

The *IGSF1*, *Wnt5a*, *FGF14*, and *ITPR1* Gene Expression and Prognosis Hallmark of Prostate Cancer

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Abstract

Background: Prostate cancer is considered as the second leading cause of cancer related death in men worldwide and the third frequent cancer among Iranian men. Despite the use of PSA as the only biomarker for early diagnosis of prostate cancer, its application in clinical settings is under debate. Therefore, the introduction of new molecular markers for early detection of prostate cancer is needed.

Methods: In the present study we intended to evaluate the expression of *IGSF1*, *Wnt5a*, *FGF14*, and *ITPR1* in prostate cancer specimens by real time PCR. Biopsy samples of 40 prostate cancer cases and 41 healthy Iranian men were compared to determine the relative gene expression of *IGSF1*, *Wnt5a*, *FGF14*, and *ITPR1* by real time PCR.

Results: Our results showed that *Wnt5a*, *FGF14*, and *IGSF1* were significantly overexpressed in the prostate cancer patients while the mean relative expression of *ITPR1* showed a significant decrease in PCa samples compared to healthy controls.

Conclusions: According to results of the present study, the combination panel of *IGSF1*, *Wnt5a*, *FGF14*, and *ITPR1* genes could be considered as potential genetic markers for prostate cancer diagnosis. However further studies on larger populations and investigating the clinicopathological relevance of these genes is needed.

Keywords: *FGF14*, *IGSF1*, *ITPR1*, Prostate Cancer, *Wnt5a*.

Introduction

Prostate cancer (PCa) is the second leading cause of cancer related death in men worldwide (1, 2). It is considered as the third frequent cancer among Iranian men and incidence of the disease is increasing in Iranian population (3). The rate of detection of PCa in Europe and the United States is higher than most Asian countries (4). Prostate cancer tends to develop in the men beyond the age of fifty and unfortunately many cases remain asymptomatic. Two-third cases of prostate

cancers are reported to grow slowly, however there are some cases of aggressive cancers (5). Prostate cancer diagnosis and adequate staging are key determinants of the clinical outcome of the disease. Despite the fact that introduction of prostate-specific antigen testing has increased the rate of PCa diagnoses and decreased the number of patients dying from PCa (6, 7), it is subject of debate mainly due to the possibility of over detection, which leads to over treatment (8). A large number of research

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have focused on finding new biomarkers for early detection and management of PCa, especially for aggressive cases. The established risk factors for PCa include age, race/ethnicity, and family history (9), however other factors such as food intake and genetics have received substantial attention. Epidemiological studies have pointed to the effects of genetic background on the incidence of PCa (10) and several studies have been conducted with the purpose of finding new biomarkers for early detection of PCa.

The immunoglobulin superfamily member 1 (IGSF1) gene is located on Xq26.2 and encodes a highly conserved plasma membrane glycoprotein in mammals. IGSF1 is present in many tissues such as brain, pancreas, testis, and muscles (11). The normal function of IGSF1 is yet to be determined. The protein is co-translationally cleaved into an amino-terminal domain (NTD) and a carboxy-terminal domain (CTD). The NTD remains in the endoplasmic reticulum while the CTD is transported to the plasma membrane (12). Pathogenic mutations in IGSF1 have been reported to inhibit the trafficking of CTD to the plasma membrane. Abnormal function of IGSF1 has been associated with testicular enlargement, prolactin hyposecretion, central congenital hypothyroidism, and delayed puberty (11). It was also reported that IGSF1 was significantly upregulated in thyroid cancer patients compared to normal tissues (13).

The Wnt family of glycoproteins consists of structurally related signaling proteins which have been implicated in various developmental processes, such as determining the cell fate during embryogenesis (14). Wnt5a is a secreted glycoprotein of the Wnt family which is involved in both canonical and non-canonical WNT pathways (15). It binds to the seven transmembrane receptors, frizzled-5, and the tyrosine kinase orphan receptor 2. Wnt5a plays important roles in organ development, cell proliferation and migration, tissue orientation and cell survival (16). The role of Wnt5a in the development of normal organs such as prostate has been documented in several studies. It has been reported that

aberrant expression/activation or inhibition of Wnt5a signaling is involved in the progression of different cancers, acting as an oncogenic or tumor suppressor factor (17). It has also been reported that as a result of hypo-methylation in the promoter of the Wnt5a gene, it is elevated in prostate cancer cells compared to normal prostate cells (18).

Inositol 1,4,5-trisphosphate (IP3) receptors are ligand-gated ion channels expressed in nearly all animal cells. They are activated by Inositol trisphosphate and intracellular calcium (19). IP3 receptors are localized to subcellular membranes, such as the endoplasmic reticulum, and their ligation results in the release of intracellular calcium (20). Inositol 1,4,5-trisphosphate receptor type 1 (ITPR1) gene encodes an intracellular receptor for IP3 and multiple transcript variants have been identified for this gene. Binding of ITPR1 to inositol 1,4,5-trisphosphate results in the release of calcium from endoplasmic reticulum. ITPR1 is mostly expressed in thyroid, brain, prostate, and other tissues (21).

Fibroblast growth factor-14 (FGF14) gene is located on chromosome 13q33 and encodes the FGF14 protein which is predicted to localize to the nucleus and belongs to the FGF homologous factors, FHF, previously known as FGF11–FGF14 (22, 23). The FHF family show high sequence identity with fibroblast growth factors (FGF) family members but do not bind to FGF receptors (24). FHFs are predominantly expressed in the nervous system and may play a role in the development and/or function of the nervous system and correlate with neurologic and psychiatric disorders (25). FGF14 is known as a key regulator of KCNQ K⁺ channels and voltage-gated sodium channels in the nervous system (26). It has been also reported that FGF14 deficiency in mice resulted in ataxia and a paroxysmal hyperkinetic movement disorder. On the other hand, patients with autosomal dominant cerebral ataxia showed a loss of function mutation in FGF14 (27, 28). According to a comprehensive analysis of transcriptome and methylation microarrays the

contribution of FGF14 in the development of cervical cancer is also relevant (29).

In the present study we intended to evaluate the relative expression of IGSF1, Wnt5a, ITPR1, and FGF14 in Iranian prostate cancer cases compared to normal controls in order to find potential new biomarkers for early detection of PCa

Materials and Methods

Selection of Patients and Tissue Sampling

To collect the required specimens, 40 prostate cancer patients under treatment at Shahid Akbar Abadi Hospital (Tehran, Iran) were selected during Feb-Dec 2018. The Biopsy test results have been taken as criteria for verification of the cancer type, while no age limitation has been considered during case selection. Smoking habits and familial history of prostate cancer encounter were documented and the severity of PCa for each and every patient was measured using TNM (tumor, node, and metastasis) staging system. As the control group, 41 healthy volunteers without any history of malignant or urological diseases were sampled.

Sample Preparation and RNA Extraction

Prostate tissue samples obtained from all cases were stored in liquid nitrogen until RNA extraction. Super RNA Extraction Kit for Tissue & Culture Cells (Favorgen Biotech Corp, Taiwan) was used to extract the total RNA according to the manufacturer's instructions. The optical density (OD) of each sample at 260 and 280 nm (measured using Ultrospec 2100, Biochrom, USA) was used as criteria to determine the amount of each sample. Reverse transcription of RNA was performed at 37 °C for 1 hour in a 20 µl reaction mixture containing: first strand buffer, 200 units of Moloney murine leukemia virus reverse transcriptase, 20 units of RNasin, 10 mM DTT, 4.75 µM random hexamers, and 500 µM deoxynucleotides (all from Promega, Madison, WI). Following cDNA synthesis, the resultant mixture was heated at 95 °C for 5 min before storage at -20 °C.

Primer/ Probe Design

We designed the TaqMan primers and probes for the Wnt5a, ITPR1, IGSF1, and FGF14 genes using the Primer Express software (PE Applied Biosystems, Foster City, CA). To minimize the DNA contamination, Primers were designed in a way to span at least one intron of the genomic sequence. The dye molecule FAM and the quencher dye TAMRA with the emission wavelengths at 518 and 582 nm respectively were used to label all the TaqMan probes respectively at the 5' and 3' ends. In order to prevent extension during PCR the 3' end of the probe was phosphorylated. These sequences were checked subsequently for their specificity, using the Check-Probe function of the Ribosomal Database Project software package and the BLAST database search program.

TaqMan real-time PCR

Real-time TaqMan qPCR amplification was performed by the Rotor-Gene 6000 real-time PCR cycler (Qiagen Corbett, Hilden, Germany) with the following program: one step at 95 °C for 5 minutes, followed by 40 cycles at 95 °C for 5s, and 60 °C for 30s. For each reaction, 20 µl of the reaction mixture was used comprising: 0.4 µl of forward primer, 0.4 µl of reverse primer, 0.4 µl of TaqMan probe, 12 µl of Probe 2x Taq (Probe qPCR) Master Mix (Takara Bio, Shiga, Japan), 1 µl of template cDNA and 5.8 µl sterilized ultra-pure water. Negative controls included all compartments of the reaction mixture except for the template cDNA. The negative controls had no detected amplified DNA products and were used during the analysis. The presented data are the mean values of triplicate Real-time PCR analysis.

Statistical Analysis

Statistical package SPSS16.0 (SPSS incorporate, Chicago) was used for all analyses. The data is presented as mean±SD. Unpaired t-test and one-way Anova (Tukey posttest) were used to compare the relative expression levels of genes. P< 0.05 was considered as the level of statistical significance.

All of subject's data were collected via

questionnaires and after estimation of clinical information, data were entered into SPSS-22 (SPSS incorporate, Chicago) for analysis. Interpretation of demographic results obtained from both patient and control groups was based on frequency. Four different age groups were defined according to the observed quartiles as following: 1) age $\leq$ 45, 2) 45<age $\leq$ 54, 3) 54<age $\leq$ 63, and 4) age>63. After approving the normal distribution of all data using the Kolmogorov–Smirnov test, Mann-Whitney test was performed to evaluate between-group differences of the oncogenes. A chi-squared

(X^2) test was used to examine if age, smoking, and family history affected the risk of cancer, and eta (η) correlation ratio was also determined to investigate the association between the oncogenes and the stage of prostate cancer. The level of statistical significance was set at $p \leq 0.05$.

Results

The stage of prostate cancer patients was summarized in Table 1 and no significant relationship were observed between smoking habitant and family history of the patients with the stage of prostate cancer.

Table 1. The frequency of different TNM staging system of prostate cancer and the corresponding relationship with family history and smoking.

TNM staging system (n=40)	Frequency (%)	Smoking habit			Family history		
		Yes (%)	No (%)	NA (%)	Yes (%)	No (%)	NA (%)
T1N0M0	1 (2.5)	1 (2.5)	-	-	-	1 (2.5)	-
T1N1M1	1 (2.5)	1 (2.5)	-	-	1 (2.5)	-	-
T2N1M0	19 (47.5)	11 (27.5)	7 (17.5)	1 (2.5)	3 (7.5)	15 (37.5)	1 (2.5)
T2N1M1	4 (10)	-	4 (10)	-	-	4 (10)	-
T2N2M1	7 (17.5)	4 (10)	3 (7.5)	-	2 (5)	4 (10)	1 (2.5)
T3N1M1	2 (5)	-	2 (5)	-	2 (5)	-	-
T4N1M1	1 (2.5)	1 (2.5)	-	-	-	1 (2.5)	-
NA	5 (12.5)	2 (5)	1 (2.5)	2 (5)	1 (2.5)	3 (7.5)	1 (2.5)

T0: In these cases, there is no evidence of tumor in the prostate tissue.

T1: The tumor cannot be detected during a digital rectal exam (DRE) and cannot be seen using imaging tests.

T2: The tumor is large enough to be detected during DRE.

T3: The tumor has grown through the prostate on 1 side and into the tissue just outside the prostate.

T4: The tumor is fixed, or it is growing into adjacent structures other than the seminal vesicles.

N0: Prostate cancer has not yet extended to the regional lymph nodes.

N1: In these cases, the cancer has extended to the pelvic lymph nodes.

M0: Prostate cancer has not metastasized.

M1: Metastasis to distant tissues has occurred.

* TNM staging system was not applicable on five samples.

The age PCs and control groups were within the range of 25 to 88 (48.70 ± 15.32) and 23 to 89 (53.63 ± 13.35) years old, respectively and as Figure 1 illustrates the X^2 test revealed no significant difference between the age of PCs group and control. Moreover, The X^2 test showed that there was a difference in the incidence of prostate cancer in different age groups ($X^2 = 9.30$; $p = 0.026$). The highest prevalence of prostate cancer was observed at

the age of ≤ 45 years and the lowest was found within the group 54<age $\leq$ 63 years (Fig. 1). 22 (25.5%) PCs individuals had a family history of prostate cancer (Fig. 2) and among the control and PCs subjects 51% and 63% of had smoking habits throughout their lifetime respectively (Fig. 3). Data analysis using X^2 test showed that family history had a significant effect on prostate cancer ($X^2 = 14.43$; $p = 0.001$), whereas smoking habit

had no significant effect on the incidence of prostate cancer ($X^2=4.67$; $p=0.097$).

The relative expression of *IGSF1*, *Wnt5a*, *FGF14*, and *ITPR1* genes is presented in Figure 4. Expression of *IGSF1* gene in prostate cancer specimens (2.308 ± 2.748) was significantly higher ($p<0.05$) than normal controls (1.242 ± 1.034). *Wnt5a* gene was also overexpressed in PCa cases (33.23 ± 23.59) compared to normal controls (1.390 ± 1.096) ($p<0.0001$). The results also showed that

expression of *FGF14* was significantly higher in PCa cases (8.740 ± 7.273) in comparison to healthy controls (1.782 ± 1.912) ($p<0.0001$). Conversely PCa samples showed a significant decrease in *ITPR1* gene (0.6414 ± 0.6560) compared to normal controls (1.366 ± 0.8699) ($p<0.0001$). Above all, analyzing the data by eta (η) test showed that a strong significant correlation between the regulations of *IGSF1*, *Wnt5a*, *FGF14*, and *ITPR1* genes and the stage of prostate cancer.

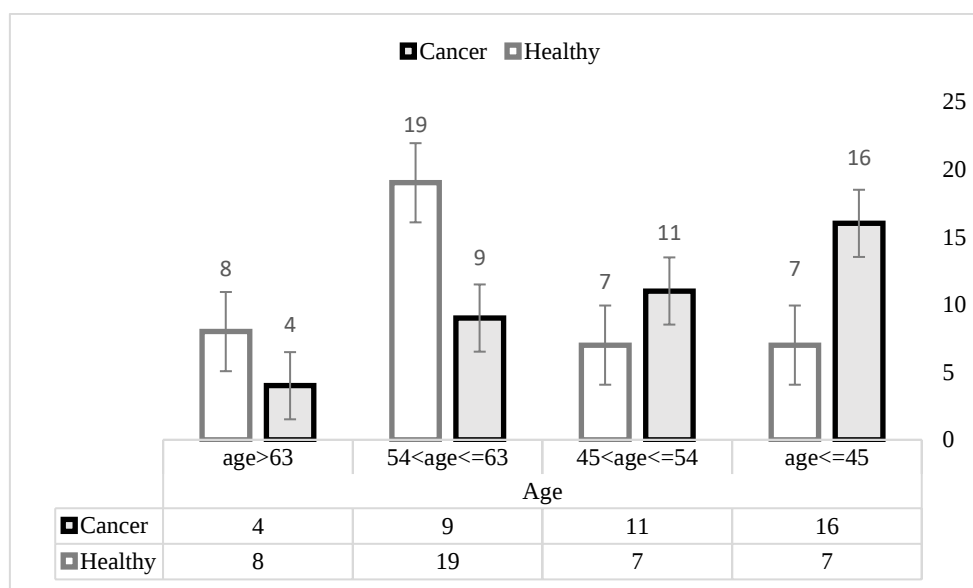


Fig. 1. The prevalence of PCS and control individuals in four different age groups.

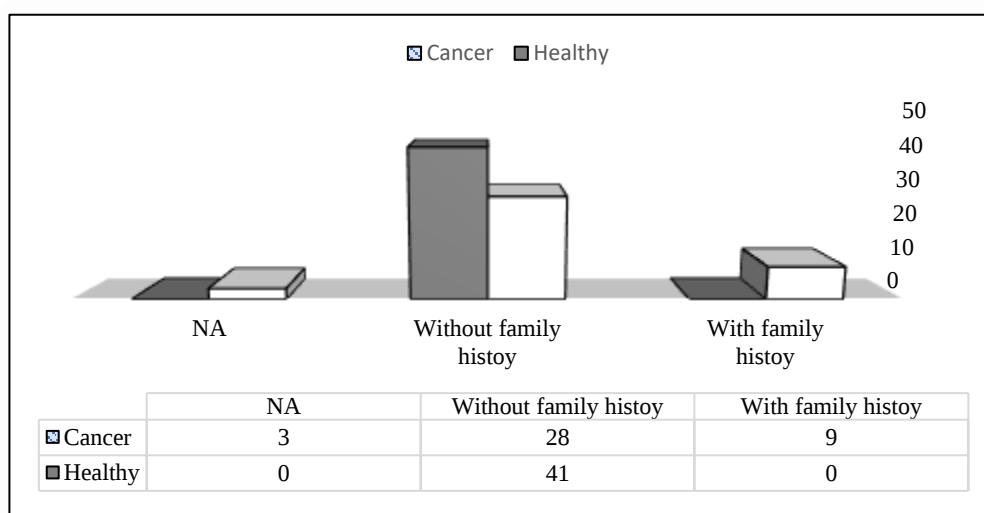


Fig. 2. Rate of family history in the two studied groups; NA: not applicable.

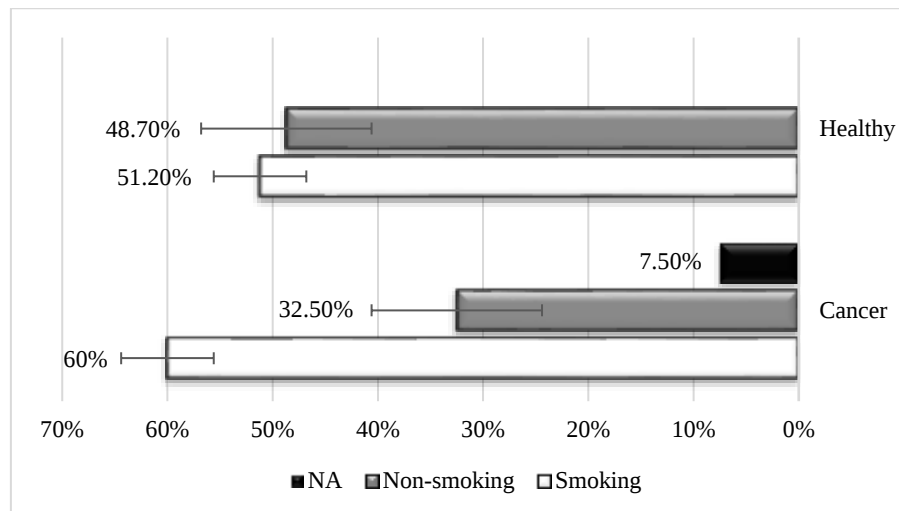


Fig. 3. Rate of smokers in patients and the control group; NA: not applicable.

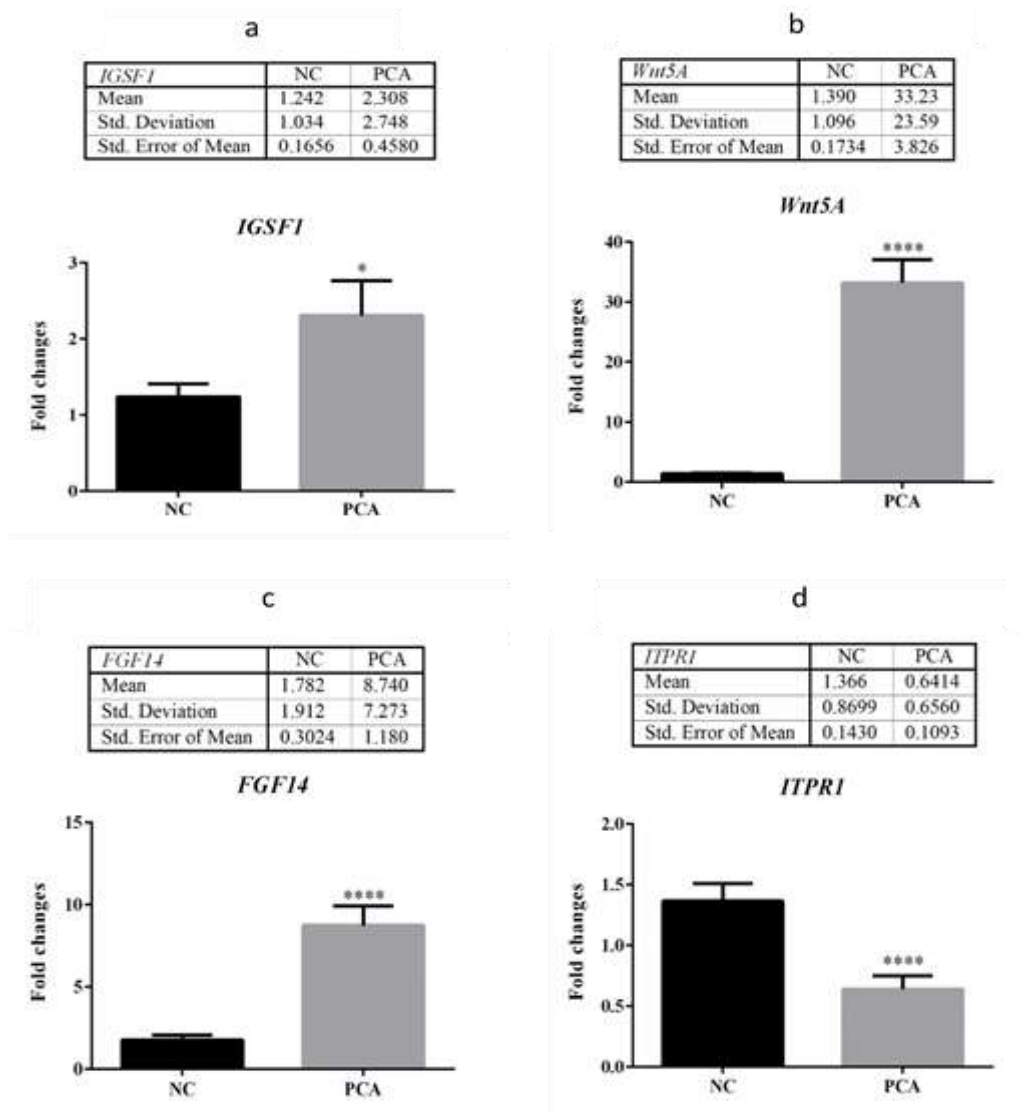


Fig. 4. The relative expression of IGSF1 (a), Wnt5a (b), FGF14 (c), and ITPR1 (d) in PCa specimens compared to normal controls, as assessed by real time PCR.

Discussion

Despite major advances in the management of Prostate cancer, it still remains the second leading cause of cancer related death in men. Although very useful, PSA screening has shown to cause over detection and over treatment without any clinical benefit in many cases. Therefore, there is an urgent need for the introduction of new biomarkers for diagnosis and progression of PCa. In the present study, we evaluated the relative expression of four candidate genes which have been involved in the development and progression of various cancers. To our knowledge this is the first report on the evaluation of IGSF1, Wnt5a, and ITPR1 mRNA in prostate cancer. Our results showed that the expression of Wnt5a, FGF14 and IGSF1 were significantly higher in PCa specimens compared to normal controls.

According to our results, an elevated expression of IGSF1 mRNA was observed in PCa specimens compared to healthy controls. It has been reported that IGSF1 is highly expressed in normal tissues including pancreas, fetal liver, testis, and adult anterior pituitary (30). It was also shown that IGSF1 mRNA was expressed at moderate levels in the prostate, heart, and small intestine. IGSF1 deficiency, as an X-linked genetic defect has been associated with GH deficiency, delayed puberty, hypo-prolactinemia, and testicular enlargement (31, 32). IGSF1 has been reported to play a role in hepatocellular carcinoma and pituitary tumor development (33, 34). To our knowledge this is the first report on the expression of IGSF1 in Prostate cancer and further studies are needed to explore the expression and function of IGSF1 in prostate cancer patients.

In the present study we also reported the overexpression of FGF14 mRNA in PCa samples compared to normal controls. In a study by Hashemi et al. they showed that the expression of FGF14 mRNA in colorectal cancer compared to the adjacent normal tissues was elevated and this increase was associated with the progression and clinicopathological features of colorectal cancer (35). However, in another study downregulation of FGF14 was

reported in colorectal cancer cell lines and specimens compared to adjacent normal tissues and a tumor suppressor activity for the FGF14 was concluded (7). Another study on the identification of key genes associated with cervical cancer showed that FGF14 was downregulated and hypermethylated in cervical cancer samples. They concluded that FGF14 might be involved in the pathogenesis and development of cervical cancer (29).

Moreover, we showed that the expression of Wnt5a mRNA increased in PCa specimens. Wnt5a has not only been previously reported to elicit both oncogenic and tumor suppressor functions in different tumors, but also tumor promoting as well as tumor suppressing activities have been attributed to Wnt5a in PCa. Although some studies have pointed to the increased expression of Wnt5a in PCa compared to benign tissues and showed that the expression of Wnt5a protein is involved in the aggressiveness of prostate cancer and correlates with relapse after prostatectomy (36, 37), other surveys reported a better clinical outcome for PCa patients with higher Wnt5a protein expression (37, 38). In a cohort of PCa patients it was shown that the expression of Wnt5a and its receptor Frizzled-5 were elevated in PCa samples and the rate of 10-year survival was higher in patients with elevated Wnt5a expression. They also showed that *in vitro* overexpression of Wnt5a resulted in the induction of apoptosis in prostate cancer cells and inhibited proliferation and migration of PCa cells. It was also reported that local growth of prostatic tumor was significantly reduced after overexpression of WNT5A in PC3 cell line. They concluded that Wnt5a could suppress the proliferation of prostatic tumor cells and induce apoptosis of these cells (39).

Our results showed a decreased expression of ITPR1 mRNA in PCa specimens compared to normal controls. Few other studies have been conducted on the expression and function of ITPR1 in pathological settings. ITPR1 mutations lead to spinocerebellar ataxia type 15, a disease associated with a heterogeneous group of cerebellar disorders. It was shown

that genetic or pharmacological inhibition of ITPR1 signal transduction could suppress the autophagy directly and indirectly (40, 41). In another study HIF2- α induced overexpression of ITPR1 resulted in the protection of renal cancer cells from natural killer cells mediated lysis by inducing autophagy.

In the current study we showed that IGSF1, Wnt5, and FGF14 mRNA expression levels were upregulated in Iranian PCa patients while the expression of ITPR1 mRNA decreased. These results emphasize the importance of

genetic biomarkers for the early detection of prostate cancer and may help find the genetic panel of PCa patients especially that of Iranian men. Further studies on larger populations and investigating the clinicopathological relevance of these genes I needed.

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