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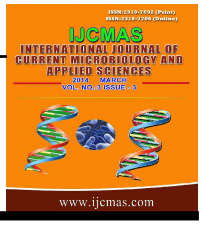


Original Research Article

A Study on PTEN Tumor Suppressor Gene Mutations in Turk Patients with Prostate Carcinoma

Meriç Tugba AKGUN[1], Nesrin TURACLAR[2*] and Hasibe Cingilli VURAL[1]

[1]Selcuk University, Department of Biology, Molecular Biology, 42079 Selçuklu, Konya, Turkey
[2]Vocational School of Health Services, Selcuk University, Konya, Turkey
Corresponding author

A B S T R A C T

PTEN is among the most commonly mutated tumor suppressor genes in human cancer. And like many other tumor suppressors, PTEN targets proteins in signaling pathways that regulate cell growth and apoptosis in healthy tissue and contributes to cancer when abnormally. Inactivation or mutation of this tumor suppressor gene is a critical step in the development of many types of cancers. This gene is important targets of investigation for the development of biomarkers and therapeutic regimes. This study would include PCR amplification of PTEN gene for the identification of heteroduplex analysis or SSCP analysis for mutation detection sequence analysis in Turk population. The present research has been examine whether there is any relation between prostate cancer cases at our working group and PTEN gene from tumor suppressor genes (exon 1 and exon 2) by using PCR/SSCP method and DNA sequence analysis. DNA isolation done through using paraffine-embedded tumor tissues of 50 patients who were diagnosed as prostate carcinoma and as control group the paraffine-embedded normal tissues of same patients, also peripheral venous blood samples of 10 patients from the same patient group. At the end of research, PTEN gene exon 2, at the end of sequence analyze, SNP (Single Nucleotide Polymorphism) at the 401st nucleotide of tumor and normal tissues was found. Literature reference number is 1903858 and determined to be found in an intronic region. Normal allele is Cytosine (C) of this sequence that's in the intronic region of PTEN gene; and it was determined that there exist Cytosine/Thymine (C/T) heterozygote in 62.5% of tumor tissues of patients and C/T heterozygote in 37.5% of normal tissues. DNA sequencing reveals that A to T insertion occurred in exon 2 of the PTEN gene. In the present study, which amplified only 1 exon at a time, we could not determine whether these mutations existed in different PTEN alleles. Patients with prostate cancer who had *PTEN* mutation had also a significantly greater GS, poorer prognosis, and higher rate of metastasis. However, this mutation cannot predict the prognosis and the GS is a more precise factor.

Keywords

TNM;
Tumour and Nodes and Metastases;
PCR-SSCP;
Polymerase Chain Reaction;
Single Strand Conformation Polymorphism.

Introduction

Prostate cancer is the most common malignancy in men and majority leading cause of cancer deaths in the Eastern world. The genetic predisposition to

prostate cancer is well established, as genomic instability is a common feature of many human cancers. Epidemiological studies have suggested that several risk factors (Li *et al*., 1997; Steck *et al*., 1997; Magnusson *et al*., 1998; Marshall, 1991).

Knowing about the genetic markers of prostate cancer in men with prostate cancer diagnosis could help. Inactivation or deletion a large number of tumor-related genes, which otherwise regulate normal cellular growth and suppression of abnormal cell proliferation, is recognized to be one of the major mechanisms of tumorigenesis (Goddard and Solomon,1883; Waite *et al*., 2002). Although phosphatase and tensin homologue deleted on chromosome 10 (PTEN) localization in the nucleus and cytoplasm is established, the mechanism is unknown. PTEN is a tumor suppressor phosphatase that causes cell cycle arrest and/or apoptosis. The strongest candidate for the target gene of 10q deletion is the PTEN gene at 10q23.3, which was designated PTEN, MMAC1. PTEN/MMAC1, a potential human tumor suppressor gene, has been found to have inactivating mutations in several types of cancer, including prostate cancer. The PTEN tumor-suppressor gene, also known as MMAC1 (mutated in multiple advanced cancers) is located on chromosome 10q23.3 (Marshall, 1991; Goddard and Solomon,1883; Waite *et al*., 2002; Dong *et al*., 1998). PTEN gene has 9 exons encoding 403 amino acids make up protein of a couple specific phosphatase with putative actin-binding and tyrosine phosphatase domains (Li *et al*., 1997). Mutations of the PTEN gene have been described in several types of tumors in different frequencies of the endometrium, brain, prostate, breast and kidney. Furthermore, it was that the PTEN gene might regulate tumor cell and metastasis (Waite *et al*., 2002).

Our objective was to identify PTEN mutations in allelic alteration at specific chromosome loci in the tissue and blood samples of familial and non-familial prostate from Turkey patients. To this end, we used single strand conformation polymorphism (SSCP), and direct sequencing methods to analyze mutations of PTEN/MMAC1 in 50 cases of prostate cancer in Turkey (Table 2). We conclude that PTEN mutation play a role in the development of the majority of transitional cell tumors of the prostat, melanoma and testes cancer. In addition, in the future studies, differential gene expression pattern is also being evaluated in these patients.

Materials and Methods

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 tissue specimens of 50 prostate cancer patients were used in this study. Age range was 35-72 years, all patients were males. 10 patients had family history of cancer and 40 patients were non family. These patients went to physicians to be demonstrate a variety of serious symptoms of prostate cancer, e.g, difficulty in voiding, urodynia, urgent and frequent urination, and hematuria. 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 1 and exon 2 spesific primers of PTEN 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 (see Table 2) 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 1 and Exon 2 of the PTEN gene were amplified using primer designed according to Risinger *et al* (7, 8). Each primer pairs of the selected exons was used for mutation detection of PTEN/MMAC1 following the PCR for the single-strand conformation polymorphism (PCR-SSCP). The PCR protocol was carried out as outlined in Table 1, Tm values or annealing Tm and primers used for each PTEN 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

Table.1 PCR primers of PTEN gene exon 1 and 2 including locales and annealing Tm values of these primers.

No	Exon	Locus	Sequence of Primers	Amplified Fragments(bp)	Tm (^{o}C)
1	**2**	PTEN-Ex2-F PTEN-Ex2-R	TGACCACCTTTTATTACTCCA TACGGTAAGCCAAAAAATGA	367	60
2	**1**	PTEN-Ex1-F PTEN-Ex1-R	TCTGCCATCTCTCTCCTCCT CCGCAGAAATGGATACAGGT	177	60,5

Table.2 TNM stages of the clinicopathological characteristics of prostate tumors. Clinical features of the 5 prostate cancer patients selected for examination of the PTEN gene are illustrated in the bottom. The clinical data include age at diagnosis in years, Gleason score of the primary tumor, prediagnosis serum prostate-specific antigen values, and other primary sites of cancer. In the table, particularly, were showed 5 patients with prostate cancer which was done Mutation determination. Case numbers, patient ages and gleason scores were illustrated in case of the quadruplets respectively in this table. NA, not available. Mutation defined patients were shown as bold colour.

Prostate cancer characteristics			**Personal history of cancer**	**Family history of cancer**
Case No	**Patient Age**	**Gleason Score**	**Other Primary Cancer**	**Other Cancers in Family**
1, 2, 3, 4	49, 58, 52, 51	5, 5, 5, 5	No, No, kidney, testes	No, No, No, Lung
5, 6, 7, 8	60, 65, 63, 75	6, 7, 6, NA	No, liver, No, kidney	No, No, No, liver
9,10, 11,12	47, 45, 55, 49	5, 5, 6, 5	No, No, testes, No	No, No, liver-pancreas, No
13, 14, 15 16	49, 58, 72, 57	5, 6, 9, 5	No, No, testes-kidney, lung	No, No, Liver, No
17, 18, 19, 20	69, 69, 59, 55	7, 7, 6, 5	Testes, testes, No, liver	Testes, stomach, No, pancreas
21, 22 23, 24	71, 73, 70, 66	9, 9, 8, 7	Lymph node, testes, kidney, liver	Testes, pancreas, kidney, No
25, 26, **27**, 28	NA, 51, **59**, 55	9, 5, **7**, 6	No, No, **testes-bladder**, No	No, No, **colon**, No
29, 30, 31, 32	58, 64, 51, 61	5, 6, 5, 5	No, colon, No, No	No, No, No, No
33, 34, 35, **36**	NA, NA, 56, **65**	NA, NA, NA, **7**	No, No, testes, **testes-colon**	No, No, No, **colon-brain**
37, 38, 39, 40	**74**, NA, 88, 83	**8**, NA, NA, NA	**Testes-melanoma**, No, testes, kidney	**Melanoma**, No, No, liver
41, 42, 43, **44**	NA, NA, 58, **61**	6, 5, 7, **8**	No, No, lung, **melanoma**	No, No, No, **No**
45, **46**, 47, 48	66, **64**, 69, 55	6, **7**, 5, 5	Kidney, **testes**, liver, No	No, **No**, No, lung
49, 50	51, 81	7, NA	No, bladder	No, No

(Advanced Biotechnologies Ltd., Fermantase Life Science). Amplification was submitted to polymerase chain reaction for exon1; Initial denaturation at 94°C 4 min, denaturation at 94°C 1 min, annealing Tm at 60,5°C 1 min, extension at 72°C 15 s, total 30 cycles and final extension at 72°C 3 min. As for exon 2 PCR amplification conditions; initial denaturation at 94°C 4 min, denaturation at 94°C 15 s, annealing Tm at 60°C 30 s, extension at 72°C 15 s following 10 cycles and at 94°C 15 denaturation, at 58°C 20 s annealing, at 72°C 15 s extension and 20 cycles, end step at 72°C 3 min final extension. For this exon 2 reaction was effected as two progressive. PCR was done on genomic DNA extracted from whole blood and parafine-embedded tissue samples using suitable primers to amplify these loci. Amplified PCR products were screened for genotyping of loci by SSCP (single stranded conformation polymorphism) using non-radioactive method. PCR products were separated by electrophoresis on 2% agarose gel in 1XTAE buffer (45Mm Tris, 1mM EDTA, pH 8), stained with ethidium bromide. In order to avoid non-specific reactions, PCR mixture should be prepared and maintained under cool conditions (4^{o}C) (Figure 1 and 2).

Heteroduplex Analysis by SSCP

Each of the primary prostate cancers was first screened for mutation by using the PCR-SSCP approach. Primers used for each PTEN exon were the same as described previously (16). The intron-exon structure of PTEN has been reported previously primers were designed to amplify each of the two exons (primer details and conditions are available on request). PCR products (5 μl) were denatured and electrophoresed through an 8% polyacrylamide gel containing 5% glycerol, using 0.5 TBE as the running buffer. Electrophoresis was at 1 watt per gel for 12-18 hours depending on the size of PCR products. DNA bands were visualised by silver staining.

Sequence Anaysis of Mutation Locies

For 50 samples received from patients with prostate cancer, genomic DNA was extracted and quantified from 10 whole blood samples and matched control samples. Two exons of PTEN genes were amplified using specific primers. None - radioactively labelled Heteroduplex gel analysis was done for identification of sequence variants of PTEN gene. Shifts in Exon 2 of PTEN gene with three visible patterns were seen in samples and controls. Sequencing later confirmed these to be polymorphism (Figure 3).

SSCP Analysis of Genomic DNA and Direct Sequencing

Since point mutations, small insertions, and small deletions of PTEN/MMAC1 have been reported in several cancers (Risinger *et al*., 1997; Sakurada *et al*., 1997; Kwabi-Addo *et al*., 2001; Butler *et al*., 2005; Scanga *et al*., 2000; Hlobilkova *et al*., 2000; Whiteman *et al*., 2003; Wu *et al*., 2003; Ronco *et al*., 1996) we performed non-isotope SSCP analysis to screen for such changes. Details of the method have been described (20, 21, 22). In brief, two sets of primers were designed to cover the full coding region of nine exons of PTEN/MMAC1 (Table 1). PCR amplification was performed with each set of primers for 30 cycles in a BioRad thermal cycler in which each cycle included denaturation at 94°C 1 min, annealing Tm 60,5°C 1 min, extension 72°C 15 s, total 30 cycles and final

Figure.1 Screening of ethidium bromide staining of the PCR products (177 bp) for exon 1 PTEN gene amplified of humans with Prostate cancer. Subtle bands are detected at a rightmost lane.

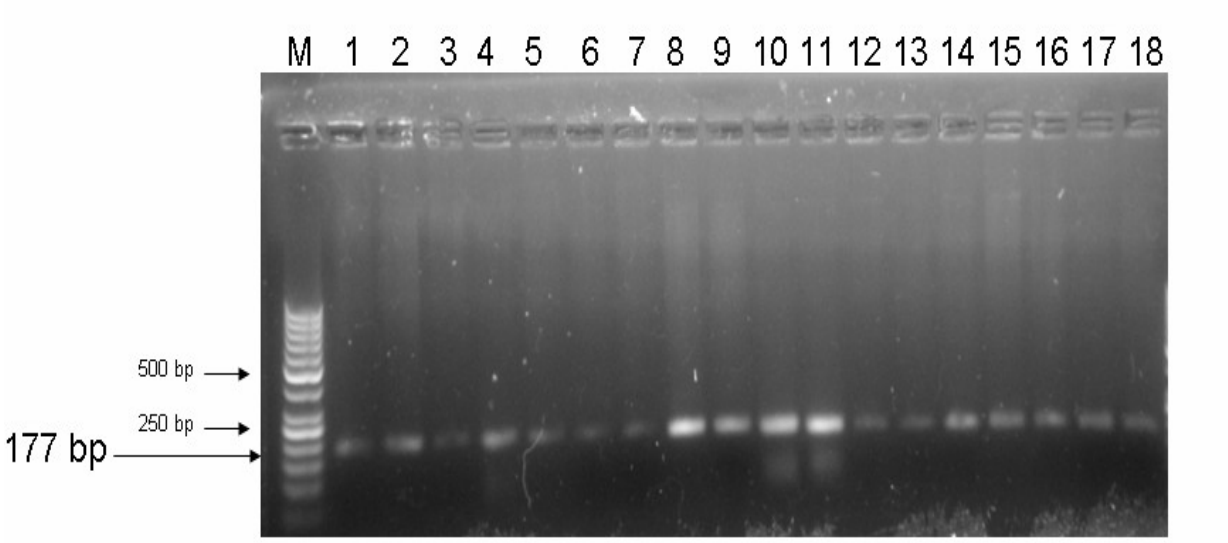


Figure.2 Screening of ethidium bromide staining of the PCR products (367 bp) for exon 2 PTEN gene amplified of humans with Prostate cancer. Subtle bands are detected at a rightmost lane. Exon 2 PTEN gene-specific primer was used for mutation detection in prostate cancer patients.

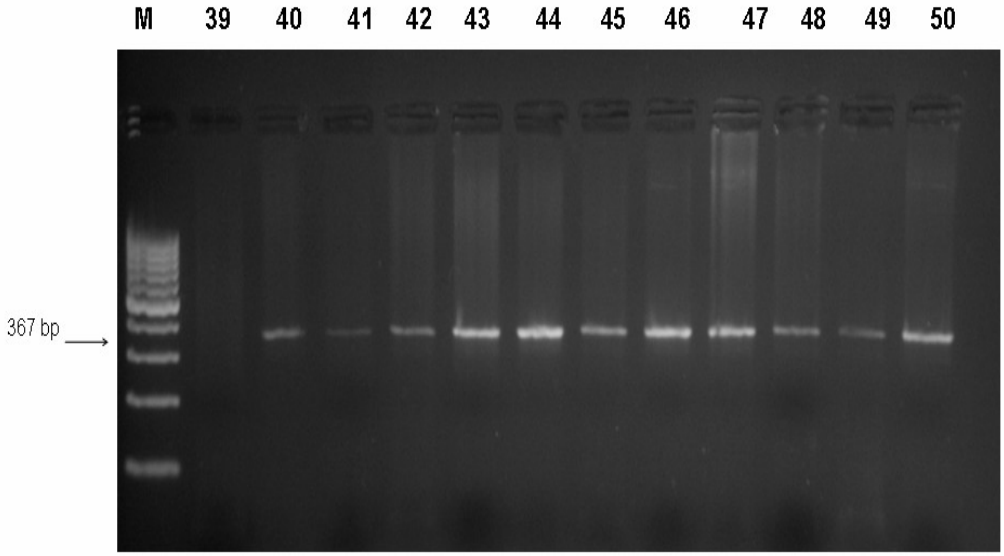
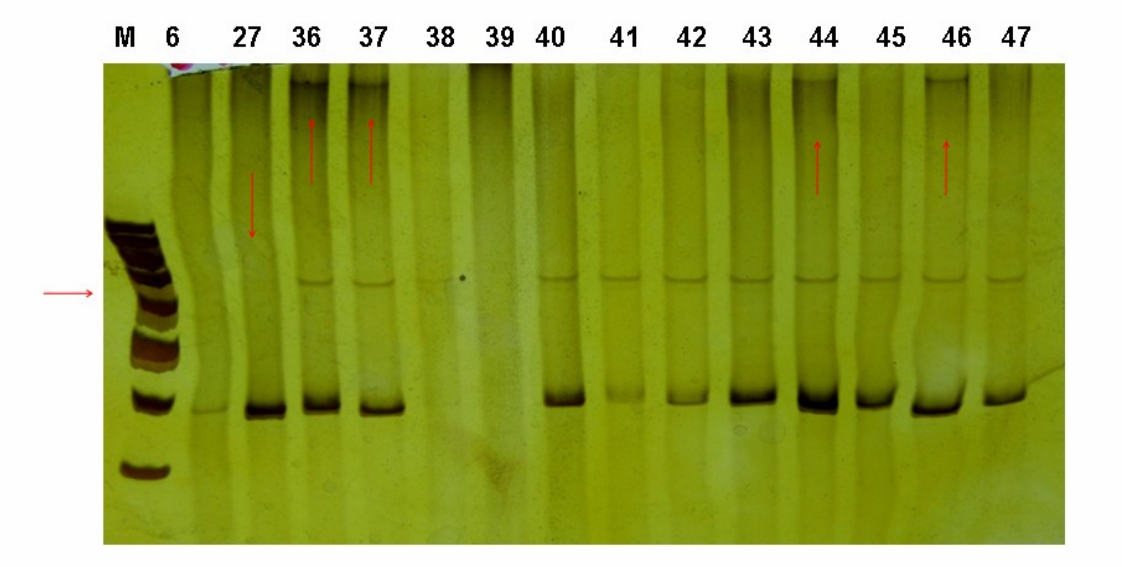


Figure.3 Single Stranded Conformation Polymorhism (SSCP) of 6 prostate tumor DNA samples for PTEN exon 2. Six DNA samples (respectively, 27, 36, 37, 44 and 46) were isolated from prostate tumors and polymerase chain reaction-amplified for exon 2. The above banding pattern represents the major single stranded conformational states of the DNA. Initial band line for exon 2 refers to a slightly shifted alternate band present in six of the samples, whereas band 367 bp refers to the more common position of this band. Namely, Heteroduplex analysis of the tumor and control samples in which shifts in exon 2 of PTEN gene were seen. Five patterns are visible.



extension 72°C 3 min. 94^{o}C for 1 min, annealing for 1 min at the temperature shown in Table 1, For exon 2 with abnormal SSCP patterns, the corresponding PCR products were sequenced by the cycling sequencing method in an ABI 310 automatic sequencer (TUBITAK-MAM, Turkey). On bands in exons 1, and 2, in which single stranded conformation polymorphism (SSCP) showed reproducible shifts , DNA sequencing was performed. That is to say, tumor DNAs were subjected to PCR-SSCP analysis to search for mutations. Aberrant bands revealed by single strand conformational polymorphism analysis (Figure 3) and this PCR products submitted to for direct sequencing. For exon 2 was screened mutations in the PTEN gene. Nonetheless, using SSCP, 5 samples of DNA isolated from tumors of patients with prostate cancer were initially screened for possible mutations, beginning with exon 2. No reproducible banding abnormalities were noted throughout the exon 1 and exon 2 for the other 45 cases.

Statistical Analysis

The difference in the frequency of PTEN mutations between primary tumors in the current study and that of our previous study (Ambrosone *et al*., 1996) was analyzed statistically by the use of Fisher's exact test. $P < 0.05$ was taken to indicate statistical significance.

Figure.4 Sequence electrogram of prostate cancer patients showing mutation heterozygote sequence C/T for PTEN gene; **4.1** SNPs genotyped across the PTEN locus in the tissues with Prostate cancer or Control Sequence for T in normal sequence; **4.2** Screening of SNP mutation or rs1903858 in PTEN gene sequence in human

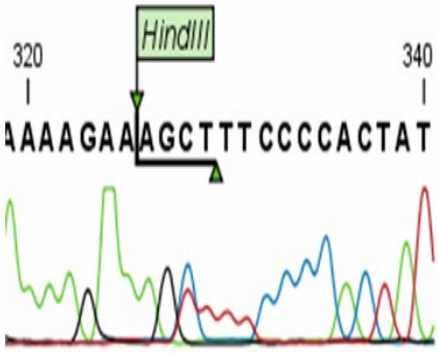


Figure 4. Heterozygote Sequence C/T

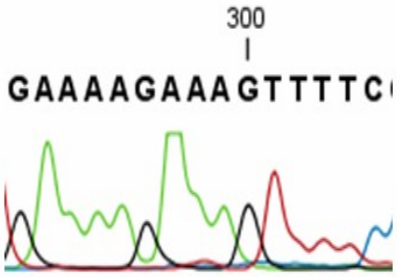


Figure 4.1. Control Sequence for T in normal sequence

Figure 4.2. Screening of SNP mutation or rs1903858 in PTEN gene sequence in human

Figure.5 Normal PTEN gene sequence in Human; **5.1** T insertion at 44. forward in patients with prostate cancer; **5.2** T insertion at the 44. reverse in patients with prostate cancer

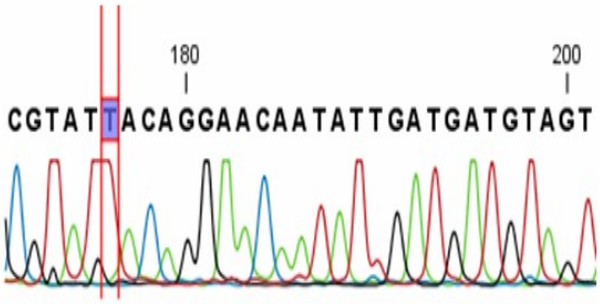


Figure 5. 1. T insertion at the 44. forward site in patients with prostate cancer

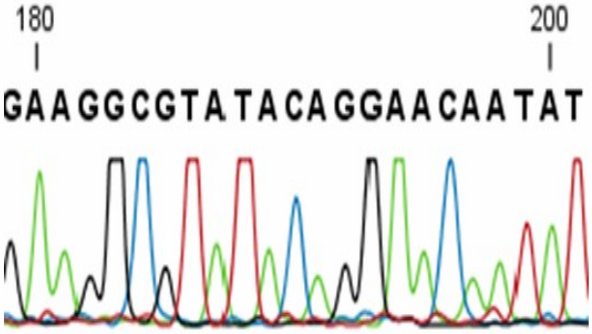


Figure 5. Normal PTEN gene sequence in Human

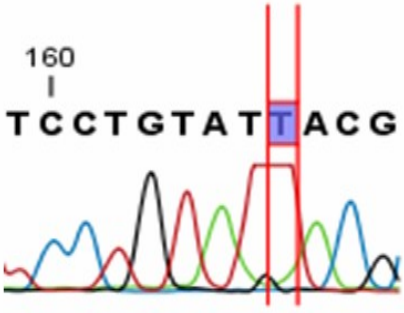


Figure 5. 2. T insertion at the 44. reverse site in patients with prostate cancer

Results and Discussion

Deletions of genomic regions involving tumor suppressor genes are thought to be important in the initiation and progression of all cancer risk. Segments of the genome frequently deleted in breast and prostate tumors and known or suspected to contain tumor suppressor genes are located on several chromosome arms, including arms such as 1p, 1q, 6q, 8p, 9p, 11p, 11q, 13q, 16q, l7p, 17q, (13, 14, 18, 20). Only a few cases have involved deletion at the 10q23.3 region, which is the location of the PTEN/MMAC1 gene (Risinger *et al*., 1997; Sakurada *et al*., 1997). Mutations of the tumor suppressor gene PTEN have been reported in patients with prostate cancer and in several malignant tumors. In the current study, we examined prostate cancer for mutations of PTEN and LOH at the loci flanking the PTEN gene to determine the origin of the synchronous tumors at the molecular level. We found frequent allelic losses at 10q23.3 and mutations of the PTEN gene in these carcinomas and identified PTEN as an important molecular marker that may contribute to a reliable differentiation between a metastatic disease and a process of two independent tumors. There were observed differences among studies on the frequency of PTEN mutation in prostate cancer patients, largely because of differences in tumor grade and stage in the study populations. For example, mutations up to 60% have been detected in studies of prostate cancer cell lines and xenografts from metastases (Whiteman *et al*., 2003). The obstacles to the development of gene-based human therapeutics are significant, but the rewards are great. Recent developments in molecular biology together with the ability to individualize molecular profiles, indicate a promising future for gene therapy for prostate cancer.

Clinical information describing the 50 patients and their family is provided in Table 2. The average prostate age of prostate cancer diagnosis in the 50 men reported here was 64 years (range, 59-74 years). Five of the cases of prostate cancer were assigned a Gleason score of 7; the remaining 13 cases were Gleason score 2 or 3 tumors. Twenty five of the 50 men had a serum prostate-specific antigen value of 10< ng/ml at the time of diagnosis of prostate cancer. 27, 36, 37, 44 and 46 patients of protocol numbers had extraordinarily high serum prostate-specific antigen value >20 ng/mg and were found to have widespread metastatic disease at presentation. The other 13 patients had at least one primary malignancy including; liver, testes, colon and kidney cancer. Furthermore, 25 of the 50 individuals reported that at least one family member had been previously diagnosed with prostate cancer. NA; 7 patients were not available. In brief, 5 cases mutation; 25 cases normal; 7 cases NA; 13 cases primary malignancy tumors (Table 2 and Figure 3). The two (exon 1 and exon 2) of all nine exons of the PTEN gene were studied in this study and screened for mutation in the 50 men with prostate cancer from 5 unrelated families.

The present research has been examine whether there is any relation between prostate cancer cases at our working group and PTEN gene from tumor suppressor genes (exon 1 and exon 2) by using PCR/SSCP method and DNA sequence analysis. DNA isolation done through using paraffine-embedded tumor tissues of 50 patients who were diagnosed as prostate carcinoma and as control group the paraffine-embedded normal tissues of same patients, also peripheral venous blood samples of 10 patients from the same patient group. At the end of research,

PTEN gene exon 2, at the end of sequence analyze, SNP (Single Nucleotide Polymorphism) at the 401st nucleotide of tumor and normal tissues was found. Is literature reference number is 1903858 and determined to be found in an intronic region. Normal allele is Cytosine (C) of this sequence that's in the intronic region of PTEN gene; and it was determined that there exist Cytosine/Thymine (C/T) heterozygote in 62.5% of tumor tissues of patients and C/T heterozygote in 37.5% of normal tissues. DNA sequencing reveals that A to T insertion occurred in exon 2 of the PTEN gene. Primer sequences of the PTEN exon 1 and exon 2 genes used in this study are shown in Table 1. Any change has been determined at exon 1. Namely, Exon 1 did not show any mutation, regardless of the presence or absence of loss of heterozygosity. The results as age, and TNM classification of patients in our present group, has been informative that it is concluded at the prostate carcinogenic epidemiology (Figure 4, 4.1, 4.2, 5, 5.1 and 5.2) and (Table 2). Consequently, No missense or nonsensegerm-line mutations were discovered in any of the 50 prostate cancer patients. There are no definitive clinical or pathological criteria that allow one to differentiate between the inherited and sporadic forms of the disease (Ambrosone *et al*., 1996). The candidate gene approach is an effective strategy to complement linkage studies. The PTEN gene is a logical candidate gene for hereditary prostate cancer for several reasons. PCR-SSCP and direct DNA sequencing analyses of these samples revealed PTEN sequence alterations in 5 cases. Examples of band shifts for tumors in SSCP assay, which indicated the existence of sequence alterations in the PTEN gene, are shown in Figure 3, and examples of DNA sequencing ladders that identify PTEN mutations are shown in Figure 4, 4.1, 4.2, 5, 5.1 and 5.2 and Table 2.

In conclusion, our study of prostate cancer patients with personal and/or family history of prostate and other cancers failed to reveal germ-line PTEN mutations. Our data, therefore, suggest that the PTEN gene is unlikely to play a significant role in the inherited predisposition to prostate cancer. We also analyzed metastases of prostate cancer from Turkey men, using the methods of PCR amplification and direct DNA sequencing. Seven cases showed PTEN mutations. A total of 50 prostate patients were analysed for PTEN gene mutations. These included the 5 patients were observed gene mutations for PTEN gene. The coding sequence of the PTEN gene was amplified in 2 single exon fragments and analysed by SSCP and ABI sequencing. Furthermore, In this study of PTEN in prostate cancer from Turkey patients, who were diagnosed with clinical symptoms but without the aid of the serum PSA screening test and the majority of tumors were high grade, PTEN mutations occured in 5 of 50 (12%) cases. Consistent with mutation studies, loss of PTEN expression has also been shown to correlate with higher grade of primary tumors. The PCR-SSCP technique is economical, convenient, fast, safe and popular in clinical research. This suggested that we can use the method paticularly for older suspicious patients for the early diagnosis of prostate cancer. In this study was identified 1 single-nucleotide polymorphisms (SNPs) in genes in PTEN exon 2. The SNPs showed evidence of an association with prostate cancer at rs1903858 model. Namely, In the tissues of patients with prostate cancer were observed PTEN SNP which was polymorphic in our sample and selected for genotyping at intron boundary or

rs1903858. Exon 1 was observed any mutation which is determined by SSCP technique. As exon 2 observed band diversity 12% in polymerase chain reaction products of DNA samples belonging to 5 patients. These varieties were band present or absent in tumor tissue. The diversity of band was determined which is single nucleotite polymorhisms in tumor and normal tisssues 401. in nucleotite as a result of DNA sequence analysis. Polymorphism was showed in the other etnic groups and detected in intronic loci that is 1903858 of reference number. We conclude that PTEN mutation play a role in the development of the majority of transitional cell tumors of the prostat, melanoma and testes cancer. Whether primary prostate tumors with PTEN mutations have a greater tendency to metastasize than those of similar grade and stage without mutations remains to be determined. In the present study, which amplified only 1 exon at a time, we could not determine whether these mutations existed in different PTEN alleles.

Since our series is small, the results should be considered as preliminary. However, it appears that survival is not only related to good care and treatment, but is also, at least in part, genetically determined.

We subsequently investigated the presence of point mutations in exon 1 and exon 2 fragments of the PTEN gene by SSCP analysis, a technique that is sensitive enough to detect more than 80% of mutations The screening of almost the entire coding sequence (98%) of PTEN revealed band migration shifts, for exon 2, in four primary tumours (see example in Figure 3).

PTEN gene mutations have several aspects for prostate cancer development. Exon 2 of the PTEN gene includes part of the DNA binding domain of this trancription factor. Analysis of the sequencing data showed significant number of variants in the tumor samples, These data have important implications for selecting appropriate technologies for cancer genome projects as well as for understanding prostate cancer progression.

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