Expression of SCUBE2 and BCL2 Predicts Favorable Response in ERα Positive Breast Cancer

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Abstract

Background: The study aimed at evaluating steroid biomarker genes (ERα, PGR, ERβ) and determining the expression level of estrogen-regulated genes (SCUBE2 and BCL2) and growth factors receptors (HER2 and IGFR1) in cancer tissue samples obtained from Iranian patients with breast cancer. Moreover, relationships with clinicopathologic aspects of tumor and response to treatment were studied.

Methods: The current study was conducted on 246 breast tissue samples. The expression levels of these genes and their relationships with clinicopathologic aspects and treatment response were evaluated.

Results: Based on immunohistochemistry (IHC) results, 12% of the ER negative patients expressed ERα. Comparing the effects of ERα and coexpression of BCL2 and SCUBE2 on the survival of the patients demonstrated remarkably poorer survival in ERα positive, SCUBE2, and BCL2 negative groups in comparison with other patients, which was statistically significant in the log-rank analysis (P = 0.01). Evaluation of the effects of coexpression of HER2 and IGFR1 on patients' survival demonstrated a worse survival rate in patients with positive expression of both receptors, which was insignificant.

Conclusion: Many studies suggest that PGR alone is not enough for the functional evaluation of ERα. Evaluation of the progesterone receptor expression as well as other genes such as BLC2, SCUBE2, and IGFR1, seems necessary to evaluate functionality.

Keywords: BLC2, ER, IGFR1, Multigene model, SCUBE2

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Introduction

Today, cancer studies are focused on finding diagnostic, predictive, and prognostic biomarkers. These biomarkers are commonly assessed in tumor tissues by categorizing patients into different subgroups leading to the selection of effective therapeutic methods. Since breast cancer (BC) is a heterogeneous disease, finding such biomarkers is of great importance to personalize the treatment.

Most of the clinically approved biomarkers are assessed using immunohistochemistry (IHC). Nevertheless, some recently developed cancer genetic panels such as Oncotype DX employ quantitative real-time polymerase chain reaction (qRT-PCR) to assess biomarkers. Several studies reported some practical biomarkers to predict the efficacy of different therapeutic approaches for BC such as hormone therapy, targeted therapy, and chemotherapy. However, none of them are approved for clinical practice yet.

In about 70% of patients with BC, estrogen receptor alpha (ERα) is expressed in the early tumor, which indicates that tumor cells growth is hormone-dependent and such patients can benefit from endocrine treatment; moreover, tamoxifen is the standard choice in most of the patients with ERα+ patients. Nevertheless, the prognosis of ERα+ cases is different, and some of them develop resistance against treatment after the therapeutic course, while the mechanism of this resistance is not entirely understood. Expression of progesterone receptor (PGR) is another factor; in other words, PGR+ indicates that the ER signaling pathway is functional in such patients. PGR+ tissue samples is a positive predictive factor and indicates the functionality of the ERα+ pathway and the patient may gain maximum advantage from blocking the pathway. Most of the studies recommend that PGR alone is not enough for the functionality of ERα. According to a variety of responses to therapeutic methods in ERα+/PGR- patients and sometimes lack of response to treatment in the early stages, the expression of signal peptides, CUB...
domain, and EGF such as domains containing 2 (SCUB2) and B-cell leukemia/lymphoma 2 (BCL2) as well as PGR is of great importance.\textsuperscript{10,11} These genes are suggested as estrogen-regulated genes.\textsuperscript{12,13} Some studies suggest measuring these markers to predict hormone therapy.

In addition to hormone therapy, the expression of biomarkers plays a significant role in responding to other therapeutic agents such as chemotherapy and targeted cancer therapy. For example, it was observed that in addition to HER2, blocking the expression of ER and insulin-like growth factor receptor-1 (IGFRI) influences response to treatment with herceptin.\textsuperscript{14} It was also observed that lower expression of IGFRI after chemotherapy is associated with better response to treatment in patients undergoing ACT (adriamycin, cyclophosphamide, and taxotere) chemotherapy.\textsuperscript{15} It is noteworthy that higher expression of IGFRI exacerbates response to chemotherapy and higher activity of IGFRI protein induces resistance to radiotherapy and chemotherapy.\textsuperscript{16}

In recent years, the expression of ERB\textsubscript{β}, the second identified estrogen receptor, is evaluated in different studies and the results are rather controversial.\textsuperscript{17-19} Tumors with higher levels of ERB\textsubscript{β} expression have a lower risk of an event such as recurrence or metastasis compared with tumors with lower expression levels in patients undergoing chemotherapy.\textsuperscript{20}

The current study aimed at evaluating steroid biomarker genes (ER\textsubscript{α}, PGR, ERB\textsubscript{β}) and determining the expression level of estrogen-regulated genes (SCUB2 and BCL2) and growth factor receptors (HER2 and IGFRI) in cancer tissue samples obtained from Iranian patients with BC using real-time PCR. Also, their relationships with clinicopathologic aspects of tumor and response to treatment were evaluated.

**Materials and Methods**

**Tissue and Sample**

The current study was conducted on 246 breast tissue samples including 123 tumors and 123 normal adjacent tissues. Sample size was calculated using an online web tool with 95% confidence level, 80% margin of error and 10% population proportion.\textsuperscript{21,22} The sample size was calculated at 62 for each group. Due to the possibility of sample attrition, 123 samples were considered in each group. Tissue samples along with clinicopathologic data were obtained from the Breast Cancer Research Center Biobank (BCRC-BB), Iran. According to the protocols followed by BCRC-BB, immediately after excisional biopsy or surgery, sample tissues were snap-frozen in liquid nitrogen and stored at -70°C.\textsuperscript{23}

Primers and TaqMan probes were designed by Gene Runner software version 3.0.5 for ER\textsubscript{α}, PGR, ERB\textsubscript{β}, SCUBE2, BCL2, HER2, and IGFRI. The list of primers and probes are available upon request. ACTB and TFRC were used as housekeeping genes.\textsuperscript{24}

**RNA Extraction and cDNA Synthesis and Gene Expression Assay**

RNA extraction was performed using 820 mg of the breast tumor and normal adjacent tissue by Rnx-Plus (Cinagen, Iran) as previously explained.\textsuperscript{24} The quality and quantity of the extracted RNA were measured by gel electrophoresis and spectrophotometry, respectively. The cDNA synthesis was performed using the cDNA synthesis Kit (Qiagen, Germany) according to the manufacturer’s protocol. Real-Time PCR was conducted using SYBR Green Master mix (Takara, Japan) and ABI 7500 version 2.0.6. Normal adjacent tissue was used as control.

**Data Analysis**

Gene expression was analyzed using the 2-∆∆CT method. Gene expression amounts >2 were considered as upregulation. Data were analyzed using SPSS version 19.0 (SPSS, Inc., Chicago, IL, USA). The student t-test was used to compare ∆CT between tumor and normal adjacent tissue. The frequency of genes expression was presented in total samples and also in ER- and ER+ positive patients separately (ER protein expression production based on IHC results). Age and follow-up time were presented as median/IQR and median/range respectively. Clinicopathologic data were presented as categorical data with frequencies and percentages. Shapiro Wilk’s and Levene tests and probability plots were used to evaluate the normal distribution of the variables and the homogeneity of variances, respectively. One-way analysis of variance (ANOVA) was employed to conduct the comparisons among the three groups.

The chi-square or the Fisher exact tests were used to determine the significance of differences between up- or downregulated gene expression and clinicopathologic variables. The correlation between gene expressions and clinicopathologic data was evaluated by following the Shapiro-Wilk’s method for normality tests and Pearson’s for correlation tests using the Pearson correlation test. The Kaplan-Meier analysis with log-rank tests was performed to calculate the cumulative survival proportion for disease free survival (DFS) based on the gene expression level. A Cox proportional hazards model was applied to investigate the univariate hazard ratio. Date of surgery was assumed as time zero. For multivariate analysis, variables with $P < 0.2$ in univariate analysis were included. $P < 0.05$ was considered to specify a statistically significant result.

**Results**

**Patients**

Demographic and clinicopathological data of the patients including age at diagnosis, IHC results of estrogen, progesterone and HER2 receptors, stage, grade, lymph nodes, and tumor size are summarized in Table 1. The number of triple negative subtypes in comparison with other subtypes is also included. The median age of the
patients was 48 years (range 29-87), and median follow-up was 48.5 months (range 1-65).

Gene Expression
The frequencies of upregulated, downregulated, and no changes in gene expressions are summarized in Table 2. It was observed that \(ER\alpha\) and \(ER\beta\) were also expressed in \(ER\) patients (based on IHC results). Twelve percent of \(ER\) patients expressed \(ER\alpha\). Also, the expression of this gene was absent in 5% of \(ER\) patients, but \(ER\beta\) expression was almost the same in \(ER\) and \(ER\) groups. This pattern was observed for \(PGR\), \(SCUBE2\), and \(BCL2\) expression as estrogen related genes. Some \(ER\) patients lacked the expression of these genes, while some \(ER\) patients expressed the abovementioned genes. There was a significant difference between tumor and matched normal tissue samples in the expression of \(BCL2\), \(ER\beta\), \(HER2\), \(PGR\), and \(SCUBE2\). \(P\) values are 0.0001, 0.0001, 0.005, 0.0001, and 0.0001, respectively.

The ANOVA analysis showed that patients with larger tumor sizes expressed higher \(HER2\) (\(P = 0.028\)) and lower \(ER\beta\) levels (\(P = 0.029\)). Moreover, patients above 50 years expressed higher \(HER2\) (\(P = 0.05\)) and lower \(ER\beta\) (\(P = 0.047\)). It was also observed that tumors with lower levels of \(ER\alpha\) expression had higher grades (\(P = 0.032\)) (data not shown).

Correlation of Gene Expressions and Clinicopathologic Data
As shown in Table 3, a significant negative correlation was observed between \(ER\beta\) expression and tumor stage (\(P = 0.006\)). Moreover, upregulation of \(HER2\) was correlated with higher stage and tumor size (\(P = 0.028\), \(P = 0.041\)).

The correlations between different genes are shown in Table 4. There was a significant correlation between \(ER\alpha\) and \(PGR\) (\(P < 0.001\)) and \(SCUBE2\) (\(P < 0.001\)), and \(SCUBE2\) and \(IGFR1\) (\(P < 0.001\)). There were also significant negative correlations between \(ER\alpha\) and \(IGFR1\) (\(P < 0.001\)), \(ER\beta\) and \(HER2\) (\(P < 0.001\)), and \(PGR\) and \(IGFR1\) (\(P < 0.001\)). The two latter correlations were strong. Other correlations were not significant. Chi-square analysis showed that upregulation of \(IGFR1\) was associated with triple negative subtype (\(P = 0.01\)) (data not shown). Analysis of \(BCL2\), \(SCUBE2\), and \(ER\beta\) showed no significant association with the type of tumor.

Survival Analysis and Prognostic Significance of Gene Expressions
Association between the selected gene expressions and patient survival was evaluated using the Kaplan-Meier analysis with a log-rank test for DFS. The median follow-up duration was 48.5 months (95% CI: 1-65 months). According to this analysis, gene expression status had no significant impact on survival of patients. Prognostic values of all gene expressions were investigated in the univariate and multivariate analyses of DFS. Although upregulation of \(ER\alpha\), \(HER2\), and \(PGR\) showed negative effects on survival and upregulation of \(BCL2\), \(IGFR1\), \(SCUBE2\), and \(ER\beta\) had positive effects on survival, the results were only significant for \(ER\alpha\). Multivariate analysis was not significant for the selected biomarkers (Table 5). Comparison of the effects of \(ER\alpha\) and coexpression of \(BCL2\) and \(SCUBE2\) on patients' survival demonstrated a remarkably poorer survival rate in \(ER\alpha^+\), \(SCUBE2^+\), and \(BCL2\) groups in comparison with other patients, which was statistically significant in the log-rank analysis (\(P = 0.01\)). Comparison of the effects of \(HER2\) and \(IGFR1\) coexpression on patients' survival demonstrated a worse survival rate in patients with positive expression for both
Table 2. Frequency of Gene Expression in ER- and ER+ Patients

| Gene | N* | Percent | N (ER Patients) | Percent | N (ER+ Patients) | Percent |
|------|----|---------|-----------------|---------|------------------|---------|
| ERα  |     |         |                 |         |                  |         |
| Down | 52  | 41.6    | 24              | 54.5    | 25               | 35.2    |
| No change | 16  | 12.8    | 9               | 20.5    | 5                | 7       |
| Up   | 57  | 45.6    | 11              | 22.7    | 41               | 57.7    |
| ERβ  |     |         |                 |         |                  |         |
| Down | 33  | 26.4    | 12              | 27.3    | 18               | 25.4    |
| No change | 8   | 6.4     | 2               | 27.345  | 5                | 7       |
| Up   | 84  | 67.2    | 30              | 68.2    | 48               | 67.6    |
| PGR  |     |         |                 |         |                  |         |
| Down | 66  | 52.8    | 26              | 59.1    | 35               | 49.3    |
| No change | 17  | 13.6    | 3               | 6.8     | 13               | 18.3    |
| Up   | 42  | 33.6    | 15              | 34.1    | 23               | 32.4    |
| SCUBE2 |     |         |                 |         |                  |         |
| Down | 86  | 68.8    | 26              | 59.1    | 52               | 73.2    |
| No change | 14  | 11.2    | 7               | 15.9    | 7                | 9.9     |
| Up   | 25  | 20.0    | 11              | 25      | 12               | 16.9    |
| HER2 |     |         |                 |         |                  |         |
| Down | 94  | 75.2    | 32              | 72.7    | 53               | 74.6    |
| No change | 9   | 7.2     | 1               | 2.3     | 7                | 9.9     |
| Up   | 22  | 17.6    | 11              | 25      | 11               | 15.5    |
| PGR  |     |         |                 |         |                  |         |
| Down | 60  | 48.0    | 18              | 40.9    | 37               | 52.1    |
| No change | 14  | 11.2    | 177             | 15.9    | 6                | 8.5     |
| Up   | 51  | 40.8    | 19              | 43.2    | 28               | 39.4    |
| HER2 |     |         |                 |         |                  |         |
| Down | 94  | 75.2    | 32              | 72.7    | 53               | 74.6    |
| No change | 9   | 7.2     | 1               | 2.3     | 7                | 9.9     |
| Up   | 22  | 17.6    | 11              | 25      | 11               | 15.5    |
| IGF1 |     |         |                 |         |                  |         |
| Down | 94  | 75.2    | 32              | 72.7    | 53               | 74.6    |
| No change | 9   | 7.2     | 1               | 2.3     | 7                | 9.9     |
| Up   | 22  | 17.6    | 11              | 25      | 11               | 15.5    |

* N, N (ER- Patients) + N (ER+ Patients) + missing.

Table 3. Pearson Correlation Coefficient for Different Gene Expressions and Clinicopathologic Data

| Gene | Stage | Grade | LN | T Size | Status | Age at Diagnosis |
|------|-------|-------|----|--------|--------|------------------|
| ERα  | -0.048| -0.086| 0.047| -0.078| 0.143  | -0.04            |
| ERβ  | -0.271**| 0.048| -0.183| -0.168| -0.065  | -0.15            |
| BC1H2| -0.041| 0.026| -0.029| 0.018| -0.10   | 0.06             |
| HER2 | 0.220*| -0.023| 0.177| 0.193*| 0.037  | 0.21             |
| PGR  | 0.084| -0.046| -0.066| 0.092| 0.064  | -0.16*           |
| SCUBE2| -0.066| 0.121| 0.001| -0.065| -0.067| -0.063          |
| IGF1 | -0.084| 0.049| 0.48| -0.083| -0.023| 0.05            |

* P value ≤ 0.05, ** P value ≤ 0.01.

Table 4. The Correlations between Different Gene Expressions

| Gene | ERαCAT | ERβCAT | PGRCAT | SCUBE2CAT | HER2CAT | IGF1CAT |
|------|--------|--------|--------|----------|---------|---------|
| ERαCAT | 1.000  |        |        |          |         |         |
| ERβCAT | 0.032  | 1.000  |        |          |         |         |
| PGRCAT | 0.315**| 0.164  | 1.000  |          |         |         |
| SCUBE2CAT | -0.469**| 0.128| -0.201*| 1.000    |         |         |
| HER2CAT | -0.099| -0.882**| -0.174| -0.089  | 1.000   |         |
| IGF1CAT | -0.388**| -0.127| -0.902**| 0.311**  | 0.145  | 1.000   |

* P value ≤ 0.05, ** P value ≤ 0.01.
receptors, but the result was not significant \((P = 0.1)\) (Figure 1). Coexpression of \(ER\beta\) and \(IGFRI\) and other combinations of gene expression was not significant.

**Discussion**

In the current study, the expression of \(ER\alpha\), \(PGR\), \(ER\beta\), \(SCUBE2\), \(BCL2\), \(HER2\), and \(IGFR1\) genes was investigated using real-time PCR. The findings demonstrated the significant effect of coexpression of \(Bcl2\) and \(SCUBE2\) on the survival of patients with \(ER\alpha\) overexpression. To the best of the authors’ knowledge, the effect of coexpression of these two genes was reported only in the recurrence score calculation in Oncotype DX along with 18 other genes, which calculated the risk of distant metastasis in

| Overall Survival | Univariate | Multivariate |
|------------------|------------|--------------|
| \(ER\alpha\)      |            |              |
| No and Down       | 1          | 1            |
| Up                | 2.90       | 0.91–9.2     | 0.07 | 2.27 | 0.685–7.583 | 0.180 |
| \(ER\beta\)       |            |              |
| No and Down       | 1          | 1            |
| Up                | 0.54       | 0.22–2.68    | 0.2  | 0.58 | 0.203–1.686 | 0.321 |
| \(HER2\)          |            |              |
| No and Down       | 1          | 1            |
| Up                | 1.40       | 0.44–4.5     | 0.54 |
| \(PGR\)           |            |              |
| No and down       | 1          | 1            |
| Up                | 1.80       | 0.65–5.3     | 0.23 |
| \(SCUBE2\)        |            |              |
| No and down       | 1          | 1            |
| Up                | 0.89       | 0.20–4.01    | 0.88 |
| \(IGFR1\)         |            |              |
| No and down       | 1          | 1            |
| Up                | 0.69       | 0.23–2.08    | 0.52 |
| \(BCL2\)          |            |              |
| No and down       | 1          | 1            |
| Up                | 0.74       | 0.23–2.3     | 0.62 |
| \(ER\) IHC        |            |              |
| Negative          | 1          | 1            |
| Positive          | 0.70       | 0.25–2.2     | 0.6  |
| \(PR\) IHC        |            |              |
| Negative          | 1          | 1            |
| Positive          | 0.80       | 0.27–2.4     | 0.7  |
| \(HER2\) IHC      |            |              |
| Negative          | 1          | 1            |
| Positive          | 1.20       | 0.38–4.1     | 0.69 |
| Stage             |            |              |
| I and II          | 1          | 1            |
| II and IV         | 3.20       | 0.82–12.4    | 0.09 | 1.05 | 0.418–2.658 | 0.911 |
| Grade             |            |              |
| I and II          | 1          | 1            |
| III               | 1.70       | 0.54–5.8     | 0.3  |
| Tumor Size        |            |              |
| I and II          | 1          | 1            |
| III and IV        | 2.60       | 0.8–8.9      | 0.10 | 2.49 | 0.692–8.396 | 0.162 |
| LN                |            |              |
| No and I-III      | 1          | 1            |
| IV-IX and >IX     | 2.87       | 0.86–9.56    | 0.08 | 1.46 | 0.413–5.195 | 0.554 |
| LN                |            |              |
| Negative          | 1          | 1            |
| Positive          | 5.10       | 0.66–40.1    | 0.1  |
| \(ER\) IHC        |            |              |
| Negative          | 1          | 1            |
| Positive          | 1.30       | 0.44–3.97    | 0.6  |
| \(PR\) IHC        |            |              |
| Negative          | 1          | 1            |
| Positive          | 1.20       | 0.40–3.6     | 0.7  |
| \(HER2\) IHC      |            |              |
| Negative          | 1          | 1            |
| Positive          | 0.80       | 0.24–2.56    | 0.7  |

CI, confidence interval
patients with stage I and II. Moreover, a negative effect of coexpression of IGFR1 and HER2 on patients’ survival was observed. Other studies show that blocking IGFR1 is helpful for response to herceptin. Moreover, patients with decreased expression of IGFR1 had fewer events in chemotherapy. Furthermore, increased expression of this protein had a negative impact on resistance to chemo- and radiotherapy. The current study did not investigate the effects of gene expression on different treatment regimens due to unavailability of this data. The results of the current study showed that upregulation of ERα, HER2, and PGR had negative impacts on survival. Also, the effect of BCL2, IGFR1, SCUBE2, and ERβ upregulation on survival was investigated. However, the results were positively significant for ERα.

The prognostic effect of ERα, PGR, and HER2 gene expression was in accordance with the effect of ER, PR, and HER2 protein by IHC. But there was no correlation between IHC and real-time PCR results of these biomarkers. It should be noted that recent studies have shown different correlations between RT-qPCR and IHC for ER. So, this discordance could be attributed to several factors, as follows. There is a lot of variation in IHC methodology. These wide variations consist of the cold ischemia time which is related to tissue processing, scoring and annotation system and using differences antibody clones, all of which are different between labs. Moreover, the lack of a proper cutoff set for IHC as a semi-quantitative technique with different scoring methodology can cause this disparity. The IHC technique targets ER protein. However, RT-qPCR evaluates ER gene expression at the RNA level. Therefore, post-translational modification of the ER gene may be responsible for this discrepancy. Tumor dissection is necessary for RT-qPCR assay. Sometimes, incorrect tumor dissection can lead to diverse results, indicating the inclusion of some parts of normal tissue which contaminates the sample. It should be mentioned that in the current study, tumor dissection was performed only on the tumor part. In the Oncotype Dx test which evaluates the ten-year risk of recurrence in breast cancer, the real-time PCR assay is used for detecting the level of hormone receptors as well as HER2 which shows mRNA expression would be a good choice for diagnostic tests.

The results of the correlation between gene expression and clinicopathologic data of the patients were in concordance or discordance with some studies, which will be discussed. According to previous studies, IGFR1 protein binds to the high-affinity insulin-like growth factor. This receptor has tyrosine activity and is overexpressed in many malignant tissues and acts as an anti-apoptosis factor, which increases the survival rate of cancer cells. In the current study, higher IGFR1 expression was a favorable prognostic factor; its expression also had a positive correlation with SCUBE2 expression and a negative correlation with ERα and ERβ expressions. The results of similar studies are in agreement with those of the current study, although some are inconsistent. Lato et al indicated a correlation between increased IGFR1 expression and higher grades of the tumor, shorter DFS, and poor prognosis. Some other studies also reported a correlation between the expression activity of IGFR1 with disease progression, increased resistance to radiotherapy, and poor prognosis. Higher IGFR1 expression in tumors >2 cm, and grade II or III was also reported by Browne et al. A study by Yerushalmi et al showed that the expression of IGFR1 was associated with lower tumor grades, ER expression, and lack of HER2 expression. Expression of IGFR1 was associated with good prognostic factors such as older age at diagnosis, lower grades, negative HER2, and higher levels of P27. In contrast, a study on 60 patients with BC showed a significant relationship between the increased IGFR1 expression and higher tumor grades. They suggested that

Figure 1. The Kaplan-Meier Plot. The Kaplan Meier plot of patient survival stratified by coexpression of BCL2-SCUBE2 (right) and IGFR1-HER2 (left) in ERα+patients; vertical marks show censored patients. The censoring was due to missing data of the patients’ follow-up (0.2 in BCL2-SCUBE2 and 0.06 in IGFR1-HER2). Censoring means the total survival duration for that patient cannot be accurately determined. Most of the time, it occurs when participants are either excluded or refuse to participate in the study.
the overexpression of IGFR1 was associated with invasive behavior of tumor cells, but indicated no relationship between IGFR1 and stage of the disease or lymph node metastasis. It was also revealed that IGFR1 expression might cause angiogenesis by vascular endothelial growth factor, and consequently, metastasis in BC cases. Nevertheless, some similar studies indicated no association between the expression of IGFR1 and clinicopathological features of the tumor; in a study on 210 paraffin-embedded early BC tumors, similar to the current study, no relationship was observed between IGFR1 expression and clinicopathological features of the disease such as age at incidence, tumor size, lymph nodes status, and hormone receptors. However, in a study by Al Sarakbi et al, a relationship was observed between the mRNA level of IGFR1 and that of lymph nodes. In a study on non-small cell lung cancer cases, increased IGFR1 expression was associated with larger tumor sizes.

The SCUBE2 protein belongs to the SCUBE protein family and is a tumor-inhibitory factor. Expression of SCUBE2 protein has been observed in different tissue such as breast ducts epithelium. Although the role of this protein is not perfectly identified in healthy cells, its expression is observed in early BC tumors, and better prognosis is reported in patients expressing SCUBE2, compared with the ones who do not express it. The SCUBE2 protein inhibits tumor growth through the bone morphogenetic pathway and beta-catenin signaling pathway. In the current study, a negative correlation was observed between the expression of ERα and PGR with SCUBE2 and also a negative correlation with the expression of ERα and PGR with IGFR1. Also, a significant correlation was observed between the increased expression of this gene and better prognosis in patients with BC. A study by Skrzypczak et al showed that the expression of this gene was reduced in cases with endometrial cancer of higher grades. It is noteworthy that the expression of this gene showed a positive correlation with the expression of PGR and ER. Another study also indicated lower recurrence and better survival rates in patients with colorectal cancer and higher expression levels of these genes; on the other hand, decreased expression of SCUBE2 was associated with progression and prognosis in such patients.

ERβ belongs to the estrogen receptors family and it is present in nucleus, cytoplasm, and mitochondria. By binding a ligand to ERβ, it forms homo- and heterodimers, which activate transcription from specific sequences of DNA. Some isoforms of this receptor inhibit the activity of other members of the estrogen receptors family. In the current study, ERβ gene showed lower expression levels in tumors with a greater size. Also, patients aged above 50 years had lower ERβ and higher HER2 expression levels. In a study by Sapino et al on BC using IHC and real-time PCR techniques, the mean age of ERβ+ patients was lower than those with ERβ-, which is in concordance with the findings of the current study. Miyoshi et al reported that the ERβ expression was associated with tumors smaller than 2 cm and higher grades. The first result is in concordance with the findings of the current study. Another study showed that smaller size tumors and higher overall survival (OS) were associated with lack of ERβ expression. In a study on ovarian cancer, ERβ expression was associated with metastasis to lymph nodes. By evaluating 508 tumor samples, no significant association was observed between ERβ expression and clinicopathological features.

In conclusion, many studies today suggest that PGR alone is not enough for the functional evaluation of ERα, based on the variety of responses to treatment in ERα+/PgR- patients and even lack of response to different treatments in the early stages. Evaluation of the progesterone receptor expression as well as other genes such as BLC2 and SCUBE2, IGFR1 (using the multigene model), seems necessary to evaluate the functionality of ERα.
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