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Investigation of Mutations in exon 7, 8 and exon 9 of FHIT Gene in Primer Prostate Cancer

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ABSTRACT

Cancer is defined uncontrolled growth that consists of the abnormality in cellular differentiation and maturation. Prostate cancer is a type of cancer, which usually occurs in men. It takes place after the lung cancer as the reason of death related with cancer among men over 50 years.
This study is planned to show the role of the gene FHIT ekson 7,8,9 which is not studied enough in prostate cancers and to emphasize the relationship between the molecular differances and FHIT protein .
In this study 50 samples of prostate cancerous solid tissues which are parafine embedded and 20 healthy control groups are used.
DNA isolation was carried out in parafine embedded tissues of the prostates followed by PCR amplification for exon 7,8,9 of FHIT gene. Then mutational analysis was carried out using SSCP technique. Our study was supported with HRM which is a process of RT-PCR .
As the result of this study, mutation was not determined in FHIT ekson 7,8,9.
KEYWORDS: Prostate cancer , FHIT, RT-PCR, HRM

INTRODUCTION

One of the urogenital cancers, prostate cancer is the most frequently found non-cutaneous malignancy in American males and ranks second among the most frequent cancers in men in the whole world with a rate of 14 % (1-2); likewise, it ranks first among cancer-related deaths in developed countries and sixth in developing countries (2). Although the cause of prostate cancer is still not known, its incidence and mortality may exhibit variation among races and ethnicities and from country to country. Several other risk factors such as old age, lifestyle, diet, impaired androgen metabolism, and environmental and genetic factors have been described for the development of the disease. Although genetic factors such as mutations and polymorphisms lead to permanent nucleotide changes in DNA, epigenetic changes cause permanent gene expression changes in DNA (3).

Relatively little information has been obtained about the molecular roots of prostate cancer although it is a common tumor (1,3-7). The transformation of normal epithelial cells into malignant cells occurs as a result of the inactivation of one or more tumor suppressor genes (TSG). The role of TSGs in prostate carcinomas has not been as well understood as in other cancers (1). One of these genes, which are also called tumor suppressor genes, is FHIT (Fragile Histidine Triad Gene). FHIT belongs to the nucleotide binding protein family from the TSG group. The FHIT gene includes human chromosome fragile zone, FRA3B, settles on 3p14.2 and is specified in many normal adult tissues and tumor cells (8,9). FHIT is a gene that is 2.5 Mb in size and involves 10 exons. This gene, which encodes the protein that plays a role in DNA replication and cell cycle control (10-13), was first identified by Sozzi et al. (1996)(14) in lung cancers.

It has been determined that inactivation of the FHIT gene results in aberrations of transcription factors in various cancers involving lung, colorectal, breast, skin cervix and head-neck cancers, and genetic mutations that include chromosomal deletions (9,15,16). In a study conducted by Fouts et al., too, it was found that FHIT gene expression in patients with prostate cancer who underwent radical prostatectomy is associated with various clinicopathological parameters. However, there are not many extensive studies regarding the fact that the FHIT gene may play a role in prostate cancers.

MATERIAL AND METHOD

Tumor Samples

Paraffine blocks of prostate pathologies were derived from the archives of the Department of Pathology in Faculty of Medicine at the University of Cumhuriyet, Turkey. Namely, Paraffine-embedded prostate cancer

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tissue specimens of 50 prostate cancer patients were used in this study. Age range was 35-72 years. 10 patients had family history of cancer and 40 patients were non family. Their prostates were examined by one or more of the following means: rectal ultrasound detection, digital rectal examination, computed tomography, and magnetic resonance imaging. Biopsy was performed for the patients who were suspected to have prostate cancer, and all specimens were from archived paraffine blocks that were collected specifically for this study. Control samples were obtained from natural or healthy tissue of the same cases. We are carried tumor, nodule and metastasis grading out as postoperative anamnesis and pathological reports of all the patients. All assays were tested as localization of tumor (T); T1, T2, T3 and T4; formation of nodule (N); N0, N1, N2, N3 and the case of metastasis (M); M0 and M1 The clinicopathological characteristics of the tumors are listed in Table 2. Furthermore, in this study we are used exon 7,8 and exon 9 spesific primers of FHIT gene for polymerase chain reaction stated in the below.

Patient Anamnesis

The study was approved by the local ethics committee, all patients gave written informed consent for participation in the study. Patients with either prostate cancer and other primary cancer, and family history of cancer in terms of patient age and gleason score and healthy individuals without prostate cancer were included in the study. In addition to working in an individual's age and eligibility criteria in terms of gleason scores already published in previous studies. Tissue samples taken from patients according to pathological findings were recruited between 2010 and 2011.

DNA Isolation Procedure

Formalin-fixed paraffin samples were cut into 10-µm sections. The sections were pulverized under liquid nitrogen condition using microdismembrator (B. Braun, Melsungen, Germany) of each sample, 0.1 g of pulverized tissue powder was resuspended in 1 ml of xylene and left for 15 minutes at 55°C. The suspension was then centrifuged at 14000 g for 5 minutes. The pellet was suspended in 0.1 ml of xylene and processed as above for the second time. The resulted sediment was mixed with 100% ethanol and processed with xylene lysis buffer (Tris, sodium dodecyl sulfate, ethylenediamine tetraacetic acid or EDTA). A lysis buffer containing 300 µg/mL of proteinase K was added to the pellet, mixed and incubated at 55°C for an overnight period. The DNA was extracted following the use of phenol-chloroform procedure, then dissolved in TE buffer (Tris-HCl and EDTA) and stored at 4°C. Furthermore, Genomic DNA was isolated from paraffin-embedded prostate cancer tissue and the normal tissue using the tissue kit (Roche).

Polymerase Chain Reaction

For polymerase chain reaction (PCR) application (Biotechnology Research Laboratory, Turkey belonging Dr. H.C.V.), increasing concentrations of extracted DNA of each specimen was tested to find out the optimum dose that resulted in good amplicon product. Exon 7,8 and Exon 9 of the FHIT gene were amplified using primer designed according to Risinger et al (4, 5). Each primer pairs of the selected exons was used for mutation detection of FHIT following the PCR for the single-strand conformation polymorphism (PCR-SSCP). Tm values or annealing Tm and primers(table 1) used for each FHIT exon were as follows:

4 µl prostate tissue genomic DNA was amplified in a mixture composed of 5 µl 10XPCR Taq buffer (pH 8.8), 2 mM $MgCl_2$, and 10 mM dNTPs (dGTP, dATP, dTTP, dCTP) at each, 0.5 mM of each primer, and 0.3 U DreamTaq polymerase (Advanced Biotechnologies Ltd., Fermantase Life Science). Amplification was submitted to polymerase chain reaction for exon7,8,9; Initial denaturation at 95°C 4 min, denaturation at 95°C 15 sec, annealing Tm at 57,5°C 30 sec, extension at 72°C 20 sec, total 35 cycles and final extension at 72°C 4 min. PCR was done on genomic DNA extracted from whole blood and parafine-embedded tissue samples using suitable primers to amplify these loci.

Imaging of PCR products

PCR products were loaded into the wells in 2 % agarose gel. Gel electrophoresis tank was attached to the power supply and electric current was applied to the gel; then, the PCR products were investigated in the gel imaging system and viewed through photographs. DNAs isolated from tissues with prostate cancer and exons 7, 8 and 9 belonging to the FHIT gene were amplified.

FHIT Gene Mutation Analysis

PCR primers

Exons 7-9 of the FHIT gene were used for mutation analysis. The oligonucleotide primers used for PCR were selected from ATQ – GenBank (Giagen firm, GERMANY). The sequences of these exons and the annealing temperatures were listed in Table 1.

Table 1. The sequences of these exons and the annealing temperatures

Locus		Primer	Tm	PCR Product
FHIT Exon-7	F	5'-TGGTCCCCATGAGAATACTATAAATTAACA-3'	60°C	304 bp
	R	3'-TTACGGCTCTAACACTGAGGGTCTCTCTGA -5'		
FHIT Exon-8	F	5'-GAGTAATTGGGCTTCATGAGAGCATCACT-3'	63°C	224 bp
	R	3'-AGGTTGATGTCATCCCACCGACAGT-5'		
FHIT Exon-9	F	5'- TTCTCCAAAGCTCCAGAAACATGACAAGGA-3'	58,5°C	119 bp
	R	3'- GTCTTTACCTGTGTCACTGAAAGTAGACCC-5'		

HRM Results

Biopsy samples were taken from 50 patients with primary prostate cancer at Sivas Cumhuriyet University Medical School Hospital Pathology Department (upon decision no. 2009/144 of Selcuk University Meram Medical School Hospital ethical board), were subjected to histopathological examination, each patient's medical history, age, TNM phase, Gleason score, cancer-proneness and family history parameter were recorded; then, 20 blood samples of healthy volunteers taken from Konya Genetikon genetic diagnosis and treatment center was subjected to HRM analysis, which is one of the RT-PCR-based techniques, for mutation analysis.

No mutation was observed in the samples analyzed in the HRM study, which was conducted to determine areas with mutation exons 7,8 and 9 of the FHIT gene of the patients with prostate cancer. When the results of the two methods we used in our study (SSCP analysis and HRM analysis) are examined, it is seen that the results confirm each other and that no mutation was identified in either method.

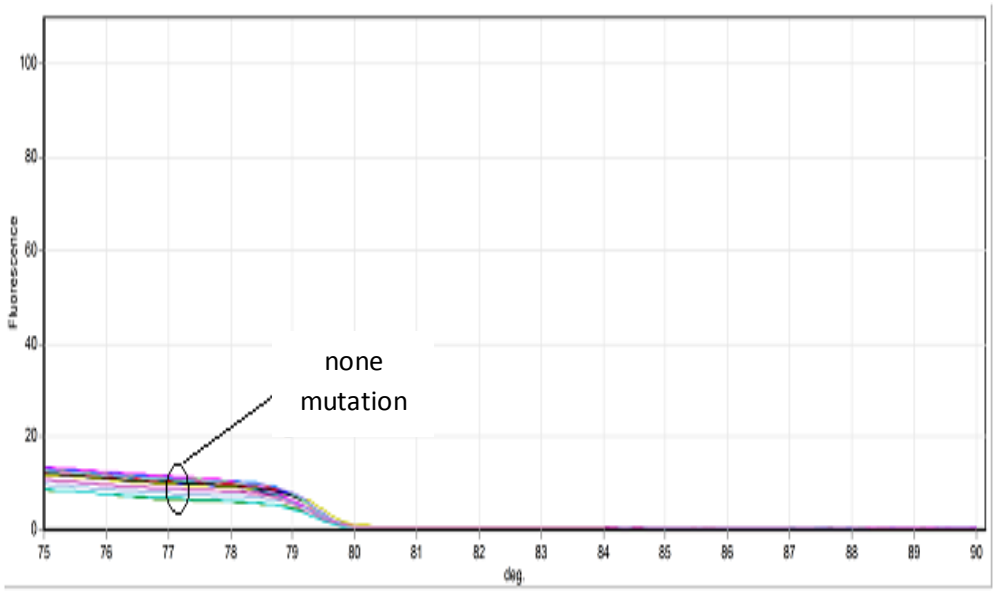

Figure 1. HRM analysis of FHIT gene exon 7

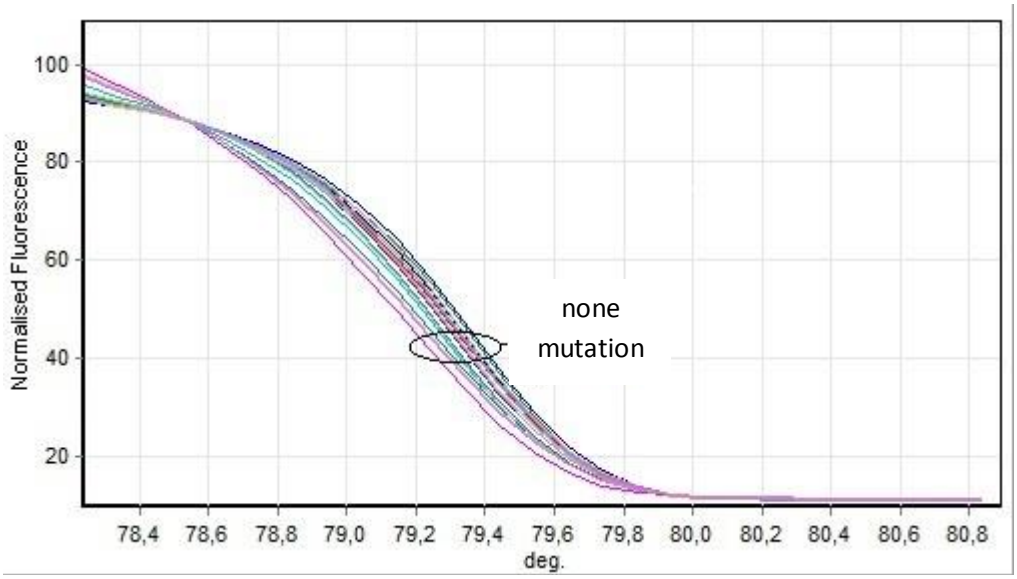

Figure 2. HRM analysis of FHIT gene exon 7

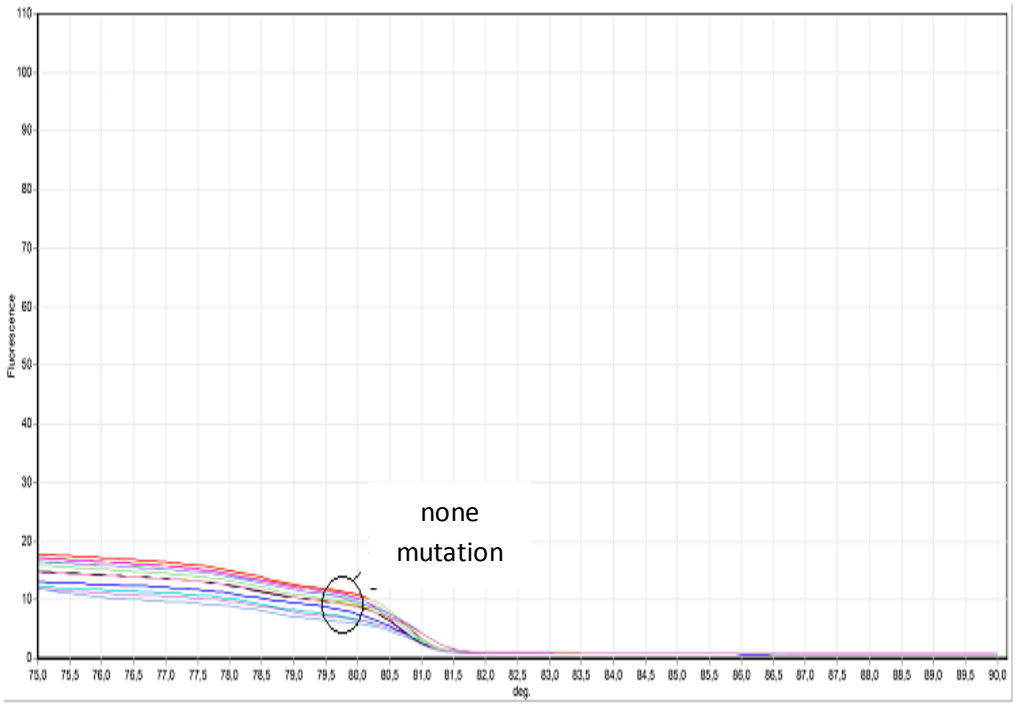

Figure 3. HRM analysis of FHIT gene exon 8

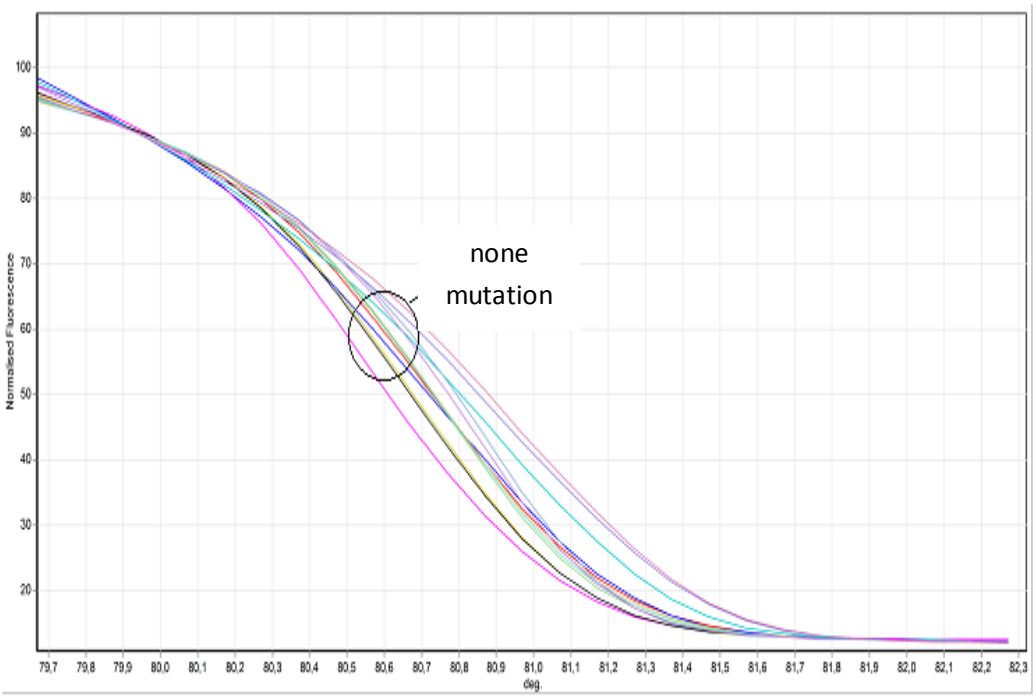

Figure 4. HRM analysis of FHIT gene exon 8

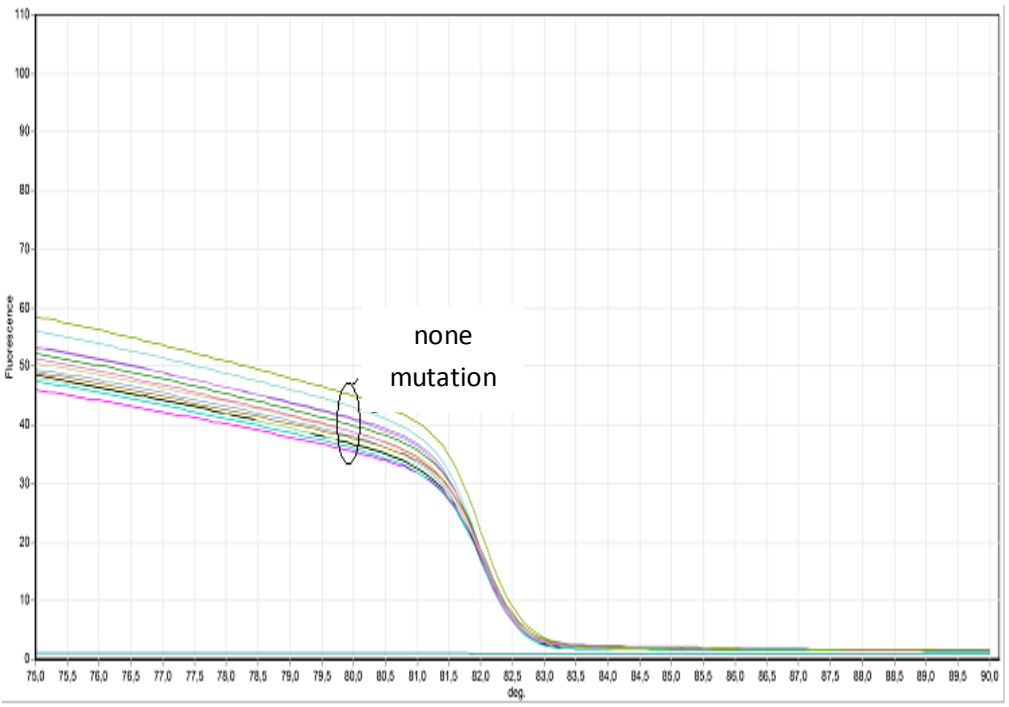

Figure 5. HRM analysis of FHIT gene exon 9

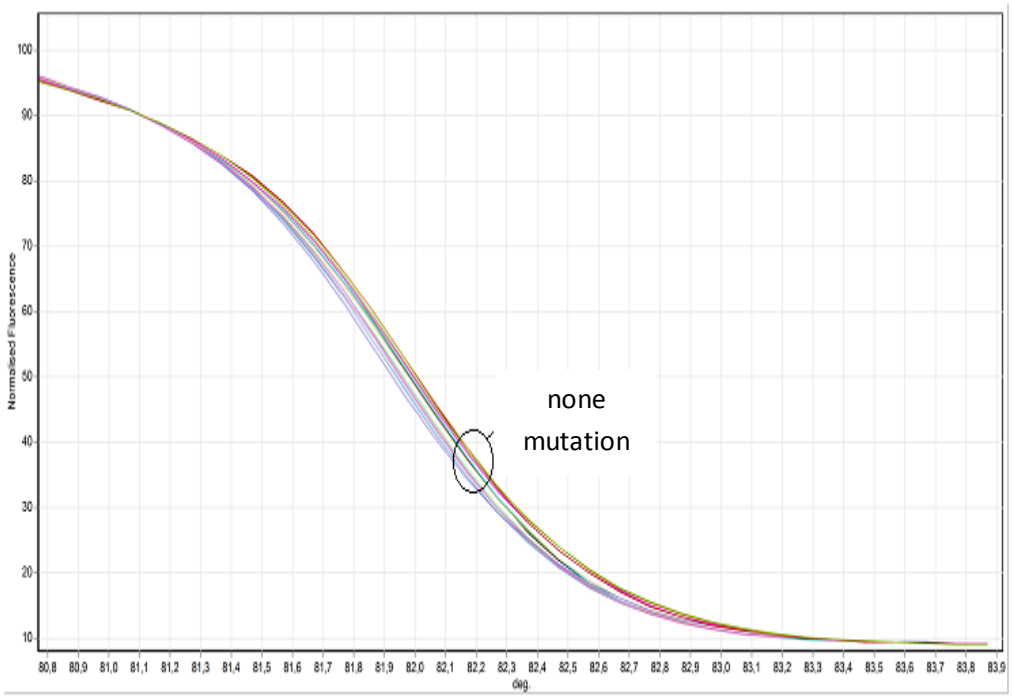

Figure 6. HRM analysis of FHIT gene exon 9

RESULTS and DISCUSSION

Prostate cancer is the top second cause of death from cancer among males and one of out of six males will develop this disease in one stage of their lives. Although it is known that it is a multifactor disease due to genetic and environmental factors, decoding of genetic factors will play a role in developing treatments for this disease through appropriate diagnosis. Large twin studies conducted in Scandinavian countries have pointed to genetic factors as important risk factors (17). However, in prevalence studies concerning this disease including ethnic groups, there was a high incidence among African Americans whereas incidence was lower among Asians, which indicated that risks for the disease were influenced by differences in locus and environmental factors (18).

As a result of successful gene replacement treatments conducted in animal models and gene transfers in tumor cell cultures, it was clearly revealed that FHIT is a tumor suppressor gene (19).

Prostate cancers include frequent genetic mutations in chromosomal and subchromosomal levels. The most frequent anomalies were identified in chromosomes 7p, 7q, 8q, Xq 8p, 10q, 13q and 16q (20).

Various studies have shown that although the FHIT gene plays an important role in human neoplasia on tumor suppressor locus of 3p chromosome in different cancer types, the branch (arm) of this chromosome has been rarely investigated in prostate cancer and some of the relevant studies focused on 3p25 in VHL locus (4,21,22).

Studies conducted in this regard revealed that mutations in the genes RNASEL (1q24-q25, HPC1), ELAC2 (17p11, HPC2), and MSR1 (8p22) were linked to prostate cancer (23-25). These studies also indicated that prostate cancer risk was also correlated with BRCA2 and CHEK2 gene mutations, which are involved in breast cancer risk and both related to DNA repair (26-28)

Mutations encountered in the FHIT gene are genetic mutations that are frequently encountered in human cancers. Potential role of the FHIT gene in human tumors has been investigated since the year 1996. The FHIT gene internal deletions have been indicated in esophagus, lung, abdomen, colon, breast, bladder, ovary, cervix, uterus, pancreas, kidney, nasopharynx and finally skin tumors. Loss of FHIT expression is linked to early result of the development of many cancers and negative prognosis of some cancers especially lung and breast cancers (29-31). There are few studies on the role of FHIT in prostate carcinomas (1).

Although working mechanism of FHIT, which is a tumor suppressor gene, is not yet fully known, it is thought to be related to apoptosis and cell cycle control (1,32). In a study they conducted, Eyzaguirre et al. (1999) (34) indicated high levels of losses in FHIT expression when they compared other renal tumor types where FHIT gene was protected with claer cell renal carcinomas. Fouts et al. (2003) emphasized, in a study they carried out on prostate carcinogenesis, that FHIT expression losses could be related to prostate cancer.

In 1998, in a study which Latil et al. conducted on 15 malignant prostate tumors and 62 normal prostate tissues in the FHIT (exon 1-10) gene using the RT-PCR method, they found mutations in two of the tumorous tissues. In another study, DeMarzo et al (2003) found that losses in tumor suppressor gene zone were related to prostate cancer. Guo et al. (2000) indicated in a study they carried out that FHIT gene mutations were early

incidents of prostate cancer. A study by Larson et al. in 2005 on prostate cancer and the FHIT (intron 5) gene implied that mutations in the FHIT gene strongly increased the prostate cancer risk.

Various studies have demonstrated that the FHIT gene on chromosome 3p has played significant roles in human neoplasm. However, the role of the FHIT gene has been studied in very few studies.

In this study, DNA amplification was performed through PCR in tissues with prostate cancer buried in paraffin and then a mutation scan was conducted in FHIT exons 7,8 and 9 using the SSCP method. Subsequent to SSCP, another mutation scan was performed using the HRM method this time. However, no mutations were encountered in either of the methods we used in our study. Since our series is small, the results should be considered as preliminary.

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