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Relationship between Immunohistochemical Expression of Insulin-like Growth Factor II with the Morphological Parameters in Breast Cancer

Ali Shafeeq Neameh[1]*, Hussein Riyadh Ali Shlaka[1], and Zeyad Swadi Obaid Al-Isawi [2]

[1-]Department of Pathology, Faculty of Medicine, University of Jabir Ibn Hayyan For Medical and Pharmaceutical Sciences, Iraq.

[2-] Department of Basic Science, Faculty of Dentistry, University of Kufa, Iraq.

***Corresponding Author Email:** hussein.r.alasady@jmu.edu.iq

ABSTRACT

Background: *The stroma of breast cancer has a high concentration of IGF-II messenger RNA, according to recent in situ hybridization studies. The purpose of this research was to analyze 90 cases of breast infiltrating ductal carcinomas (IDC) to see if there was a correlation between the expression of the IGF-II protein and other prognostic variables.* ***Methodology:*** *Immunohistochemistry was used to examine the expression of proliferative activity, IGF-II protein, endoplasmic reticulum (ER), progesterone receptor (PgR), p53, and p21 in tissue sections. We measured the extent of stromal proliferation. There was knowledge of menopausal status, involvement of axillary lymph nodes, and nuclear grade. There were 45 premenopausal patients (50%) and 47 patients (53.3%) with lymph node metastases, of the tissues examined.* ***Result:****40 (44%) showed stromal proliferation, and 22 (24%) had a high nuclear grade. There was a lack of IGF-II in 20 tumors (22.2%). 15 specimens (16.7%) had high IGF-II, 19 (21.1%) had low, and 35 (38.9%) had intermediate levels. High proliferative activity was noted in 26 tumors (29%). ER was positive in 57.8% and PgR in 41.1%. p21 was present in 40 tumors (44.4%) and p53 in 11 (12.2%). IGF-II had no association with ER, p53, or other factors, but was linked to PgR (p=0.03), stromal proliferation (p=0.008), and p21 (p=0.01). The study indicates IGF-II is expressed in IDC and associated with stromal quantity, PgR, and p21.* ***Conclusion:*** *The findings indicate that IGF-II may contribute to breast cancer progression through paracrine mechanisms within the tumor microenvironment.*

Keywords: p21-ras, Oncogenes, IGF-II, Estrogen Receptor, Progesterone Receptor, Breast Cancer.

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INTRODUCTION

There is a class of polypeptide hormones called insulin-like growth factors (IGFs) that are structurally and functionally similar to insulin. It is unclear what IGF-II does physiologically, and its action is not highly reliant on growth hormone activity, in contrast to IGF-I (1). The IGF binding proteins (IGFBPs) stabilize IGF2. The type I IGF receptor (IGF1R) is the primary binding site for IGF2, which activates downstream signaling pathways including the PI3K kinase and mitogen-activated protein (MAP) kinase pathways (2). Over the last ten years, several research groups have investigated the IGF-II is significantly more common in

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cancer. Specifically, it is expression and secretion have been observed in cancer cell lines derived from solid tumors (3,4).

Elevated IGF-2 expression in tumors, which encourages the development of tumor cells. IGF-2 overexpression can cause serious growth and development problems in both the embryonic and adult stages, as well as the development of tumors (5). According to earlier research, CAFs secrete a number of soluble molecules, including TGF-β and IGF-2, which accelerate the growth of cancer(6). The course of breast cancer may be predicted by a number of prognostic variables. We postulated that IGF-II expression in stroma and epithelial cells may correspond with any or all of the predictive indicators for breast cancer development, given its strong mitogenic potential.

In this study, we used immunohistochemistry to examine the IGF-II protein expression in a number of breast IDCs. We also hoped to learn more about how this protein relates to nuclear grade, estrogen receptor (ER), progesterone receptor (PgR), lymph node involvement, menopausal status, and other morphological and clinical variables that might indicate tumor proliferative activity and prognosis. The involvement of IGF-II in stromal-epithelial interactions in breast cancer is supported by our early data.

METHODS

Patients and samples

In this study, we looked at breast cancer samples taken from 90 women who had IDC surgery; the samples were formalin-fixed and embedded in paraffin. Of the patients, 45 (50%) were premenopausal, and their mean age ± SD was 53.3±15.2 years, with a range of 30-80 years. Suspected palpable breast nodules or abnormalities on mammograms have recently appeared in some individuals. They had all had surgery without having previously undergone combination chemotherapy. Every single instance did not have a history of breast cancer in the family. Table I summarizes the primary breast tumor features.

Lymph node involvement and nuclear grade

All patients had known information on the regional lymph node status and nuclear grade. In order to do statistical analysis, the patients were split into two groups based on whether they had lymph node metastases or not. the severity of nuclear cancer was evaluated (7). The level of nuclear differentiation was graded from 1 to 3, with higher scores indicating stronger differentiation. Scores 1, 2, and 3 were used to categorize the tumors for statistical analysis.

Stromal proliferation

According to Fisher et al. (7), the stromal component was considered modest when it was less than the tumor's cellular component, moderate when the proportions of stromal and tumor cells were similar, and conspicuous when it was believed to be more than the cellular component. In order to do statistical analysis, the tumors were arbitrarily classified as either slight/moderate or marked based on the quantity of stroma (8).

Proliferative activity immunohistochemistry

To retrieve the Ki-67 nuclear antigen, slices that had been fixed in paraffin were dewaxed and rehydrated with ethanol. Then, they were subjected to microwave irradiation using the MIB-1 mouse monoclonal antibody (Immunoteck S.A., Marseilles, France). A nuclear antigen is produced by cells that are proliferating but not by cells that are quiescent; the MIB-1 antibody responds with this antigen. Immunostaining in paraffin sections with MIB-1 was comparable to that in frozen tissues stained with Ki-67 (9,10). Overnight, the sections were left at room temperature to incubate with MIB-1 antibody. The avidin-biotin combination containing diaminobenzidine was used as a chromogen by Vector Laboratories of Burlingame, CA, USA, to observe the immunoreaction. High proliferative activity was defined as values over 10%. We have a big series of breast cancer

specimens, and the median proliferative activity value of these tissues is 10.

IGF-II immunohistochemistr

We used a rabbit polyclonal antibody that had been specifically produced to target intact human recombinant IGF-II peptide. Recombinant IGF-I caused less than 10% cross-reactivity while recombinant epidermal growth factor (EGF) had no effect, confirming the antibody's specificity by means of enzyme-linked immunosorbent assay (ELISA). This antibody, which was affinity-purified from an intact recombinant peptide, exhibited in the ELISA the same staining and reactivity as polyclonal antiserum. In order to rehydrate the paraffin sections, a stepwise ethanol series was used for deparaffinization. In a nutshell, after a PBS wash, the slides were left to incubate at room temperature with standard goat serum for 20 minutes. Before overnight incubation at 4°C, tissue slices were treated with a diluted primary IGF-II antibody in a solution containing PBS, 1% BSA, and 1% sodium azide. Next, the slides were immersed in PBS twice for a duration of three minutes each (11). The BioGenex Multilink system (BioGenex, San Ramon, CA, USA) was used to observe the reaction. The labeled secondary antibody was identified after a 10-minute incubation with the chromogen Fast Red. Cells of the breast cancerous epithelium and stroma were found to be immunostained. More than 10% stained epithelial and/or stromal cells was used to arbitrarily classify tumors as positive. When 10–20% of cells were positive, immunostaining was considered moderate; when 20–30% of cells were positive, it was considered marked; and when more than 30% of cells were positive, it was considered marked. Sarcastically classified as "IGF-II negative" for the sake of future statistical analysis were tumors that showed no, weak, or moderate staining, as well as tumors that showed strong positivity for IGF-II.

TABLE I - Main characteristics of the 75 breast cancers.
(mean age ± SD of the patients: 53.3 ± 15.2 years)

Parameters	Number	%
Node status Positive	48	53.3
Negative	42	46.6
Nuclear grade 1	25	27.7
Nuclear grade 2	41	45.5
Nuclear grade 3	22	24.4
Stromal proliferation: Slight or moderate	50	55.5
Marked	40	44.4
Proliferative activity High	26	28.8
Low	55	71.
IGF-II expression Absent	20	22.2
Slight	19	21.1
moderate	36	40

Parameters	Number	%
marked	15	16.6
ER status ER+	52	57.8
ER-	38	42.2
PgR status PgR+	37	41.1
PgR-	53	58.2
p21-ras positive	40	44.4
Negative p53	50	55.5
positive	11	12.2
negative	58	87.7

Estrogen and progesterone receptor immunohistochemistry

The wax was removed and the paraffin portions were rehydrated. The next step in retrieving the ER and PgR was to subject the tissue to microwave irradiation. The outcomes of this therapy are comparable to those of frozen section analyses (unpublished data). The sections were heated in a microwave for 30 seconds, then treated with anti-mouse biotinylated serum. Following this, they were incubated for an hour with anti-ER 1D5 and anti-PgR 1A6 monoclonal antibodies diluted 1:10 in appropriate buffer (Dako, Glostrup, DK). By using avidin-biotin-peroxidase with diaminobenzidine as the chromogen, the immunoreactivity could be seen. The main monoclonal antibodies were not included in the negative control groups. Tumors were deemed ER+ and PgR+ when 10% or more of the neoplastic cells showed specific nuclear staining.

Oncogene protein product (p21, p53) immunohistochemistry

The sections were exposed to a diluted 1:200 Y13-259 monoclonal antibody from Oncogene Science in Cambridge, MA, USA, for one night at room temperature after dewaxing and paraffin embedding. This antibody uniquely identifies the p21 protein products of the H-K-N ras oncogenes. Their immunoreactive agent was a mixture of avidin and biotin, with diaminobenzidine serving as the chromogen, developed by Oncogene Science of Cambridge, MA, USA. The cells that were found to be positive for epithelial carcinoma contained cytoplasm that was heavily stained. after the methods outlined before (12,13), the presence of nuclear p53 protein was confirmed after microwave irradiation with a diluted 1:200 solution of the DO7 mouse monoclonal antibody produced by Novocastra Laboratories Ltd of Newcastle upon Tyne, UK. It was determined that p21-ras and p53 expression were both positive when values exceeded 10% of stained epithelial cells.

Statistical analysis

An exact test known as Fisher's was used to examine the outcomes. For statistical significance, a probability value below 0.05 was used.

RESULTS

A total of 53.3% of the 90 patients tested showed positive regional lymph nodes, and 27.7%, 45.5%, and 24.4% of the tumors had nuclear grade, respectively. Some 40 patients (44.4%) had severe stromal proliferation, whereas 55.5 patients (50%) had minor or

moderate stromal proliferation. A total of 26 breast cancer specimens, or 28.8% of the total, showed signs of high proliferative activity. Out of 90 tumors, 22.2% did not express IGF-II protein, 21.1% shown modest expression, and 40% substantial expression. fifteen instances 16.65% showed staining for IGF-II. The smooth muscle component of tiny blood artery walls and stromal fibroblasts, as well as the cytoplasm of epithelial cancer cells, were the usual locations for IGF-II protein inside tumors (Fig. 1c; Fig. 1a,b). The ER positive (ER+) tumor count was 57.8%, whereas the PgR positive (PgR+) tumor count was 41.1%. Cancerous epithelial cells were the only ones to have nuclear immunostaining (Fig. 1d). In the tumor stroma, ER and PgR were not detected. forty cancers (44.4%) tested positive for the p21-ras protein (Fig. 1e), whereas 11 (12.2%) tested positive for the p53 protein (Fig. 1d)

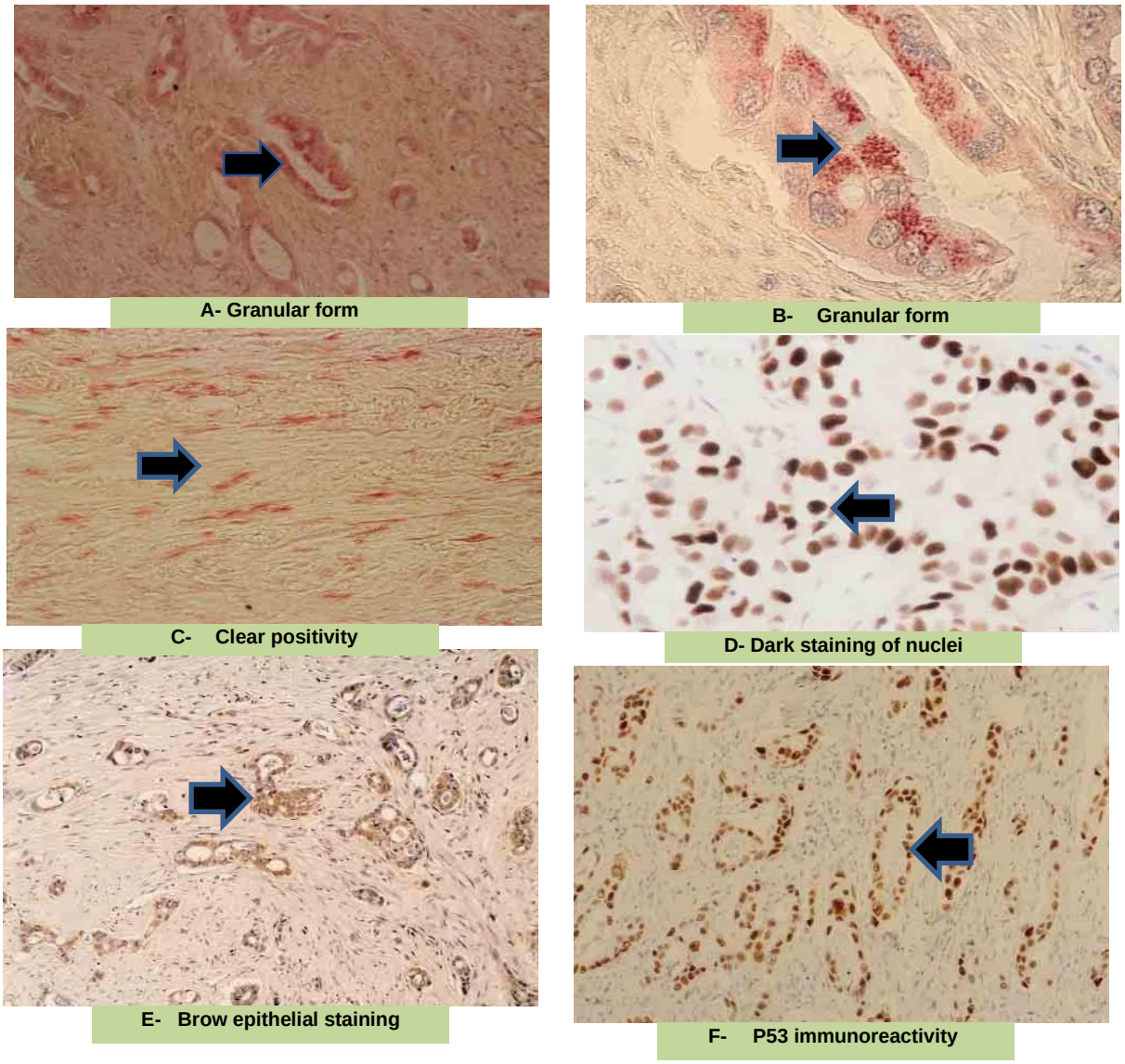

Fig. 1 - Protein expression in infiltrating ductal carcinoma of the breast, including IGF-II, PgR, p21, and p53. *A and (b) Immunohistochemistry using IGF-II in a rat model of chronic inflammatory bowel disease (IDC) reveals a dense accumulation of the immunoreactive substance in the tumor epithelium's cytoplasm, arranged in a granular form (black arrow); the stroma remains unstained. c) The stroma shows clear positivity for IGF-II, indicating that this protein is expressed in infiltrating ductal carcinoma. d) Immunohistochemistry (IHC) for PgR reveals dark staining in cancer cell nuclei (black arrow) but no staining in the surrounding stroma. e): IDC-induced p21 protein expression: brown staining in epithelial cancer cell cytoplasm (black arrow). f*) Immunoreactivity seen in the nucleus of cancerous epithelial cells (black arrow) p53 expression in IDC.

Correlation studies

Tumor proliferative activity, menopausal status, nuclear grade, or axillary node involvement were not associated with IGF-II protein expression. Protein IGF-II in opposition to stromal proliferation: Eleven out of forty breast tumors (29%) that had a significant stromal component had elevated levels of the IGF-II protein when immunostained. However, when it came to specimens with mild or moderate stromal proliferation, significant IGF-II positivity was seen in only one out of 50 specimens (2%). There was an abundance of stroma in all but one of the eleven breast tumors (91.6% of the total) that showed strong IGF-II immunostaining. Table II shows a very substantial (p=0.0008) correlation between IGF-II protein expression and fibrosis degree. Contrasting IGF-II protein with endoplasmic reticulum and progranulin receptors: twelve out of fifteen specimens with detectable levels of IGF-II were PgR+, whereas 92.6% of PgR- tumors exhibited no, weak, or moderate IGF-II expression. There was a statistically significant correlation (p=0.03) between PgR and the expression of IGF-II protein (Table III). On the other hand, immunostaining for IGF-II did not reveal any correlation with ER. Protein products of oncogenes vs IGF-II: p53 expression was unrelated to IGF-II immunostaining. The p21-ras gene was positively expressed in 13 out of 15 (86.6%) breast cancers that had a high IGF-II level. Out of 50 tumors that tested negative for p21-ras, only two (4%) had strong immunostaining for IGF-II. There was a significant correlation (p=0.01) between the IGF-II protein and the expression of p21 (Table IV).

TABLE II - Fibrosis and levels of insulin-like growth factor II (IGF-II) protein are positively and statistically correlated in breast cancer samples. p-value = 0.0008.

		IGF-II protein	
		Neg.	Pos.
Fibrosis	posa	29	11
	negb	49	1

TABLE III - In breast cancer samples, there is a statistically significant relationship between IGF-II protein and PgR (p=0.03).

		IGF-II protein	
		Neg.	Pos.
PgR	pos	34	3
	neg	41	12

TABLE IV - In breast cancer specimens, there is a positive correlation (p=0.01) between IGF protein and p21-ras.

		IGF-II protein	
		Neg.	Pos.
p21-*ras*	neg	48	2
	pos	27	13

DISCUSSION

Numerous institutes have conducted extensive research on the role of IGF-II in stromal-epithelial interactions associated to breast cancer (14,15). Research has connected IGF-II to mitogenic effects on the breast cancer epithelium, and studies have shown that conditioned media derived from cultures of breast cancer fibroblasts may significantly activate MCF-7 cells (16). The fibroblasts found in breast cancer tumors have the ability to synthesize IGF-II messenger RNA on their own, according to in vitro studies. Additionally, growth factors, cytokines, and chemokines secreted by the breast cancer associated fibroblasts (CAFs) help fuel tumor growth (17). Previous in situ hybridization research has shown that IGF-II is very abundant in the stroma just outside the malignant epithelial cells, which provides strong evidence that the stroma is the source of IGF-II in breast cancer (18).

An effective estrogenic regulation mechanism and a dependable marker of hormone sensitivity for prognosis are both suggested by the presence of PgR in breast cancer (19). Previous studies have shown a substantial correlation between stroma quantity and PgR expression and that ER and PgR expression in breast cancer was only identified in epithelial cells (20,21). Consistent with previous research, growth factor release may facilitate a paracrine pathway in breast cancer that links tumor and stromal cells. Recurrence of tumors and overall survival rates in breast cancer patients are best predicted by determining the axillary lymph node status (22). A significant number of patients with node-negative breast cancer showed that the proliferative activity of cancer cells was a separate predictor, and the nuclear grade of cancer cells is believed to be an additional prognostic factor in breast cancer (23).

A missense mutation increases the half-life of TP53, which is involved in negative cell cycle control; immunohistochemistry reveals that cells accumulate this protein (13). In 30-60% of breast tumors, the active ras oncogene encodes a p21 protein. One possible function of ras proteins in breast cancer cell proliferation regulation is the strong correlation between p21 protein expression and proliferation rate (24).

Several biochemical, morphological, and clinical variables were used in this study to establish a connection between the expression of IGF-II protein and the prognosis of breast cancer. In 16.6% of specimens, IGF-II was strongly expressed, but in 22.2%, it was not. The remaining 58.7 percent of tissues showed moderate to modest levels of IGF-II immunostaining. The site of IGF-II was often found in stromal fibroblasts and small blood vessels. It was shown that malignant epithelial cells possess IGF-II protein, in contrast to the stroma, which often expresses IGF-II mRNA in breast cancers. A robust correlation was found between the quantity of stroma and the expression of IGF-II. Recently, we found that there is a strong relationship between stromal IGF-II mRNA and IGF-II protein in a large

group of breast cancers (3). This might be the reason why epithelial cancer cells have IGF-II in their cytoplasm.

Furthermore, it is possible that the cells took in some stromal IGF-II from the surrounding blood, even if no such synthesis was occurring locally. There was no correlation between patients' menopausal state and IGF-II protein in this research. There was no correlation between IGF-II levels and nucleolar grade of cancer cells, tumor proliferative activity, or involvement of metastatic axillary nodes. Immunostaining was limited to the nuclei of cancerous epithelial cells in ER and PgR-positive breast cancers; the tumor stroma was not immunostained. Earlier studies shown, via a series of trials, that stroma quantity is significantly related to PgR epithelial expression in breast cancer, in contrast to ER(25). The study found a favorable link between ER expression and PgR, but no relationship with IGF-II protein. Stromal IGF-II may have a role in the production of epithelial PgR, in addition to its participation in ER activity, according to these properties.

We found that the IGF-II peptide is strongly associated with the production of the p21-ras oncogene protein, which plays a role in the aberrant control of breast cancer progression. Finally, our data demonstrate a favorable correlation between stroma, epithelial PgR content, and p21-ras oncogene product expression and IGF-II protein levels in breast cancer. Independent of lymph node involvement and tumor proliferative potential, our data indicate that IGF-II may play a role in the genesis and progression of breast cancer epithelial cells. Thoroughly monitoring this cohort of patients might provide light on the role of IGF-II in breast cancer prognosis.

CONCLUSION

This study emphasizes the important role of IGF-II in stromal–epithelial interactions in breast cancer. IGF-II was predominantly localized in stromal fibroblasts and showed a significant association with stromal abundance. A positive correlation was observed between IGF-II expression, epithelial PgR expression, and p21-ras oncogene product levels, suggesting involvement in hormonal and proliferative pathways.

However, no association was found with menopausal status, tumor grade, proliferative activity, or lymph node involvement. These findings indicate that IGF-II may contribute to breast cancer progression through paracrine mechanisms within the tumor microenvironment. Further studies are needed to clarify its prognostic and therapeutic significance.

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