RESEARCH ARTICLE

The Expression, Morphology, and Clinical Characteristics of Fibroblast Growth Factor-10 in Breast Cancer

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Abstract

Background: Fibroblast growth factor-10 (FGF-10) is a member of a superfamily with characteristics of epithelial cell proliferation and embryonic development and is assumed to have a role in a phenomenon called the epithelial-mesenchymal transition (EMT). The previous study has revealed the critical role of FGF-10 in type III EMT in breast cancer cell lines. The mentioned finding, demonstrates the possible role of this factor in type III EMT in cancers with different origins such as breast. The present study investigated the expression of FGF-10 as a mitotic-inducing growth factor, normally has a low expression in breast tissues amongst breast cancer patients. **Materials and Methods:** 67 breast cancer tissues and 8 normal breast tissues were randomly selected from the Iran national tumor bank. The FGF-10 gene expression analysis was performed after the RNA expression using the real-time RTPCR, which was followed by the Student's t-test statistical analysis. **Results:** The findings revealed that the relative expression of FGF-10 was elevated in tumor tissues as compared with normal breast tissues, and the higher expression had a direct correlation with the progression of clinical and pathologic staging. The expression was also significantly higher in triple-negative breast cancer and p53 null tissues. **Conclusion:** Taken together, it is suggested that, although in some variables it was not significant but generally the invasion and migration in tumor tissues are the same as in-vitro analysis as indicated before, and the expression has a direct relationship with the molecular presentation and clinical -pathological progression.

Keywords: Breast Cancer- FGF-10- triple negative breast cancer- P53

Introduction

Fibroblast Growth Factor (FGF), a member of a growth-inducer family, is a heparin-binding protein and plays a role in various phenomena including proliferation, development, angiogenesis, and embryonic development [1]. FGF has almost 22 members [2]. The related receptors in mammalian cells are identified as FGFR1-4 [3]. FGF-10 is a member of this family with characteristics of epithelial cell proliferation and embryonic development and is supposed to have a role in a phenomenon called the epithelial-mesenchymal transition (EMT) [4].

EMT is a harmonic orchestra that can induce mesenchymal markers and features [5]. Embryonic development is associated with type I EMT, which leads to organogenesis [6]. Several functions have been considered for FGF-10 in the early stage of the embryonic development [7, 8]. In contrast, type III EMT is accompanied by a bizarre disturbance in differentiation pathways and is finally associated with the invasion and metastasis [5]. The present researchers' previous study has revealed the critical role of FGF-10 in type III EMT in breast cancer cell lines. The mentioned finding demonstrates the possible role of this factor in type III EMT in cancers such as breast with different origins [4].

Breast cancer is the most common cause of cancer deaths among women and has an increasing incidence rate in Iran [9]. Although recent technologies developed

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for screening this cancer have increased the survival rate of breast cancer, there is yet a long way ahead to manage and control the outcome of patients suffering from breast cancer [10]. Specification of the factors affecting the prognosis would be very critical to achieve the mentioned goal.

Cell morphology findings and clinical staging are two different indicators that can be employed for the prognostic estimation and clinical management of breast cancer [11]. The comparisons made between these indicators and gene profile would provide a better insight for obtaining a more precise prediction in this regard [11]. In recent years, the molecular classification has helped to identify the prognosis and outcome of breast cancer more precisely by the administration of drugs that can directly target critical molecules in different signaling pathways. In this case, the classification of molecular subtypes according to the hormone receptor expression (luminal A, luminal B, Her2-enriched, and basal-like) has become an initial routine management of breast cancer [12]. The present study has investigated the expression of FGF-10 as a mitotic-inducing growth factor, which normally has a low expression in breast tissues amongst breast cancer patients.

Materials and Methods

Patients and tumor preparation

The present cross-sectional study involved 75 patients that were randomly selected from Iran national tumor bank (Cancer institute of Iran, Tehran University of Medical Sciences, Tehran, Iran). The mentioned sample included 67 breast tumor tissues as well as 8 breast tissues from patients that referred for mammoplasty without a breast cancer history. The clinical morphologic and immunohistochemical (IHC) information was received from tumor bank data base (including lymph node and perineural invasion, metastasis, grade, stage, estrogen, progestron, Her2 receptor and p53 expression). All fresh tissues were stored at -80 for further procedures. An informed consent was obtained from all the patients.

Demographic, morphologic, and clinical information of patients were entered into the excel file (Microsoft 2010, USA).

Gene primer design

Primers were designed using primer3 that is an online primer designer (http://bioinfo.ut.ee/primer3-0.4.0/primer3/). Primer sequences were 5'-ATGTCCGCTGGAGAAAGCTA-3' and 5'-CCCCTTCTTGTTCATGGCTA-3' as forward and reverse primers, respectively. GAPDH was also designed as a housekeeping gene following the same method with 5'-TCACCAGGGCTGCTTTTAAC-3' and 5'-GACAAGCTTCCCGTTCTCAG-3' sequences as forward and reverse primers, respectively. Primer-blast online tool was used to confirm the specific product and predict to avoid non- specific primer-annealing products (https://www.ncbi.nlm.nih.gov/tools/primer-blast).

RNA extraction

RNA was extracted using easy-BLUE (iNtRON, South Korea) according to the manufacturer's instruction. The quantity of the extracted RNA was analyzed using the NanoDrop spectrophotometer (Thermo, USA).

Real time RT-PCR

The cDNA was constructed based on the construction manual of cDNA synthesis kit (Takara, Japan). The 1ug of the extracted RNA was used for cDNA synthesis. The cDNA synthesis was checked by PCR for GAPDH gene, and the following procedures were performed. 2 ul of cDNA was transferred to SYBER Green Master Mix to perform the real-time PCR (Takara, Japan). Real-time PCR was performed using the Rotor-Gene Q thermocycler (Qiagen, USA), which has been described previously [4].

Data transfer and statistics

Data for the gene amplification was analyzed by Rotor-Gene Q software (Qiagen, USA). The fold change of expression was analyzed using REST software (Qiagen, USA) to perform dct and ddct calculations. To compare tumor and normal tissue gene expression dct of tumor and normal tissue was analyzed. In case of clinical and morphologic correlation, data of ddct was used. The data of the normal tissues was pooled, and the average value was calculated. The association was also addressed by calculating the p-value and employing the Student's t-test

Results

Demographic analysis

A total number of 67 breast cancer tumor tissues were studied for the expression of FGF-10. The mean age of patients was 47.52 with the maximum and minimum range of 74 and 31 years old, respectively. The pathologic distribution presented as the number (percentage) was 8 (11.98%), 33 (49.25%), 21 (31.34%), and 5 (7.47%) for grades I, II, III, and X (unknown), respectively. Moreover, the pathologic distribution presented as the number (percentage) was 2 (2.99%), 39 (58.22%), 25 (37.31%), and 1 (1.48%) for clinical stages I, II, III, and IV, respectively.

Expression of FGF-10 in tumor tissues

Dct comparison made between tumor tissues and pooled normal tissues revealed the higher expression of FGF-10 in tumor tissues (p=0.00008). The expression comparison is illustrated in Figure. 1. a.

FGF-10 expression and clinical/morphological findings

The relative expression of FGF-10 was found to increase by decreasing the differentiation of tumor. Moreover, the expression was higher in grade II as compared with grades I and III; however, it was not significant among different grades (Figure. 1. b). The same pattern was observed concerning the stages of the disease, and the expression of FGF-10 was higher in stage II as compared with the other clinical stages (Figure.1. c).
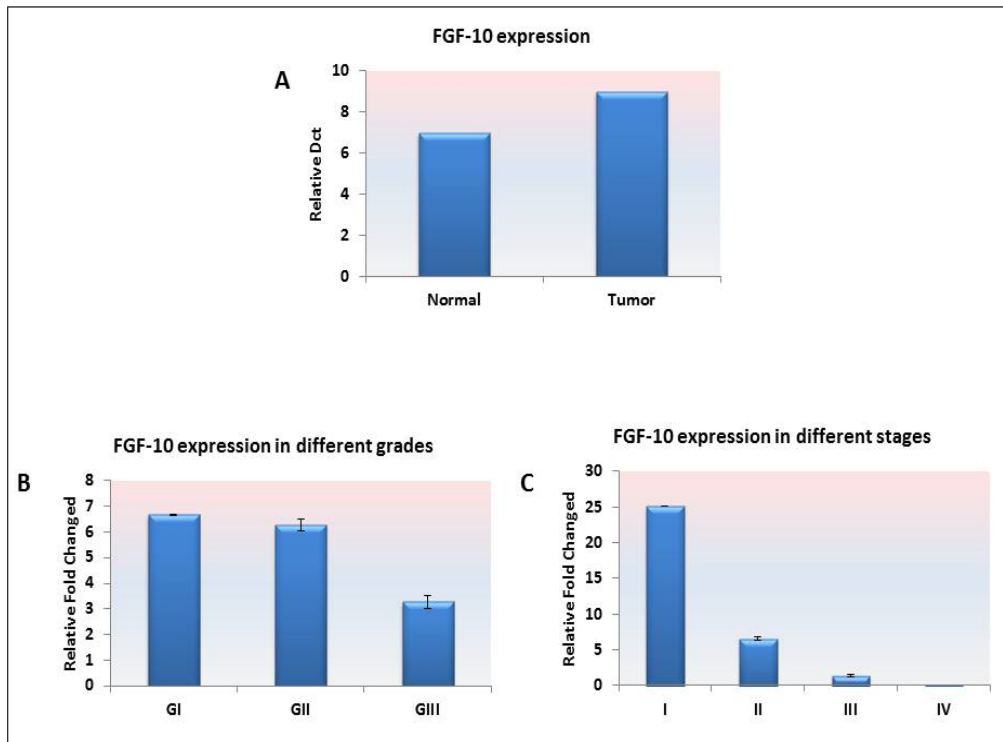
Figure 1. A. The Average of the FGF-10 Expression was Significantly (*) Higher in Tumor Tissues as Compared with Pooled Normal Breast Tissues (p=0.00008). B. The expression of FGF-10 was increasing with the progression of the pathologic grade and was higher in grade II; however, there was no statistical significance in this regard. C. The same pattern reported for the grade was observed for the clinical stage and was also more evident in stage II; however, there was no statistical significance among stages.

FGF-10 expression and invasion

The relative expression of FGF-10 was found to be higher in tumors with perineural (Figure. 2. a) and vascular (Figure. 2. b) invasion; however, it was not significant.

FGF-10 expression and molecular pattern

Number of 19 samples was triple negative based on immunohistochemistry report. Also 13 samples were expressing HER2 receptor expression. The findings have shown that the relative expression of FGF-10 was significantly lower in triple-negative tumor tissues

as compared with tumors with at least one receptor expression (Figure. 3. a) (p=0.04). However, there was no significant difference concerning the expression of the HER-2 receptor (Figure. 3.b). In contrast, the expression of FGF-10 was significantly higher in tumor tissues with no expression of p53 (Figure. 3. c) (p=0.03).

Discussion

The present researchers have previously demonstrated that the FGF-10 up-regulation has a positive effect on the invasion and migration in breast cancer cell lines [4]. The
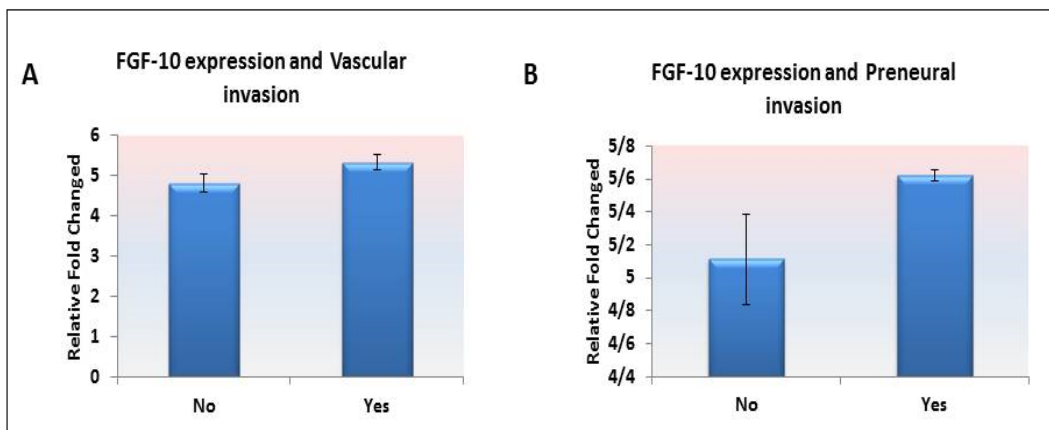

Figure 2. The Relative Expression of FGF-10 was Higher among Tissues with (A) Vascular Invasion and (B) Perineural Invasion; However, This Association was not Significant.
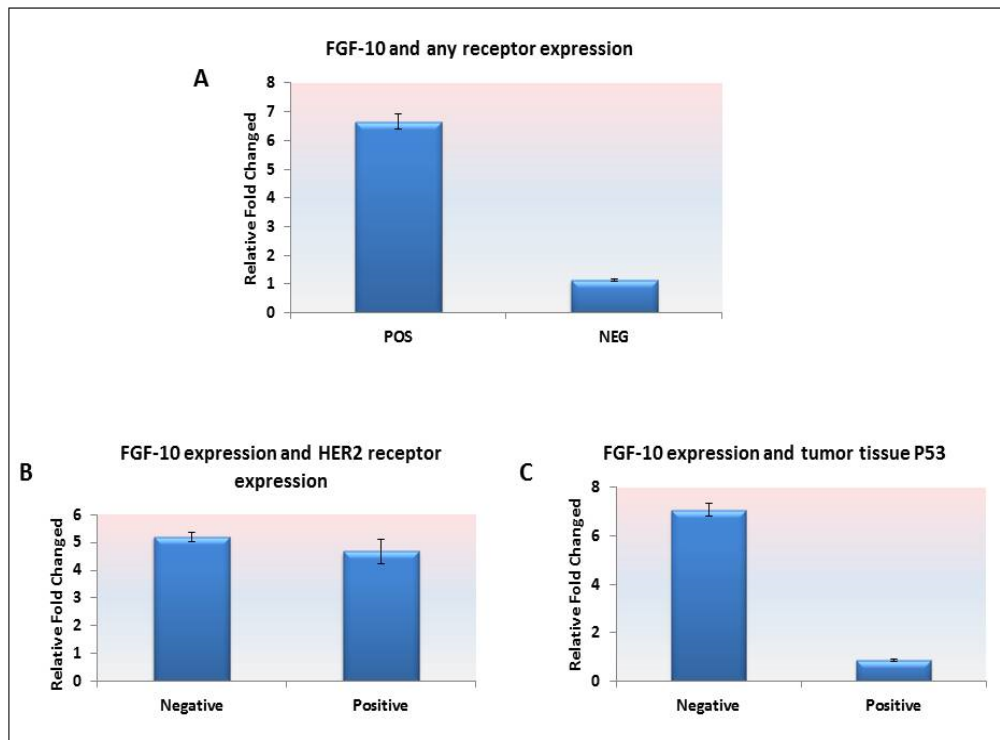
Figure 3. A. The Relative Expression of FGF-10 was Significantly (*) Higher among Tissues with Triple-negative Receptor (p=0.04). B. Comparison made between HER2- positive and -negative tumor tissues did not show a significant difference in the relative gene expression of FGF-10. C. The relative expression of FGF-10 was higher among tumors without P53 expression (*) (p=0.03).

higher expression of FGF-10 means that the expression of this critical protein in type I EMT may have a role in a considerable number of breast cancer tumor tissues [4]. In type I EMT, the expression of FGF-10 during the gastrulation and kidney development is very critical [13]. Meanwhile, the present researchers' previous findings have revealed that the regulation of this protein can change the cancer cell behavior [4]. The EMT is a harmonic orchestration, which is a critical process in the early stage of life [5, 14]. In this phenomenon, uniform and regular epithelial cells morphologically and functionally change to the spindle-shaped mesenchymal cells with the ability of movement and migration [14]. During the first stages of life, this phenomenon is a serious process for the development and organogenesis that is recognized as type I [6]. Although type II is also important during the inflammation, type III is known to happen during metastasis [6]. In this case, there are several studies showing that embryonic factors also have a role in the cancer progression. The mentioned finding makes the possible role of FGF-10 in breast cancer more prominent [5]

The present study revealed that the expression of FGF-10 was higher in breast cancer tumor tissues. The expression of FGF-10 was higher in grade and stage II as compared with other clinical and morphological characteristics. As we have previously indicated in colorectal carcinoma cell lines and tumor tissues, the expression of FGF-10 increased by increasing the grades and stages (specially stage III) [15].

There are several recognized signaling pathways for

FGF-10 including Ras/MAPK, AKT/mTOR, TGFb, and wnt signaling pathways through the phosphorylation of GSK3b [16-19]. The invasion indexes in tumor tissues have indicated a slight increase in the expression of FGF-10 in more invasive tumor cells. The mentioned finding has been confirmed with respect to breast cancer cell lines, as well. Concerning the correlation with FGF-10 expression and invasion in tumor tissues, one reason for insignificant results in comparison with the cell line might be because of the tumor heterogeneity [20]. There are different variables including the percentage of normal tissues or lymphocytic infiltration, the duration of tumor tissue preparation after excision, and environmental factors such as hypoxia that can affect the results of the relative gene expression. In such cases, the gene expression in tumor excision might be different from that of the community of unique cells that are derived from a cloned stable cell line [21].

The classification of breast cancer types based on immune-phenotyping (ER or Estrogen Receptor, PR or Progesterone Receptor, HER2 or Human Epidermal Growth Factor Receptor 2) helps to the better management of the disease and presentation of a breast cancer targeted therapy [12, 22]. In this case, the tumor tissues that have no immune-phenotypic feature are called triple-negative or basal-like breast cancer. The findings of the present study have shown that the expression of FGF-10 is higher in tumors with at least one receptor-positive as compared with a triple-negative. Triple-negative breast cancer that is the most aggressive type accounts for approximately 20% of breast cancer subtypes [23].

The FGF/FGFR system can be activated aberrantly in a ligand-dependent or -independent manner in breast cancer as various kinds of cancers and has a different role in invasion and drug resistance. Considering that FGF-10 usually binds to FGFR2, it has been revealed that the amplification and overexpression of FGFR2 could be detectable in only 4% of TNBC cases, which might explain the downregulation of FGF-10 expression in our study [24].

On the other hand, several studies have indicated the correlation between FGFR overexpression and hormone receptors in the cancer development and proliferation. Studies have shown the correlation between the expressions of different hormone receptors and the expression of other family members of FGF superfamily. For example, with respect to the HER2 receptor, there was a direct correlation between FGF-14 and Her2 receptor and the invasion in breast cancer [25]. Moreover, it has been already revealed that the FGF/FGFR inhibition could promote the endocrine therapy [26]. The findings of the present study did not demonstrate any significant difference among hormone receptor positive tumors and the expression of FGF-10.

The expression of FGF-10 was lower in tumor tissues without the expression of p53. Studies have shown that almost 80% of TNBC cases have the loss of p53, which correlates with the poor prognosis. In our study, 76% of TNBC cases have lost the expression of p53. Some studies have shown that p53 tumor suppressor has a role in the inhibition of other members of FGF family such as FGF-13 [27]. It can be conjectured that other members such as FGF-10 that has a different signaling pathway to the cell proliferation and invasion might have a possible correlation with the loss of p53 [28].

In conclusion, taken together, it is suggested that the invasion and migration in tumor tissues are the same as in-vitro analysis as indicated in the present researchers' previous study, and the expression has a direct relationship with the molecular presentation and clinical-pathological progression. Investigations with sufficient tissue samples might pave the way to shed light on the mentioned difference statistically.

Acknowledgements

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