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 \( 1p36.2-3 \) 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, \( p73 \alpha \) and \( \beta \); the latter lacks exon 13 at the COOH terminus. Simultaneously, an analysis using the yeast two-hybrid system showed data of strong interaction between \( p73 \beta \) and \( p53 \) but insignificant interaction between \( p73 \alpha \) and \( p53 \).

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.

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 \( \mu \)g 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 \( \alpha \) and \( \beta \) variants.

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, \( p73 \alpha \) and \( \beta \); the latter lacks exon 13 at the COOH terminus. Simultaneously, an analysis using the yeast two-hybrid system showed data of strong interaction between \( p73 \beta \) and \( p53 \) but insignificant interaction between \( p73 \alpha \) and \( p53 \).

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 splicing 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.

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References

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