Nakagawara. Genetic analysis of *p73*, a gene related to *p53*, in esophageal carcinoma, submitted for publication.

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 (glyceraldehyde-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.

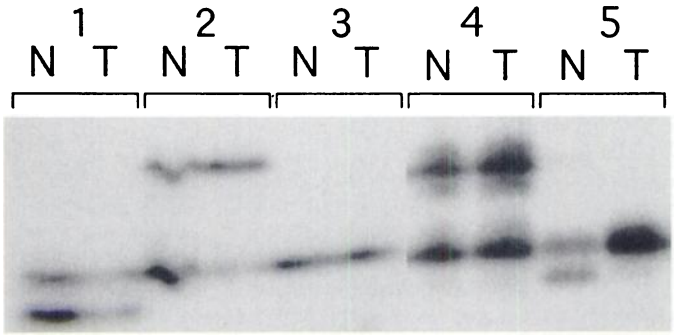

Fig. 1. Example of LOH analysis of prostatic carcinoma using a polymorphic dinucleotide repeat marker in the *p73* gene. The autoradiogram of gel electrophoresis comparing PCR products with tumor (*T*) and corresponding normal (*N*) DNA samples reveals LOH in *Lanes 2* and *5*.

Table 1 *Summary of clinicopathological and molecular biological data*

	Gleason's score[a]						
Age[a]	2–4	5–6	7	8–10	*p53* mutation[b]	*p73* mutation	LOH in *p73*
56–91	5	18	15	22	4/30	0/106	2/38
70.8[c]		6.95[c]			13.3[d]	0[d]	5.3[d]

[a] $n = 60$.
[b] Data by Watanabe *et al.* (13).
[c] Average.
[d] 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*, expressions in normal prostates. *Lanes 3–5*, expressions in prostatic carcinomas. *Lanes 3* and *2* correlate as tumor and corresponding normal tissue, respectively. *p73* α and β variants are identified as 207- and 113-bp PCR products, respectively.

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