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Opposite Pattern of *ERBB4/HER4* Gene Expression in Triple-Negative and Non-Triple Negative Breast Cancer

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Abstract

Purpose: Previous studies have drawn controversial conclusions for the expression of erb-b2 receptor tyrosine kinase 4 also known as Human Epidermal Growth Factor Receptor 4 (*ERBB4*/*HER4)* in breast cancer. Accordingly, in the present study, *ERBB4*/*HER4* expression was analyzed in healthy breast, invasive ductal triple-negative breast cancer (TNBC), and non-TNBC tissues. Materials and Methods: The expression level of *ERBB4*/*HER4* was evaluated in 10 normal breast, 20 TNBC, and 20 non-TNBC tissue samples using real-time polymerase chain reaction (Real-Time PCR). Results and Discussion: The *ERBB4*/*HER4* expression level was downregulated in TNBC compared to the healthy tissue (P value, < 0.0001; fold change, -2.1); in contrast, upregulated in non-TNBC versus the healthy tissue (P value, < 0.0001; fold change, 1.7). Noticeably, without categorization of breast cancer samples, *ERBB4*/*HER4* was not significantly dysregulated (TNBC + non-TNBC vs normal; P value, 0.9816). What's more, according to the area under the curve (AUC) and receiver operating characteristic (ROC) curves analysis, *ERBB4*/*HER4* is significantly and strongly able to distinguish TNBC from healthy and non-TNBC from healthy. To put it in a nutshell, downregulation of *ERBB4*/*HER4* in TNBC *vs* healthy tissue and its upregulation in non-TNBC *vs* healthy tissue may explain contentious findings in previous studies. This study sheds new lights on expression pattern of *ERBB4*/*HER4* in breast cancer.

Keywords: *ERBB4/HER4*, Breast Cancer, Triple-Negative Breast Cancer.

Introduction

Breast cancer (BC) with 1.6 million new cases annually is the most common type of malignancy among women worldwide (Dey, 2014). Triple negative breast cancer (TNBC), 15-20% of all BC cases, has neither expression for estrogen/progesterone receptor (ER/PR) nor human epidermal growth factor receptor 2 (*HER2*) which is defined as the worst prognostic and most aggressive type of BC. Therefore, TNBC has become an attractive subject for BC studies (Onitilo et al., 2009; Telli, 2016).

The family of the human epidermal growth factor receptor (*HER*) consists of four members including, EGFR/*ERBB1*/*HER1*, *ERBB2*/*HER2*, *ERBB3*/*HER3*, and *ERBB4*/*HER4* (Witton et al., 2003). Generally, *HER* family members activate the *MAPK* signaling pathway and are involved in cell proliferation and survival and their dysregulation can cooperate in cancer initiation. Likewise, their overexpression has been reported in many types of cancers including lung, pancreas, breast, and glioblastoma and can be considered as biological biomarkers or treatment aims in different kinds of cancer (Stern, 2008; Jones et al., 2018). For instance, higher levels of *ERBB2*/*HER2* and *ERBB3*/*HER3* expression are associated with worse prognosis in BC (Tsutsui et al., 2002; Bieche et al., 2003) and also there are several studies presenting *ERBB2*/*HER2* as an important treatment target in BC (Arteaga et al., 2012; Slamon et al., 2001). Nevertheless there are unclear aspects of choosing the right treatment procedure and targeted treatments for HER family in each breast cancer patient (Nuciforo et al., 2015). Although upregulation of *ERBB2*/*HER2* has been very well established in breast carcinogenesis, there is much less consistent information about *ERBB4*/*HER4*. *ERBB4*/*HER4* was associated with BC cell growth and also mammary carcinogenesis in mice (Lynch et al., 2007; Määttä et al., 2006; Wang et al., 2016). On the other hand, it was reported that *ERBB4*/*HER4* is associated with good prognosis and also correlated with ER-positive, PR-positive, and *HER2*-negative BC (Bieche et al., 2003; Knowlden et al., 1998; Salimi et al., 2016).

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Similarly, Co-expression of *ERBB4*/*HER4* and ER has favorable outcome for BC patients compared to ER-positive BC with low level of expressed *ERBB4*/*HER4* (Kim et al., 2016). In addition, we previously found that polymorphisms with potential effects on *ERBB4*/*HER4* expression have inconsistent association with BC (Salimi et al., 2016; Bagheri et al., 2016; Moradi et al., 2016). Therefore, investigations focused on *ERBB4* expression changes in BC have reported inconsistent results and more studies are required to clarify prognostic role of this gene in BC.

While Kim *et al.* evaluated the mRNA expression levels of *HER* family genes in term of TNBC patients' prognosis (Kim et al., 2016), to our knowledge, the expression of *ERBB4*/*HER4* has not been compared between normal breast ductal tissue, invasive ductal TNBC, and non-TNBC. Alongside with our previous reports focused on *ERBB4*/*HER4* gene polymorphisms (Salimi et al., 2016; Bagheri et al., 2016; Moradi et al., 2016), here, *ERBB4*/*HER4* expression levels in the normal ductal tissue, invasive ductal TNBC, and invasive ductal non-TNBC were compared.

Materials and Methods

Ethics statement

The samples were collected in accordance with the guidelines issued by the Ethics Committee of the Payame Noor University of Taft, Yazd, Iran. All individuals gave written informed consent to participate in research studies.

Tissue samples

Ten normal ductal breast tissue samples from women who had undergone reduction mammoplasty and 40 invasive ductal breast carcinoma tissue samples consisting of 20 TNBC and 20 non-TNBC BC samples were collected from three independent pathological laboratories in Isfahan, Iran between years 2016 and 2017. All tissue specimens including healthy and cancerous have been histologically confirmed by an experienced pathologist. Standard immunohistochemical (IHC) staining tests using standard procedures were performed to analyze ER/PR expression and *ERBB2*/*HER2* overexpression. Notably, patients with ductal carcinoma in suit, recurrent, and metastatic BC were exclude from the present study. Tissue samples and biopsies were stabilized in RNAlater (QIAGEN, Hilden, Germany) and stored at -20°C.

Total RNA isolation and reverse transcription

Total RNA was extracted from all samples using RiboEx reagent (GeneAll, South Korea), following the manufacturer's instructions. The quantity and purity of total RNA was assessed by NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). To remove DNA contamination, RNA-free DNase (TaKaRa, Japan) was used. Complementary DNA (cDNA) was synthesized by RNA reverse transcription using Random Hexamer Primers and RevertAid™ First Strand cDNA Synthesis Kit (Fermentas , Ontario, Canada) and then stored at -70 for further investigations.

ERBB4/HER4 expression using real-time quantitative PCR

Primers were designed and checked by NCBI Primer-Blast Tool (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). Primer sequences for *ERBB4*/*HER4* and GAPDH transcripts amplification were F: GTTCAGGATGTGGACGTTGC, R: CTGCCGTCACATTGTTCTGC and F: GAAAGCCTGCCGGTGACTAA and R: GCATCACCCGGAGGAGAAAT, respectively. The length of the amplified products of *ERBB4*/*HER4* and GAPDH are 112 bp and 113 bp, respectively. Real Time PCR was performed in a DA 7600 real-time PCR system (Applied Biosystems, California, USA) and using the BioEasy SYBR-Green kit (Hangzhou Bioer Technology Co., Ltd., Hangzhou, China). Reactions were conducted in the standard condition with three times repeats to eliminate experimental errors. Average Ct values from triplicate experiments were used for measuring relative gene expression. The relative gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method defined by Livak and Schmittgen (Livak et al., 2001).

Statistical analysis

Statistical analysis was conducted using GraphPad Prism 6 software (GraphPad Prism Software Inc., San Diego, CA, USA). Mann Whitney test and receiver operating characteristic (ROC) curve analysis with area under the ROC curve (AUC) were employed for examining the statistical significance of observed *ERBB4*/*HER4* expression differences between the groups, and also to evaluate whether they have enough sensitivity and specificity to distinguish the groups (Figure 1 and 2).

Results

Characteristics of participants

A total of 50 individuals, including 10 healthy women, 20 TNBC, and 20 non-TNBC patients were included in the present study. The median age at the time of sampling was 41.3, 43.1, and 45.6 for the healthy, TNBC, and non-TNBC cohorts, respectively. The proportion of stage I/II and stage III was almost similar in TNBC and non-TNBC cohorts.

Cross pattern between ERBB4/HER4 expression in TNBC and non-TNBC

Comparing the mRNA expression pattern in normal, TNBC and non-TNBC, the *ERBB4*/*HER4* gene was expressed differently (Figure 1). The *ERBB4*/*HER4* expression level was downregulated in TNBC (P value, < 0.0001; fold change, -2.1); however, upregulated in non-TNBC (P value, < 0.0001; fold change, 1.7). Moreover, *ERBB4*/*HER4* was differentially expressed between TNBC and non-TNBC (P value, < 0.0001). Interestingly, without categorization of BC samples, *ERBB4*/*HER4* was not significantly dysregulated (TNBC + non-TNBC vs normal; P value, 0.9816).

The specificity and sensitivity of ERBB4/HER4 expression level in discriminating normal, TNBC, and non-TNBC

ROC curves were generated by plotting sensitivity vs. (1-specificity), and AUC was measured and used to evaluate the potential predictive value of *ERBB4*/*HER4* to discriminate TNBC from healthy and non-TNBC from healthy (Figure 2).

ERBB4/*HER4* showed significant capability for differentiating TNBC from healthy (Area, 0.9933; Std. Error, 0.01090; 95% confidence interval, 0.9720 to 1.015; P value, < 0.0001) and non-TNBC from healthy (Area, 1.000; Std. Error, 0.0; 95% confidence interval, 1.000 to 1.000; P value, < 0.0001).

Discussion

In this study, we focused on the mRNA gene expression differences of a *HER* family member, *ERBB4*/*HER4*, in invasive ductal TNBC and non-TNBC compared with healthy mammary tissue. Our finding suggested that the pattern of *ERBB4*/*HER4* expression is completely different in TNBC and non-TNBC.

ERBB4/*HER4* is a member of epidermal growth factor receptor (EGFR) family which is less studied in comparison with other members of this family. *ERBB4*/*HER4* can have both oncogenic and proapoptotic roles at molecular levels (Mill et al., 2011; Nielsen et al., 2013). There are contradictory expression data about *ERBB4*/*HER4* in BC patients. It has been found that *ERBB4* overexpression in BC is associated with ER and PR positivity (Fujiwara et al., 2014) and upregulation of *ERBB3* (Sundvall et al., 2008). Moreover, it has been very well reviewed that *ERBB4* overexpression is associated with favorable outcome in BC patients (Thor et al., 2009), even stronger in the ER-positive (Sundvall et al., 2008) and *HER2*-positive cases (Suo et al., 2002). In addition an increased expression level of *ERBB4* is shown to have prognostic importance for overall survival (OS) and low histological grade in BC (Wang et al., 2016; Koutras et al., 2010; Machleidt et al., 2013) whereas some studies showed decreased tumor cell progression by downregulation of *ERBB4*/*HER4* (Hollmen et al., 2009; Junttila et al., 2005; Tang et al., 1999). Han *et al.* has shown the important role of *ERBB4* in interfering with estrogen receptor and consequently causing breast cancer cell proliferation and deregulation of cell cycle. In this study 90 percent of patients with enhanced levels of ER expression showed increased expression levels of *ERBB4* (Han et al., 2014) Conversely, Bieche *et al.* found that *ERBB4* expression is associated with unfavorable outcomes in BC (Bieche et al., 2003). Kim *et al.* has reported that *ERBB4* overexpression is a poor prognostic marker in ER-negative BC not in ER-positive group (Kim et al., 2016). Furthermore, our lab has shown that polymorphisms with predicted influences on regulation of *ERBB4*/*HER4* gene expression have inconsistent association with BC (Salimi et al., 2016; Bagheri et al., 2016; Moradi et al., 2016). The present study indicated that *ERBB4*/*HER4* expression is incomparable in all BC cases because it has opposite expression pattern in TNBC and non-TNBC groups.

Here, *ERBB4*/*HER4* transcript expression was compared among normal breast tissue, TNBC, non-TNBC, and BC (TNBC + non-TNBC) by real-time PCR. Our results have shown that *ERBB4*/*HER4* expression is changed when BC samples are categorized into TNBC and non-TNBC. In other words, although the expression of this gene remained constant between the normal and BC samples, the gene expression of *ERBB4*/*HER4* was significantly changed in TNBC and non-TNBC versus healthy breast tissue. Intriguingly, *ERBB4*/*HER4* gene expression was oppositely dysregulated in TNBC and non-TNBC (Figure 1). It has been established that TNBC shows more aggressive and poorer BC compared with non-TNBC; hence, this work reinforces meta-analysis of Wang et al. which suggested favorable prognosis for patients with overexpressed *ERBB4*/*HER4* (Wang et al., 2016). Moreover, according to the AUCs of ROC curves, *ERBB4*/*HER4* showed significant capability to distinguish normal breast tissue from TNBC and non-TNBC (Figure 2).

Notably, in the present work, sampling was conducted on newly diagnosed patients; hence, relapse-free survival analysis was not possible to perform. Furthermore, the sample size is another limitation of this work.

Conclusion

In conclusion, the current study suggests that *ERBB4*/*HER4* expression pattern is arbitrary in BC samples; however, its expression is associated with hormones receptors and *HER2* expression. Therefore, by categorizing BC samples into TNBC and non-TNBC groups, two distinct patterns of *ERBB4*/*HER4* expression revealed. This study shows that *ERBB4*/*HER4* expression levels increase in non-TNBC patients and conversely decrease in TNBC patients. Our results show that *ERBB4*/*HER4* may be used as a prognostic biomarker in non-TNBC patients which has the worst prognostic and also is the most aggressive kind of BC.

Conflict of Interests

N/A

Abbreviations:

Breast cancer (BC); Triple negative breast cancer (TNBC); estrogen receptor (ER); progesterone receptor (PR); human epidermal growth factor receptor (HER); immunohistochemical (IHC); Complementary DNA (cDNA); receiver operating characteristic (ROC); area under the ROC curve (AUC)

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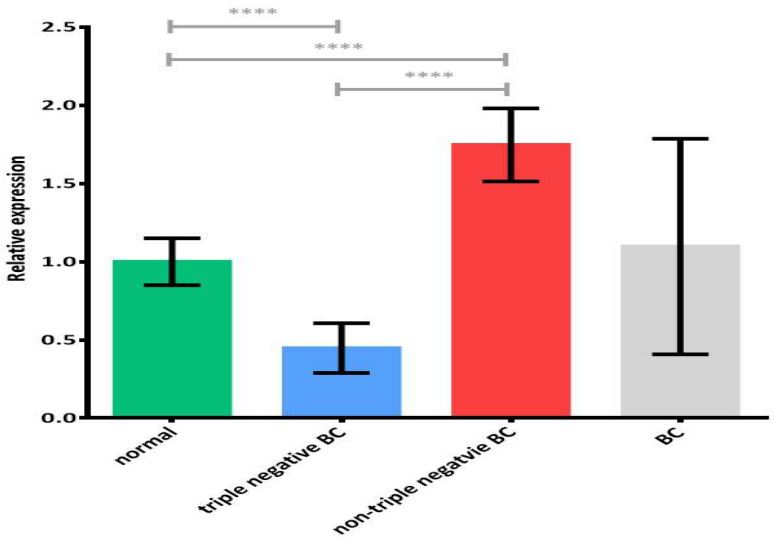

Fig. 1 Relative expression level of ERBB4/HER4

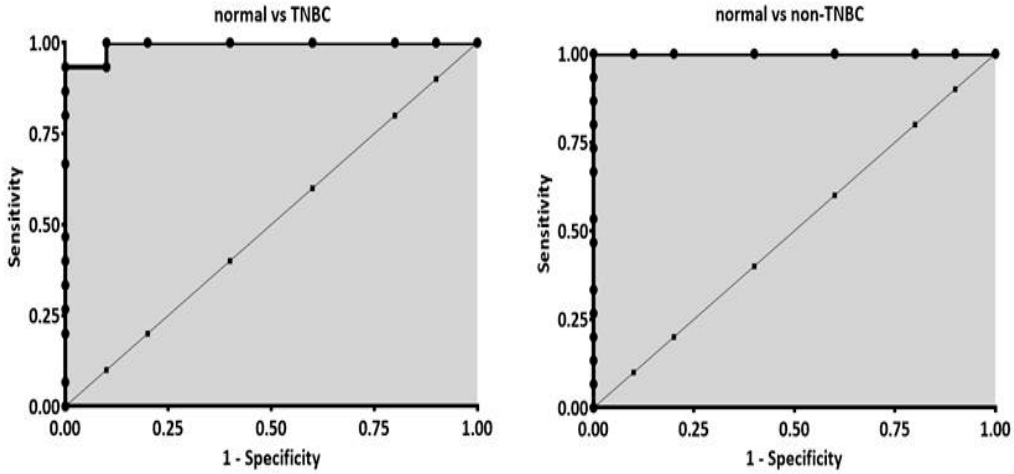

Fig. 2 ROC curves are drawn to show the capability of *ERBB4*/*HER4* to discriminate healthy tissue from TNBC (A) and non-TNBC (B).