Clinicopathological significance of insulin-like growth factor-1 receptor expression in breast cancer

Ann. Ital. Chir., 2020 91, 6: 583-591
pii: S0003469X20031486

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OBJECTIVE: *Insulin-like growth factor 1 receptor (IGF1R) is a receptor protein tyrosine kinase that is claimed to be related with tumor development and progression of breast cancer with some conflicting results in the literature. The aims of the study are to investigate expression of IGF1R, and correlate with clinicopathological parameters to clarify the significance of IGF1R on breast cancer.*
MATERIAL AND METHODS: *IGF1R and Ki67 were applied immunohistochemically to the tissue microarray sections of 370 female breast cancer patients. The results were correlated with clinical, prognostic, histopathological features, and other immunohistochemical findings [ER, PR, HER2, CK5/6, and CK14] statistically.*
RESULTS: *IGF1R overexpression showed direct correlation with Ki67 index (P=0.028), HER2 positivity (P=0.001), mitotic count (P=0.004), tumor grade (P=0.015), and geographic necrosis (P=0.023); and negative correlation with ER positivity (P=0.003). There was statistically significant difference between IGF1R expression and the molecular subtypes (P<0.001), mostly HER2+ phenotype. IGF1R expression was found to be higher in invasive ductal carcinoma (IDC) than invasive lobular carcinoma (ILC) (P=0.036). Both IGF1R and Ki67 expression were negatively correlated with disease-free survival (DFS) (P=0.020, P=0.023, respectively) and overall survival (OS) [P<0.001, each] rates. The inverse association between IGF1R overexpression and OS rate was also supported by multivariate analyses (P=0.025).*
CONCLUSIONS: *Overexpression of IGF1R was found to be directly correlated with shorter DFS and OS as well as some clinicopathological features associated with adverse prognosis such as higher Ki67 index, mitotic count, tumor grade, presence of geographic necrosis, HER2 positivity, ER negativity, HER2+ molecular subtype, histological tumor type of IDC rather than ILC. Thus, IGF1R might be considered as an useful target for comprehensive future anti-tumor therapy investigations. Additionally, using IGF1R as well as Ki67 as a part of routine pathology practice might be fruitful in breast cancer therapy and prediction of prognosis.*

KEY WORDS: Breast carcinoma, IGF1R, Insulin-like growth factor-1 receptor, Immunohistochemistry, Prognosis

Introduction

Breast cancer is one of the most common cause of cancer-related death in females [1]. The heterogeneity and variability in treatment and survival response, underline the necessity to clarify the biological mechanisms promoting breast cancer [2]. Targeting some molecules involved in the pathogenesis and the prognosis of breast cancer are being investigated to discover more effective therapies seriously. Insulin-like growth factor-1 (IGF1) and its receptor insulin-like growth factor-1 receptor (IGF1R) are among the most attractive molecules widely evaluated in the literature, due to their biological functions particularly in cell survival and tumorigenesis [2-8]. IGF1 is a 7.7 kDa single-chain polypeptide that is a necessary mitogen in the breast [2]. IGF1 binds to its receptor,

Pervenuto in Redazione Settembre 2019. Accettato per la pubblicazione Ottobre 2020
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IGF1R, and initiates a signaling cascade leading to epithelial-mesenchymal transition, regulate differentiation, cellular proliferation, invasion, metastasis, angiogenesis and protection from apoptosis [2-6,9-10]. IGF1R is implicated in the development, and progression of many cancers, including breast cancer [2-3,5-8,10-11]. In addition, overexpression of IGF1R has recently been reported to be associated with resistance to the treatment options of tamoxifen, chemotherapy, Herceptin, and radiotherapy for breast cancer [5]. However, the exact clinical and prognostic significance of IGF1R on breast cancer are still not clarified.
The goals of this study were to find out the expression of IGF1R that is thought to play a role in the breast cancer prognosis and treatment with some conflicting data in the literature by immunohistochemistry, and evaluate the association with the prognostic and histopathological features, and other immunohistochemical antibodies [ER, PR, HER2, CK5/6, CK14, and Ki67].

Materials and Methods

After obtaining informed consents and ethic committee approval, 370 patients with consecutive breast cancer diagnosed by resection specimens at Department of Pathology, Gazi University School of Medicine between 2006-2010 that did not recieve neoadjuvant therapy were included. The tissue microarray paraffin blocks containing 4 tumor samples about 0.1 cm in diameter (about filling the objective of x20 of the light microscope, Olympus BX53F, Tokyo, Japan) prepared for a thesis [1] previously from each patient were used in the study. Paraffin blocks were cut into 4-µm sections, deparaffinized and dehydrated according to standard protocols. The antibodies of Ki67 (7 ml RTU, mouse anti-human monoclonal antibody, clone K2, Leica Biosystems, Danvers, MA, USA), and IGF1R (1: 200 dilution, 1.0 ml concentrated, mouse monoclonal, clone BC10, Biocare medical, Pike Lane Concord, CA, USA) were applied to the 4µm-thick sections prepared from tissue microaaray blocks by immunohistochemistry at the Department of Pathology, Bozok University School of Medicine, in an automatised stainer (Leica Bond-Max, Leica Biosystems, United Kingdom). Citrate buffer, pH 6.0, was applied as epitope retrieval solution for 20 minutes. Diaminobenzidine (DAB) detection kit was used as chromogen, and hematoxylin as a counter-stain. Primary antibody was omitted for negative tissue control. Colon cancer was used as positive tissue control. The immunohistochemical staining in the whole sections were evaluated by a pathologist under a light microscope (Olympus BX53F, Tokyo, Japan). IGF1R expression was scored as follows: [score 0= no staining observed or staining observed <%10 of tumor cells; score 1= a faint or barely perceptible membrane staining in ≥10% of tumor cells, the cells are only stained in part of their membrane; score 2= a weak to moderate complete membrane staining in ≥10% of tumor cells; score 3= a strong complete membrane staining in ≥10% of tumor cells], similar to Sun et al [5]. According to IGF1R expression, the cases were divided into two groups as "none/low expression (score 0 and 1)" and "overexpression (score 2 and 3).
Ki67 expression was evaluated by counting the number of the positively stained nuclei in the 4 tissue microarray samples each filling the objective of x20 of the light microscope (Olympus BX53F, Tokyo, Japan). The positively stained nuclei for Ki67 were scored as follows; score 0: negative, score 1: ≤10 nucleus/nuclei, score 2: 11-50 nuclei, score 3: 51-100 nuclei, score 4: 101-200 nuclei, score 5: 201-400 nuclei, score 6: 401-600 nuclei, score 7: ≥601 nuclei similar to our recent study [12]. The immunostaining results were correlated statistically with the clinicopathological features [patient's age, histological tumor type, tumor size, mitotic count, presence of distant metastasis, nipple invasion, breast skin invasion, fascia invasion, presence of geographic necrosis, number of metastatic lymph node, tumor grade, pathological tumor stages (pT) and pathological lymph node (pN) stages, molecular tumor subtypes, disease free survival (DFS) and overall survival (OS) rates] and the expressions of other immunohistochemical antibodies (ER, PR, HER2, CK5/6, and CK14) performed previously for a thesis [1]. Cytoplasmic staining of ≥1% tumor cell(s) for CK5/6 and CK14 had been considered as positive. The cases that showed no staining had been considered as negative. Nuclear staining more than 1% for ER and PR had been considered as positive. HER2 status had been scored using the system as scores 0 to 3 [1, 13]. The HER2 status of the cases that had showed score 2 by immunohistochemistry had been evaluated by florescent in situ hibridization (FISH).

Statistical Analysis

Data analysis was performed using PASW (Predictive Analytics Software) Statistics version 18.0 (SPSS Inc. Chicago. IL. USA). The demographic variables were detected using descriptive statistics. The compliance of data with normal distribution was evaluated with the Kolmogorov–Smirnov and Shapiro-Wilk tests. Kruskal-Wallis H and Mann-Whitney U tests were used for comparisons between the groups. The Chi-squared test, Fisher's exact tests, and Spearman's Rho correlation analysis were used for investigating the association between immunoexpressions of antibodies and the clinicopathological parameters. The survival rate was estimated using the Kaplan-Meier method, and differences in survival curves were analyzed using the Log-rank tests. The effects of associated variables detected by univariate analysis were studied by multiple linear regression analysis with a backward elimination. P-value <0.05 was considered as significant.

Results

The total of 370 patients included in the study were women. The mean age of the patients was 52.9±11.4 years (range: 19-86 years). The operation material was mastectomy in 345 (93.2%) patients, and lumpectomy in 25 (6.8%) patients. The number of mastectomy was higher than lumpectomy specimens due to the clinical stage of the patients related to the size of the tumors, and the lymph node status. The tumor size ranged from 0.4 cm to 20 cm (mean: 2.6±1.59 cm). Lymph node metastasis were found in 202 of 362 cases performed lymph node dissection. The mean number of metastatic lymph nodes was 2.5±4.8. Nipple invasion was detected in 31 tumors, skin involvement was present in 13, and fascia invasion was found in 32 tumors. There were 348 (94.05%) invasive ductal carcinomas (IDCs), and 22 (5.05%) invasive lobular carcinomas (ILCs). The mean number of mitosis in 10 high power fields was calculated as 11.4±8.7 (range: 1-60). Disease-free survival (DFS) and overall survival (OS) were evaluated in 222 (60%) patients. DFS ranged from 5 months to 84 months (mean: 42.3±14.5 months), and OS ranged from 13 months to 84 months (mean: 44.4±13.9). Among 222 patients whom current status were achieved, 11 were dead, and 211 were alive. Pathological tumor stage was pT1 in 153 cases, pT2 in 202 cases, pT3 in 15 cases. Pathological lymph node stage was pN0 in 160 cases, pN1 in 125 cases, pN2 in 53 cases, pN3 in 24 cases. Distant organ metastasis was detected in 26 of 222 patients. According to modified Bloom-Richardson Classification, 103 patients had grade 1, 131 had grade 2, and 136 had grade 3 tumors [14]. Clinicopathological features of the patients are shown in Table I. ER-positivity was present in 290 cases, PR-positivity was present in 275 cases, HER2 positivity was present in 133 cases. There were 200 cases of luminal A (ER+, PR+, HER2-), 106 cases of luminal B (ER+, PR+, HER2+), 27 cases of HER2+ (ER-, PR-, HER2+), 37 cases of basal-like triple negative (ER-, PR-, HER2-, CK5/6+ and/or EGFR+) molecular subtypes according to the immunhistochemical and FISH results [1,5]. 188 cases were positive for CK 5/6, and 30 cases were positive for CK14.

The slides of 366 cases (344 IDCs and 22 ILCs) were suitable to examine the staining of IGF1R among 370 cases. In 4 cases, IGF1R expression could not be scored due to the artifacts. All of the 366 cases showed staining, at least focally, for IGF1R. 216 cases showed score 0, 67 cases showed score 1, 46 cases showed score 2, and 37 cases showed score 3 for IGF1R. According to this data, 283 (77.3%) cases showed "low expression", and 83 (22.7%) cases showed "overexpression" for IGF1R. Ki67 expression was evaluated in 352 of 366 cases. In 14 cases, Ki67 expression could not be evaluated due to the artifacts. 187 of those cases exhibited score 0, 51 cases exhibited score 1, 40 cases exhibited score 2, 22 cases exhibited score 3, 21 cases exhibited score 4, 17

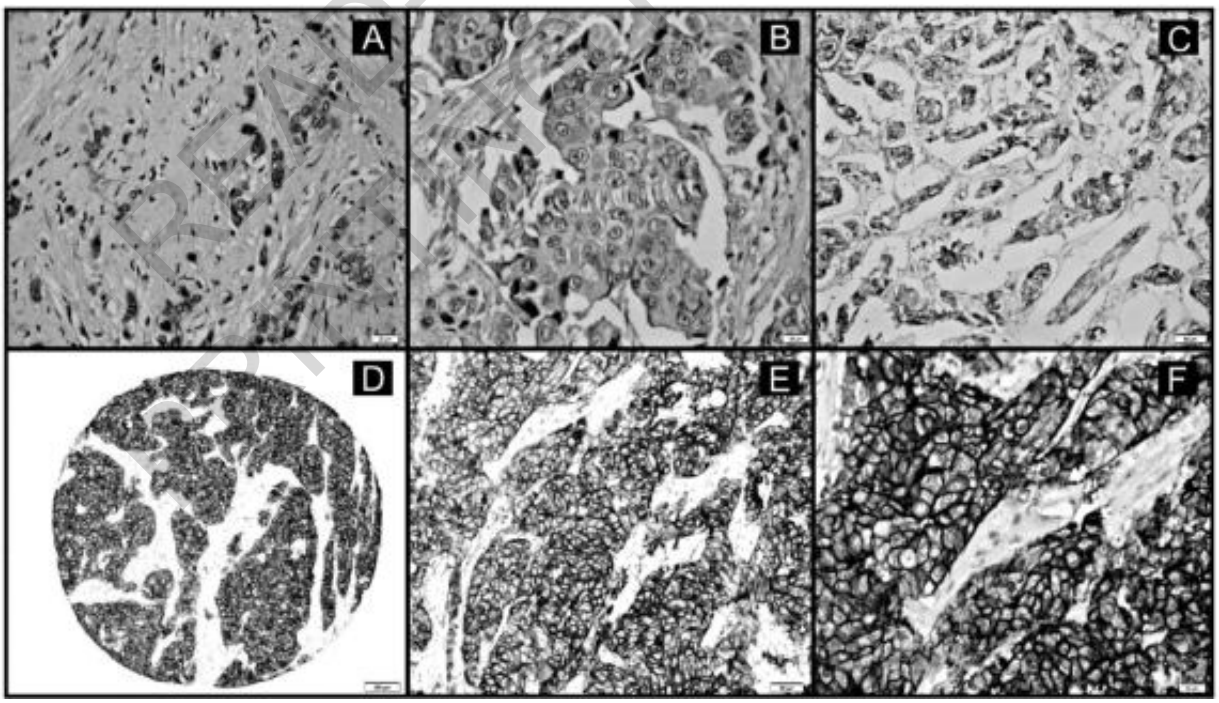

Fig. 1: Photomicrographs of IGF1R immunostaining scores in breast cancer samples. A) Score 0 in invasive lobular carcinoma, (Streptavidin-biotin peroxidase method, x40); B-C) Score 1 and score 2 in invasive ductal carcinoma, respectively (Streptavidin-biotin peroxidase method, x40, x200, respectively); D-E-F). Score 3 in invasive ductal carcinoma, (Streptavidin-biotin peroxidase method, x100, x200, x400, respectively).

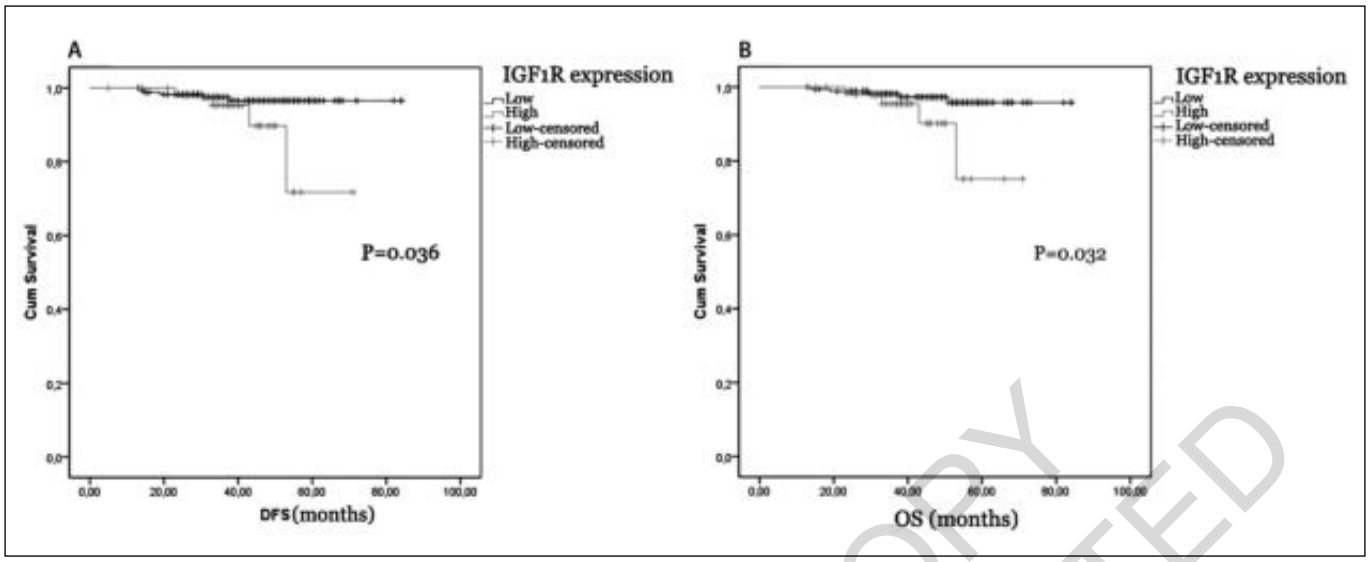

Fig. 2: Kaplan-Meier survival curves related with low vs. high IGF1R expression 4/173 (2.3%) deaths vs. 7/49 (14.2%) deaths, respectively). A. Disease-free survival (DFS) curves showing inverse correlation with IGF1R expression (P=0.036). B. Overall survival (OS) curves showing inverse correlation with IGF1R expression (P=0.032).

cases exhibited score 5, 8 cases exhibited score 6, 8 cases exhibited score 7 for Ki67.

The data about the immunohistochemical, clinicopathological and prognostic parameters that showed statistically significant correlation according to both univariate and multivariate analyses are given in Table II. IGF1R expression was detected to be higher in IDCs than ILCs (P=0.036). IGF1R (Fig. 1A-1F) expression showed direct correlation with expression of Ki67 (P=0.028), HER2 (P=0.001), mitotic count (P=0.004), tumor grade (P=0.015), and geographic necrosis (P=0.023). There was negative correlation with ER positivity (P=0.003). There was statistically significant difference between IGF1R expression and the molecular subtypes of the tumors (P<0.001). According to the molecular subtypes, the tumors with HER2+ phenotype showed higher IGF1R expression than with luminal A (P<0.001), luminal B (P=0.014), and triple negative (P=0.044) phenotypes. The tumors with Luminal B phenotype demonstrated higher IGF1R expression than those with Luminal A phenotype (P=0.019). There was no statistical significant difference between the tumors with basal-like phenotype and non-basal-like phenotypes (P=0.087). Ki67 expression was inversely correlated with DFS (P=0.023) and OS (P<0.001), statistically.

During follow-up, we detected that 4 (2.3%) of 173 cases with low IGF1R expression were deceased, while 7 (14.2%) of 49 cases with high IGF1R expression were deceased. Mean OS was 81.9±0.9 months in cases with low IGF1R expression, while it was 64.8±3.0 in cases with high IGF1R expression. Mean DFS was 81.9±0.8 months in cases with low IGF1R expression, while it was 64.1±3.4 in cases with high IGF1R expression. It was detected that when IGF1R expression increased, DFS (P= 0.020) and OS (P=0.005) tended to decrease, by univariate analysis (Table II). According to multiple linear regression analysis; IGF1R was found to be inversely correlated with OS (P=0.025, β= -0.148, t= -2.252). Furthermore, the Kaplan-Meier survival curves showed negatively association of DFS (P= 0.036) and OS (P= 0.032) in respect to IGF1R expression (Fig. 2). Associated parameters with IGFR expression other than OS in univariate analysis did not show any significant correlation by multiple linear regression analysis.

Discussion

IGF1R is a tetrameric receptor, composed of two α-identical and two α- identical subunits [2]. After binding to its ligand IGF1 and subsequent phosphorylation, IGF1R promotes the activation of two major signaling cascades via the insulin receptor substrate (IRS-1): the phosphatidylinositol 3-kinase/AKT kinase (PI3K/AKT) pathway and the RAF kinase/mitogen activated proteinkinase (RAF/MAPK) pathway [2,15]. Those pathways are well-known to stimulate proliferation and inhibit apoptosis [2,15]. A family of six IGF-binding proteins (IGFBP1-6), mostly IGFBP3, bind to IGF1 that regulates the bioavailability and half life of circulating IGF1 [2,16]. Although some of the data in the literature are controversial, many components of the IGF1 system (IGF1, IGF1R, and IGFBP1-6) are claimed to be modified during breast cancer development and progression [2, 17,18].

Table I - *Clinicopathologic features (n = 370).*

Age, years, mean ± SD (range)	52.9±11.4 (19-86)
Gender (female/male)	370/0
Operation materials, n	
Mastectomy	345
Lumpectomy	25
Tumor size, cm, mean ± SD (range)	2.6±1,59 (0.4-20)
Tumor types, n	
Invasive ductal carcinoma	348
Invasive lobular carcinoma	22
Nipple involvement, n	
Present	31
Absent	313
Unknown	26
Skin involvement, n	
Present	13
Absent	357
Fascia involvement, n	
Present	32
Absent	319
Unknown	19
Metastatic lymph nodes, n	
Present	202
Absent	160
Unknown	8
Distant metastasis, n	
Present	26
Absent	196
Unknown	148
Geographic necrosis, n	
Present	55
Absent	315
The number of mitosis /10 high power fields, mean ± SD (range)	11,4±8.7(1-60)
Tumor grade, n	
Grade 1	103
Grade 2	131
Grade 3	136
Overall survival, months, mean ± SD (range)	44.4±13.9 (13-84)
Disease-free survival, months, mean ± SD (range)	42.3±14.5 (5-84)
The current status of patients, n	
Alive	211
Dead	11
Unknown	148

Abbreviations: SD, standard deviation.

The anti-apoptotic and tumorigenic effects of IGF1 are mediated by IGF1R. Therefore, IGF1R is reported to be frequently overexpressed in breast cancer, in the literature [2]. Some studies have claimed that there is a direct association between the IGF1R expression and breast cancer establishment [2, 19-21]. Overexpression of IGF1R in epithelial cells in benign terminal duct lobular units of breast biopsies have been found to be related with up to 15 times increased breast cancer incidence [2,21]. It is reported that once the breast cancer established, elevated IGF1R levels have been demonstrated, most often regardless from cancer subtype, ER, PR or HER2 status [2]. IGF1R overexpression has been reported to be an unfavorable prognostic factor in some studies, while some others have claimed it is a favorable prognostic factor or stated that there is no association with prognosis, in the literature [2,5,22-23]. Thus, there is still no consistency about the clinical significance of IGF1R overexpression across

Table II - *Statistically significant associations between immunohistochemical and clinicopathologic characteristics (P<0.05).*

	Low IGF1R Expression n (%)	IGF1R Overexpression n (%)	Univariate Analysis (P-value)
HISTOLOGICAL TUMOR TYPE (n=366)			
IDC	262 (76.2%)	82 (23.8%)	P=0.036
ILC	21 (95.5%)	1 (4.5%)	
TUMOR GRADE (n=366)			
Grade 1	85 (83.3%)	17 (16.7%)	P=0.015, dir
Grade 2	104 (81.3%)	24 (18.8%)	
Grade 3	94 (69.1%)	42 (30.9%)	
GEOGRAPHIC NECROSIS (n=366)			
Absent	247 (79.4%)	64 (20.6%)	P=0.023, dir
Present	36 (65.5%)	19 (34.5%)	
MITOTIC COUNT/10 HPFS (N=366)			
<8	133 (85.3%)	23 (14.7%)	P=0.004, dir
7-14	74 (74.7%)	25 (25.3%)	
≥15	76 (68.5%)	35 (31.5%)	
Ki 67 (n=352)			
Score 0	152 (82.2%)	33 (17.8%)	P=0.028, dir
Score 1	36 (70.6%)	15 (29.4%)	
Score 2	34 (85.0%)	6 (15.0%)	
Score 3	17 (77.3%)	5 (22.7%)	
Score 4	15 (71.4%)	6 (28.6%)	
Score 5	9 (52.9%)	8 (47.1%)	
Score 6	4 (50.0%)	4 (50.0%)	
Score 7	5 (62.5%)	3 (37.5%)	
ER (n=366)			
Negative	52 (65.0%)	28 (35.0%)	P=0.003, inv
Positive	231 (80.8%)	55 (19.2)	
HER2 (n=366)			
Negative	194 (82.6%)	41 (17.4%)	P=0.001, dir
Positive	89 (67.9%)	42 (32.1%)	
MOLECULAR SUBTYPES (n=366)			
Luminal A	167 (84.3%)	31 (15.7%)	P<0.001
Luminal B	76 (73.1%)	28 (26.9%)	
HER2+	13(48.1%)	14 (51.9%)	
Triple negative	27 (73.0%)	10 (27.0%)	
DISEASE-FREE SURVIVAL, (N=222, months)	81.9±0.8	64.1±3.4	P=0.020, inv
OVERALL SURVIVAL (N=222, months)	81.9±0.9	64.8±3.0	P=0.005, inv

Abbreviations: dir, directly correlated; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; inv, inversely correlated; IGF1R, insulin-like growth factor 1 receptor; HPFs, high power fields; ER, estrogen receptor.

studies [2,5]. In the present study, overexpression of IGF1R has been found to be directly correlated with HER2 positivity, in contrast to some studies in the literature [3,5,20,24]. In addition, statistically significant difference between IGF1R expression and different molecular subtypes has been detected. Thus, it seems to point out the correlation of HER2 positivity and IGF1R expression. According to this, the tumors with HER2+ phenotype (ER-, PR-, HER2+ tumors) exhibited higher IGF1R expression than the others. Additionally, tumors with Luminal B (ER+, PR+, HER2+) phenotype expressed higher IGF1R than those with Luminal A (ER+, PR+, HER2-) phenotype in our study. HER2-positive tumors is well-documented to have worse outcome in the literature. In addition, Yerushalmi et al. have reported that IGF1R expression is a an unfavorable prognostic factor for the patients with HER2+ molecular phenotype [23]. ER-positivity is a world-wide well-known favorable prognostic factor [2]. Although the exact mechanism is still unknown, it is suggested that there is a cross-talk

between the IGF-system, ER and the cognate ER ligand 17β-estradiol (E2) [2]. There are some conflicting results in the literature between ER-positivity and IGF1R expression. Some studies have demonstrated direct correlation between IGF1R expression and ER-positivity, while others found no correlation between IGF1R expression and ER status [3,5,23-24]. Similar to a recent study, we have detected an inverse correlation between IGF1R overexpression and ER-positivity [25]. They have reported that decreased estrogen might increase IGF1 dramatically [25]. In addition, they have suggested that the inhibition of the IGF pathway might be used as an effective strategy for ER-positive breast cancer therapy, even in hormone therapy-resistant cases [25]. On the other hand, Wu et al. have reported inhibition of IGF1R signaling, and induction of DNA damage might show synergistic effect for the treatment of triple-negative and ER-negative breast cancer [26]. Those inconsistent results may have been obtained due to an unknown antagonistic affect of ER ligand E2 on IGF1R, a claim that should be clarified by further investigations.

Shimizu et al. reported a study conducted on evaluation of IGF1R expression in 210 breast cancer by immunohistochemistry, similar to our study [3]. They have found no correlation between IGF1R expression and age, tumor size, lymph node metastasis, tumor grade, hormone receptor status and OS rates [3]. Similar to that study, we have not detected any association between IGF1R expression and tumor size and lymph node metastasis. Whereas, we have documented direct correlation between IGF1R overexpression and HER2 positivity and tumor grade; and inverse correlation with ER positivity, DFS and OS. They have evaluated IDC, ILC, and some other rare variants of breast cancer, however no association between the histological tumor types and IGF1R expression has been mentioned in their study [3]. Nevertheless, we have detected statistically significant difference between the expressions of IGF1R among the histological tumor subtypes of IDC and ILC, for the first time in the literature, to the best of our knowledge. IDCs showed higher expression than ILCs for IGF1R. It is well documented that IDCs are more agressive tumors than ILCs, therefore this finding might support the negative effect of IGF1R on clinical outcome.

In the literature, a growing evidence is present that indicates high Ki67 expression is associated with poorer outcome in breast cancer [27,28]. Similar to the literature, we have detected an inverse correlation between higher Ki67 expression and both lower DFS and OS. In addition, we have demonstrated a direct correlation between higher Ki67 expression and IGF1R overexpression. As a support to this finding, higher mitotic count has also been found to be associated with IGF1R overexpression. However, a study has demonstrated an inverse correlation between IGF1R expression and Ki67 [23].

In the literature, it has been claimed by a study that there is no correlation between IGF1R and tumor grade [3], while another study has found an inverse correlation between them [23]. However, we have detected that the tumors with higher histological grade show statistically significant higher IGF1R expression, indicating poorer prognosis.

As mentioned previously, the results about IGF1R expression on breast cancer show discrepancy among the different studies in the literature. Several factors may be responsible for those contradictory results. For instance, there is no concensus on methodological approaches, cut-off points or mode of reporting IGF1R. In addition, presence of different molecular subtypes, tumor heterogeneity, different therapeutic, genetic and particularly ethnic features in distinct study groups are stated to cause those conflicting results [2]. There are some studies that have pointed out the possible impact of ethnic differences on IGF1 and IGF1R expression in breast cancer [2,5]. In the present study we have investigated the significance of IGF1R expression on breast cancer among Turkish women for the first time in the literature, to the best of our knowledge. Thus, the findings of our study may contribute an additional data about the ethnic differences on IGF1R expression in breast cancer.

In order to prevent the development of breast cancer and inhibit the adverse prognostic effect of IGF1R, some drug investigations have been conducted that targeted it, either with monoclonal antibodies causing internalization of the receptor, or by blocking the receptor's tyrosine kinase domain activation using receptor tyrosine kinase inhibitors (RTKIs) [2]. Ganitumab (AMG-479), figitumumab (CP-751, 871), cixutumumab (IMC-A12), teprotumumab (R1507), (RO4858696), TKI (NVP-ADW742), RTKI (NVP-ADW742), NVP-ADW742, OSI-906 (linsitinib), dalotuzumab (MK-0646), are some drugs of IGF1R inhibitor created with phase I and II clinical trials [2,29]. However, it has been estimated that most of these drugs does not make significant differences in solid tumor prognosis as breast cancer, even some drugs as dalotuzumab shorten OS and progression-free survival (PFS) by a meta-analysis [29]. Only one study have claimed that IGF1R inhibitors (AMG-479) have active trend to improve OS or PFS in advanced solid tumors [29]. Some side effects including glucose dysregulation and diabetes due to the inhibition of hybrid IGF1R/IR receptors and neutropenia could not be prevented, thus some of those drugs have been turned out to be harmful rather than healer [2,29-30]. It is suggested that futher investigations should be carried out to clarify the conflicting therapeutic effects of IGF1R inhibitors.

In summary, overexpression of IGF1R has been found to be correlated with many unfavourable prognostic parameters such as: higher tumor grade, presence of geographic necrosis, positivity for HER2 and negativity for ER by immunohistochemistry, HER2+ molecular subtype, histological tumor type of IDC rather than ILC, higher mitotic count and higher Ki67 expression, lower DFS and lower OS rates by univariate analyses, in our study. However, none of those factors other than OS

turned out to be significant on multivariate analysis. These inconsistant results between univariate and multivariate analysis might be attributable to the fact that many of these factors are linked and might necessarily not related with IGF1R. For instance, the majority of ER-positive cases are likely to be HER2-negative, and the majority of ILC will be ER-positive and higher tumor grade is likely to be associated with higher Ki67 index. In addition, poor prognosis might be related to overexpression of HER2.

In conclusion, our study has demonstrated that overexpression of IGF1R is possibly associated with poorer prognosis in breast cancer of Turkish women. Future investigations that especially focus on specific ethnic groups, targeting the IGF1R expression patterns and distinct molecular mechanisms might lead the development of effective prevention and treatment strategies against breast cancer. In addition, performing IGF1R and also Ki67 by immunohistochemistry in routine practice of pathology while diagnosing breast cancer is strongly recommended in order to improve the data about them in the literature, achieve standardisation, and elucidate their exact impacts on breast cancer pathogenesis and prognosis.

Acknowledgment

We thank to Turan Erdogdu, for his contribution for preparation of the illustrations used in the study.

Riassunto

Il recettore del fattore di crescita insulino-simile 1 (IGF1R) è un recettore della proteina tirosin chinasi che si afferma essere correlato allo sviluppo del tumore e alla progressione del cancro al seno con alcuni risultati contrastanti in letteratura. Gli obiettivi di questo studio sono di indagare l'espressione di IGF1R e correlare con i parametri clinicopatologici per chiarire il significato di IGF1R sul cancro al seno.

Materiale e Metodi: IGF1R e Ki67 sono stati applicati immunoistochimicamente alle sezioni di microarray di tessuto di 370 donne affette da cancro del seno. I risultati erano stati posti in correlazione statistica con le caratteristiche cliniche, prognostiche, istopatologiche e altri risultati immunoistochimici [ER, PR, HER2, CK5 / 6 e CK14].

Risultati: La sovraespressione di IGF1R ha mostrato una correlazione diretta con l'indice Ki67 (P = 0,028), la positività HER2 (P = 0,001), la conta mitotica (P = 0,004), il grado del tumore (P = 0,015) e la necrosi geografica (P = 0,023); e una correlazione negativa con positività ER (P = 0,003). C'era una differenza statisticamente significativa tra l'espressione di IGF1R e i sottotipi molecolari (P <0,001), principalmente fenotipo HER2 +. L'espressione di IGF1R è risultata più elevata nel carcinoma duttale invasivo (IDC) rispetto al carcinoma lobulare invasivo (ILC) (P = 0,036). Sia l'espressione di IGF1R che di Ki67 erano correlate negativamente con i tassi di sopravvivenza libera da malattia (DFS) (P = 0,020, P = 0,023, rispettivamente) e di sopravvivenza globale (OS) [P <0,001, ciascuno]. L'associazione inversa tra la sovraespressione di IGF1R e il tasso di OS è stata supportata anche da analisi multivariate (P = 0,025).

Si conclude che la sovraespressione di IGF1R è stata trovata direttamente correlata a DFS e OS più brevi, nonché ad alcune caratteristiche clinico-patologiche associate a prognosi negativa come l'indice Ki67 più alto, conta mitotica, grado tumorale, presenza di necrosi geografica, positività HER2, negatività ER, HER2 + sottotipo molecolare, tipo di tumore istologico di IDC piuttosto che ILC.

Pertanto, IGF1R potrebbe essere considerato un obiettivo utile per future indagini complete sulla terapia antitumorale. Inoltre, l'utilizzo di IGF1R e Ki67 come parte della pratica patologica di routine potrebbe essere fruttuoso nella terapia del cancro al seno e nella previsione della prognosi.

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