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Expression Profiles of *IGF1*, *EGF*, and *FGF2* Genes in Patients With Prostate Cancer in Isfahan Province, Iran

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ABSTRACT

Background: Prostate cancer is the second most prevalent cancer among men all over the world. Over the past 10 years, prostate cancer prevalence has increased in Iran. Growth factors have an important role in the regulation and growth of malignant and normal prostate cells. Therefore, the purpose of this investigation is to examine the association of the expression profile of *IGF1*, *EGF*, and *FGF2* with prostate cancer in an Iranian male population.

Materials and Methods: In this investigation, the quantitative real-time RT-PCR technique was applied to evaluate the expression profiles of IGF, *EGF*, and *FGF2* in the peripheral blood samples of 40 patients with prostate cancer and 40 healthy individuals. Moreover, the relative expressions of *IGF1*, *EGF*, and *FGF2* in various stages of disease were evaluated.

Results: Our obtained data indicated a significant increase in the expression of *EGF* and *FGF2* in patients with prostate cancer compared with the healthy subjects (P=0.02 and P=0.009, respectively). In contrast, the expression level of *IGF1* was not significantly different between the patients and controls (P=0.052), but the expression level of *IGF1* was lower in the patients' group. Additionally, it has been observed that *IGF1*, *EGF*, and *FGF2* expression were directly associated with the stage of disease.

Conclusion: Our results suggest that *EGF* and *FGF2* probably have important role in prostate cancer and were consistent with what had previously been reported. On the other hand, our data revealed no association between the expression of *IGF1* and prostate cancer in the population studied.

Introduction

Cancer is the third leading cause of death in Iran. There are several estimates of prostate cancer prevalence rates in various regions of Iran [1]. Prostate cancer is one of the most common cancers among males in the US and Western industrialized nations and a leading cause of cancer mortality. Nearly 3% of all males will demise of this malignancy, although the fatality has decreased by 31% over the past

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13 years [2, 3]. The interpatient molecular heterogeneity of prostate cancer is well known. It would be helpful to recognize its predictive biomarkers to explore and provide the more exact treatment for prostate cancer [3]. In prostate cancer patients whose cancer has been progressed, there are remedial options such as prostatectomy, radiation, and androgen-deprivation therapy [4].

Epidemiological investigations consistently indicate the familial clustering of prostate cancer. A man's lifetime risk of prostate cancer increases two to eight times if one of his first-degree relatives has this disease [5]. According to GLOBOCAN 2012, prostate cancer is one of the most prevalent cancers among Iranian men [6]. Proteins that regulate cellular growth and apoptosis are called peptide growth factors. Multiple oncogenes that are associated with the malignant alteration of a cell are receptors for growth factors. Growth factors have an essential role in the regulation of both malignant and normal prostate cells.

The most critical Epidermal Growth Factor (*EGF*) family includes the Fibroblast Growth Factor (FGF) and the Insulin-like Growth Factor (IGF) [7]. The associated Binding Proteins (IGFBP) of IGF are correlated in the arrangement of cell proliferation and apoptosis. Also, there has been considerable interest in the IGF family role in the metastasis of prostate malignancy. It has been proposed that aberrant IGF signaling is associated with many cancers such as colon, prostate, pancreatic cancers [8, 9].

EGF is one of the well-studied oncogenes, and it is the ligand of the Epidermal Growth Factor Receptor (*EGF*R). Although the expression of *EGF* is under androgen control in ordinary prostate, it has shown overexpression in prostate cancer's epithelium [10, 11]. Previous investigations have been indicated the linking changes of the FGF system to the initiation and development of a vast diversity of malignancies such as prostate cancer. FGFs have a significant role in the growth and preservation of the normal prostate. FGF is a potentially serious mitogen in prostate cancer because can binds the various isoforms of FGF receptor. Moreover, *FGF2* (basic FGF) is expressed by several human malignant cells, such as prostate cancer [12, 13].

Because of limited studies on the correlation of growth factors such as *IGF1*, *EGF* and *FGF2* and prostate cancer in Iran, this study aimed to investigate mRNA expression of *IGF1*, *EGF*, and *FGF2* in prostate cancer among Iranian male population (Isfahan Province), to explore any difference in the patients with prostate cancer and healthy subjects.

Materials and Methods

The Ethics Committee of the Medical Genetics Research Center of Genome approved this study. Before taking the blood sample of individuals, written informed consent was taken from each person.

Sample collection

A total of 40 patients with prostate cancer, diagnosed in the Isa Ibn Maryam Hospital, Isfahan, Iran (Mean±SD age: 67.05±1.85, range: 44-80 years) and 40 controls (Mean±SD age: 59.37±1.95, range: 40-78 years) were chosen for the present study. The controls had no family history of prostate cancer and did not receive any specific medicine. Prostate Specific Antigen (PSA) blood levels were measured for all participants. Nine patients had received chemotherapy, 12 patients hormone therapy, and 17 patients radiotherapy. Of them, six patients had received all therapies, and two patients had received no therapy for at least a month. Blood samples were collected from patients and healthy subjects (as controls). Four milliliters of peripheral blood was collected in EDTA anticoagulant from every participant and was quickly transferred to the laboratory on ice.

RNA extraction

The RNeasy Kit (Qiagen, Germany) was applied for RNA extraction and tested by a NanoDrop spectrometer (WPA Biowave II, Biochrom, USA) for the value of RNA quality with the absorbance of 260/280 nm.

cDNA synthesis

cDNA synthesis for *IGF1*, *EGF*, and *FGF2* was fulfilled on a total RNA using a cDNA Synthesis Kit (Qiagen, Germany) according to the kit protocol. Then, the synthesized cDNA was stored at -20° C until the next step.

Real-time reverse transcriptase-polymerase chain reaction assay

The device tested was a Rotor-Gene 6000 system (Corbett Life Science, Australia) for real-time quantitative PCR to perform a total volume of 10 µL by specific primer pairs (Table 1) [7]. The product of cDNA was mixed with a master mix, including *IGF1*, *EGF* and *FGF2* primers, 0.5 µL separate from each primer (forward and reverse), 3 µL of diethylpyrocarbonate-treated water, and 5 µL of SYBR Premix Ex Taq II (TaKaRa, Kusatsu, Japan). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was applied as a housekeeping gene for gene expression studies using real-time PCR. Three independent RT-PCR experiments were done for all genes studied in each sample mRNA level.

Thermal steps of PCR were as follows: 95° C for 10 min, 40 cycles of 95° C for 10 s, 60° C for 20 s (annealing) and 72° C for 25 s (extension). Negative controls were used in all reactions. The efficiency of *IGF1* (1.14), *EGF* (1.22), *FGF2* (0.97), and GAPDH (0.84) primers were examined at various concentrations (Figure 1).

Statistical analysis

The data obtained were analyzed by the $2^{-\Delta\Delta CT}$ statistical method in GraphPad Prism version 5.01 (GraphPad, San Diego, USA). The Kolmogorov–Smirnov test examined the normality of data distribution. The independent samples t test was used to analyze the data among groups. In all analyses, $P \leq 0.05$ was considered as statistically significant.

Results

Characteristics of the patients

A study population, including 40 patients, were enrolled in this investigation. Patients and controls were matched in terms of age (P=0.12). Of the 40 prostate cancer patients, 13 patients had a family history of cancer, and 26 of them were smokers. Table 2 presents clinical characteristics of the patients.

Expression profile of *IGF1, EGF,* and *FGF2*

In this study, we examined the mRNA levels of *IGF1*, *EGF*, and *FGF2* in healthy subjects and patients with prostate cancer to find a correlation that may be beneficial in clinical diagnosis. The quantitative real-time PCR method was assessed for the expression of *IGF1*, *EGF*, and *FGF2* in the two groups (40 controls and 40 patients). The $2^{-\Delta\Delta CT}$ method was applied for the analysis of real-time PCR data where CT is the cycle threshold. Our data have shown that the Relative Quantification (RQ) was different in both groups. A significant increase in expression appeared in our data analysis for *EGF* (Figure 2) and *FGF2* (Figure 3) in patients with prostate cancer compared with the healthy subjects (P=0.02 and P=0.009, respectively). Our findings may indicate the role of *EGF* and *FGF2* and their use as an approach to control and manage prostate cancer. These factors might represent an additional marker of potential clinical relevance.

In contrast, although the expression level of *IGF1* was not significantly different between patients with prostate cancer and controls (P=0.052), the relative expression level was slightly lower in patients with prostate cancer compared with the healthy subjects (Figure 4). The results suggest that *IGF1* probably lacks any major role in this cancer, at least in our studied population. However, to understand the role of *IGF1* in prostate cancer, more studies are warranted. On the other hand, the relative expression of each of these genes was analyzed according to the stage of cancer.

Our obtained data demonstrated that gene expression was directly related to the stage of disease. Higher statistically significant relative expression of *EGF* in stage I (1.82), stage II (2.74), and stage III (3.36) were observed (P=0.023, P=0.003, and P= 0.009, respectively). There was significant increase in the expression of *FGF2* in stage I (1.03),

Table 1. Primer sequences used for real-time PCR (5′-3′)

Gene	Forward Primer	Reverse Primer	Size (bp)
EGF	CTTGTCATGCTGCTCCTCCTG	TGCGACTCCTCACATCTCTGC	118
FGF2	CTGGCTATGAAGGAAGATGGA	TGCCCAGTTCGTTTCAGTGå	149
IGF1	CCTCCTCGCATCTCTTCTACCTG	CTGCTGGAGCCATACCCTGTG	166
GAPDH	CCACTCCTCCACCTTTGACG	CCACCACCCTGTTGCTGTAG	107

Table 2. Clinical characteristics of prostate patients

Samples	No. (%) / Mean±SD				Number of Family History
	Average PSA Free (ng/mL)	Average PSA Total (ng/mL)	Stage	Disease Duration (y)	
Patients	6.61±1.67	22.26±2.62	Stage I: 18 (45) Stage II: 13 (32.5) Stage III: 9 (22.5)	5.42±0.67	13
Controls	1.83±2.15	10.37±1.52	-	-	-

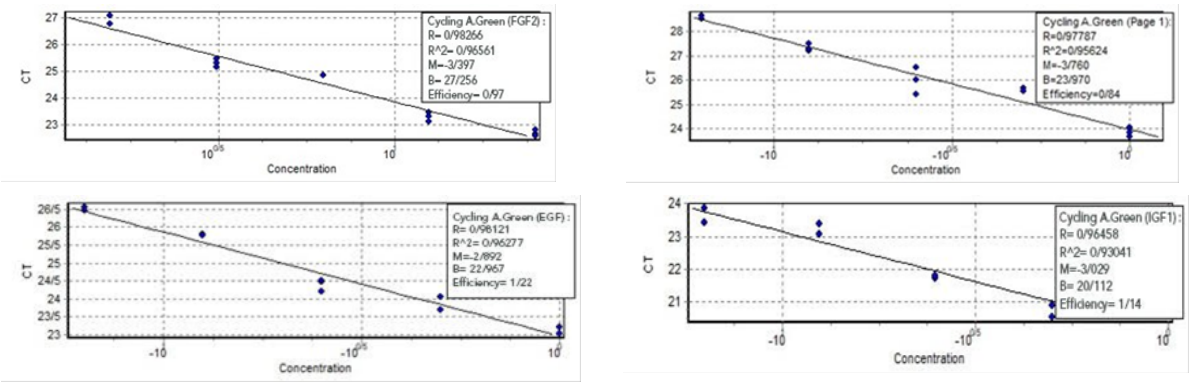

Figure 1. The efficiency of *IGF1*, *EGF*, *FGF2*, and GAPDH primers at different concentrations

stage II (1.37), and stage III (1.76) (P=0.005, P=0.004, and P=0.0002, respectively). Although there was no significant correlation between relative expression of *IGF1* and stage I (P=0.11), significant differences were detected for *IGF1*expression in stage II (0.83) and stage III.

Discussion

In this investigation, the association of the expression profiles of *IGF1*, *EGF*, and *FGF2* with prostate cancer was examined with RT-qPCR in the Iranian population. We observed a higher expression profile of both genes studied (*EGF* and *FGF2*) in patients with prostate cancer than controls. Our obtained data demonstrated that prostate cancer patients had a statistically significant elevation in the expression level of *EGF* and *FGF2* compared with the healthy subjects. However, we observed no statistically significant difference between patients with prostate cancer and healthy subjects in the expression level of *IGF1*, but the relative expression of *IGF1* was slightly lower in patients with prostate cancer compared with the healthy subjects. Further analysis based on the stage of disease showed that *IGF1*, *EGF*, and *FGF2* expression were directly correlated with the stage of disease.

Geographic alternations in prostate cancer outbreak rates and explanations of potential environmental factors have been hampered by diversity in the PSA experiments. Genetic and environmental factors probably have a key role in the ongoing geographical outbreak differences over the three decades despite various levels of PSA experiments [14]. It seems that the investigations of underlying genetic factors in this cancer in different populations with various geographic and environmental factors are essential.

Thus, this study examined the association of the expression profile of *IGF1*, *EGF*, and *FGF2* with prostate cancer in Iranian population. The growth factors, includ-

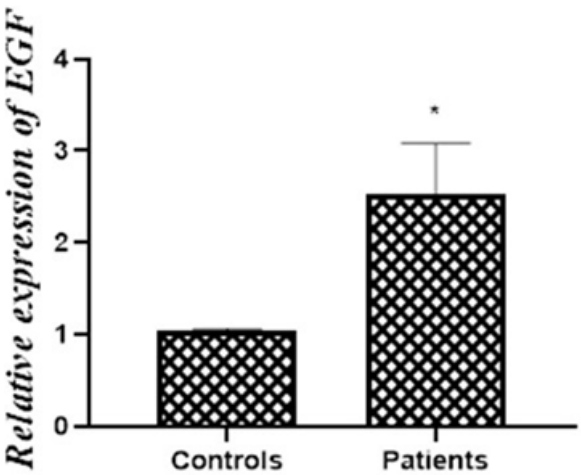

Figure 2. The expression profile of *EGF* in patients and controls had a significant difference.

*P<0.02)

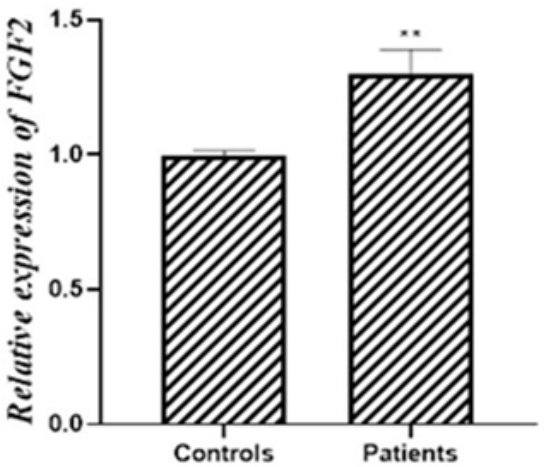

Figure 3. The expression profile of *FGF2* in patients and controls had a significant difference.

**P<0.009

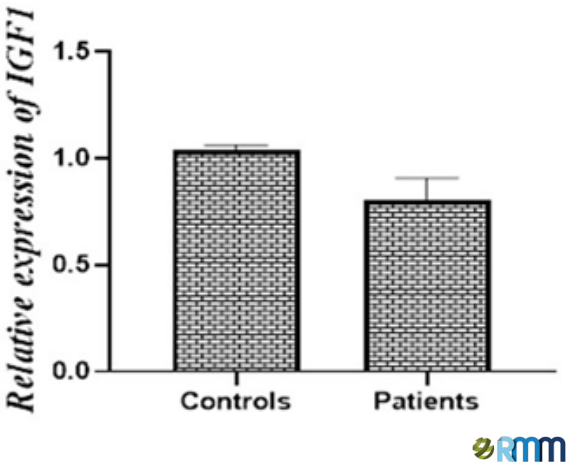

Figure 4. The expression profile of *IGF1* in patients and controls had a significant difference

P<0.052; P=0.03 and P=0.012, respectively

ing V*EGF*, *FGF2*, TGFB1, *EGF*, and *IGF1* are the most prevalent envoy of five significant growth factor families, which are associated with approximately all intracellular processes and so influence cells significantly. Soulitzis et al. reported the overexpression of growth factors, including V*EGF*, *EGF*, and *FGF2* in prostate cancer, but underexpression of TGFB1 and *IGF1* mRNA levels [7]. Their results are consistent with our results. The associated binding proteins of IGF are related to the regulation of cell proliferation and apoptosis.

The role of IGFs in prostate carcinogenesis was supported by investigations that have recognized several cancer-promoting attributes of IGFI, such as mitotic and antiapoptotic impacts. That is why researchers have focused on the role of *IGF1* in the development of prostate cancer [15]. Also, *EGF* family interacts with their receptors, such as *EGF*R. It is shown that the expression of *EGF*R is down in ordinary prostate tissues, while it is overexpressed in prostate cancer tissues [16]. *FGF2* (basic FGF) is expressed in many malignancies such as breast, pancreas, lung, head, and neck, prostate cancer and so on. ELISA studies have shown that *FGF2* is found in human prostate cancer tissues in significantly higher concentrations compared with the normal prostate [17-19].

Trojan et al. reported that the number of V*EGF*- positive and *EGF*-positive cells in prostate cancer tissue was significantly elevated compared with the normal tissue [20]. Our study further supports the hypothesis that *FGF2* and *EGF* may be clinically useful for therapy of prostate cancer. Future investigation should recruit larger samples of prostate cancers with and without history of medication to investigate the exact association of *IGF1*, *EGF*, and *FGF2* expression with prostate cancer.

However, this study was limited to the findings obtained from the whole blood; therefore, research and comparison of *IGF1*, *EGF* and *FGF2* expression in prostate tissues, as well as their evaluation in the early-stage prostate cancer or benign prostatic hyperplasia and healthy subjects, probably yield different results. Moreover, future studies are required to investigate the perception of gene-gene and gene-environment interactions, splicing, etc. to achieve a comprehensive understanding of the relation of *IGF1*, *EGF*, and *FGF2* with prostate cancer.

In conclusion, *EGF* and *FGF2* deregulation are probably associated with prostate cancer. The findings of the current study agree with what had previously been reported, and therefore, it can be concluded that *EGF* and *FGF2* are also upregulated in this investigation population. These genes are potential biomarkers for prostate cancer in future studies. However, our results suggested no association between *IGF1* expression and prostate cancer; thus, further studies at a broader level are required to understand the role of this gene.

Ethical Considerations

Compliance with ethical guidelines

The Ethics Committee of the Medical Genetics Research Center of Genome approved this study

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Authors contribution's

Contributing to conception and design: Mansoor Salehi; Contributing to all experimental work and molecular experiments, statistical analysis, and interpretation of data: Farinaz Khosravian, Fatemeh Ketabchi, Faezeh Namazi, and Nasrin Hadi; Contributing to sample collection: Nasrin Hadiand and Faezeh Namazi; Financial support: Mansoor Salehi; Discussion the results and the manuscript: Mansoor Salehi and Farinaz Khosravian; Reading and approving the final manuscript: All authors.

Conflict of interest

The authors declare no conflict of interest.

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