

ORIGINAL ARTICLE

Correlation of the fibroblast growth factor-inducible 14 receptor and progranulin as prognostic biological markers in ductal invasive breast cancer: Immunohistochemical study

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Abstract

Background: The Fn14 fibroblast growth factor-inducible 14 (Fn14) can stimulate cell migration and promote cancer lesions. Progranulin (GP88) protein has been identified as an epidermal growth factor and participates in many biological processes. The aim of the present work was to investigate the immunohistochemical expression of Fn14 and GP88 proteins in relation to the clinical parameters in women's invasive ductal carcinoma (IDC) and to explore their role as novel prognostic biomarkers.

Methods: The qualitative and quantitative immunohistochemical techniques were used to evaluate the expression levels of Fn14 and GP88 in 100 fresh samples of Egyptian women who had breast lesions. They were divided into three groups: control healthy tissues (10 samples from woman lesions), benign group (30 cases), and IDC group (60 cases).

Results: The histopathological results of 60 cases with IDC have been reported with 45 cases being grade II and 15 cases being grade III. The immunohistochemical results showed that the degree of strong positive staining for both markers was increased in grade III compared to that in grade II. The integrated optical density was significantly increased in grade III ($p < 0.05$). Also, the result revealed a highly significant correlation between the two markers and the tumor size, grades, and lymph node metastasis, as well as a correlation to normal and benign breast lesions.

Conclusion: The quantitative immunohistochemistry of Fn14 and GP88 proteins revealed the correlation between the two markers and clinical parameters. Therefore, the two markers may be serviceable as prognostic and therapeutic markers in IDC patients.

KEYWORDS

immunohistochemical technique, integrated optical density (IOD), Fn14 protein, GP88 protein, invasive ductal carcinoma

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INTRODUCTION

Cytokines are a diverse group of proteins and peptides that are associated with the plasma membrane or secreted proteins and peptides. Also, they are glycoproteins which act as hormonal regulators or signaling molecules that regulate many important biological processes, and their stability is important in regulating and keeping up cellular functions [1]. The cytokine receptor subgroups are considered as a promising reservoir of therapeutic targets for the treatment of complex human diseases [2]. Fibroblast growth factor-inducible 14 (Fn14) was classified as a member of the tumor necrosis factor receptor (TNFR) [3]. Also, Fn14 is a type I trans-membrane receptor. The TNF-like weak inducer of apoptosis (TWEAK) has been found to bind to the Fn14 group, and this binding leads to the activation of intracellular signaling cascades including mitogen-activated protein kinase (MAPK) and nuclear factor κ B (NF- κ B) [4]. The Fn14 protein is expressed primarily on the surface of non-lymphoid cells such as epithelial, endothelial, and nonhematopoietic cells [5]. It is triggered by a range of factors, including fibroblast growth factor 1 (FGF-1), FGF-2, and platelet-derived growth factor in marine and human fibroblasts. It is known that the expression level of Fn14 is elevated in injured tissues against low levels of expression in normal tissues. It plays a role in tissue remodeling [2] and invasiveness of some tumors [6], including breast [7], pancreas [8], esophagus [9], brain [10], and glioma cell lines [11].

Growth factors are multifunctional polypeptides that have an important role in the growth, maturation, and maintenance of tissues [12]. They promote cell proliferation, migration, invasiveness, survival, and the formation of new blood vessels [13]. Progranulin (GP88) was cloned from human leukocytes and identified as an epidermal growth factor, also called proepithelin or epithelin precursor [14]. GP88 is present in epithelial cells and immune cells [15]. GP88 signaling may interact with integrin signaling pathways via focal adhesion kinase (FAK), and FAK plays a crucial role in cellular motility regulated by growth factors and integrin [16]. It is involved in various biological processes, including embryogenesis, inflammation, and wound healing [17]. Also, it contributes to host defense [18], cartilage development, and degradation [19]. GP88 has a role in reducing inflammation and promoting the progression of atherosclerosis [20]. The increased GP88 expression is correlated with aggressive tumors that occur in the breast, brain, kidney, and cervix [21]. However, growth factors and cytokines are signaling molecules that exert their biological effects through binding to specific receptors and triggering the activation of signaling pathways that regulate gene transcription in the nucleus and stimulate biological responses [22]. Dysregulated or excessive production of growth factors and cytokines may lead to a variety of diseases, including cancer [23], liver fibrosis [24], and

bronchopulmonary dysplasia [25]. In the past, the growth factors were thought to exert a positive influence on cell growth and proliferation, whereas cytokines were deemed to elicit an immunological or hematopoietic response [26]. Therefore, this study aimed to explore the relationship between the expression of Fn14 and GP88 using qualitative and quantitative immunohistochemical techniques in invasive ductal carcinoma (IDC), in order to evaluate their potential as new prognostic biomarkers in human IDC.

METHODS

Subjects

The prospective samples were collected from patients who had been diagnosed with breast tumors in the Pathology Department at Medical Research Institute, Alexandria University, Alexandria, Egypt. One hundred samples were processed to prepare formalin-fixed paraffin-embedded (FFPE) sections. The subjects were divided into three groups. In group I, 10 control samples were taken from normal tissues adjacent to the breast tumor tissues. Group II, including 30 patients, was diagnosed with benign breast tumor, and group III, including 60 patients, was diagnosed with IDC according to the Elston-Ellis modification of the Scarff-Bloom-Richardson grading system [27, 28]. All cases were asked to voluntarily participate in the study, and informed consent was obtained before their inclusion in the study in accordance with the ethical guidelines of the Medical Research Institute, Alexandria University (IORG#: IORG0008812). Samples from all studied cases were subjected to the following procedures.

Clinicopathological evaluation

The gross examination and clinical parameters of the specimens include patients' age and tumor size, and the excised specimens were measured in three dimensions in centimeters. The number of axillary lymph node metastasis (LNM) and lymph/vascular invasion were recorded. Then fresh samples from all subjects were fixed with neutral buffered formalin at room temperature. They were processed to investigate their histopathological stages.

Histopathological examination

Different samples were chosen from the focus cases under the virtual examination and proceeded to prepare the FFPE sections. Sections with a thickness of 5 μ m were prepared and placed on glass slides. They

were stained by routine hematoxylin and eosin (H&E) stains [29] in the Histochemistry and Cell Biology Department, Medical Research Institute, Alexandria University. The samples were investigated and diagnosed by three pathologists, who classified the present collected cases into benign and IDC groups, and also, differentiated the IDC group into grade II IDC and grade III IDC.

Immunohistochemical protocol for Fn14 and GP88 evaluation

The immunohistochemical technique employing the avidin-biotin complex (ABC) protocol was applied to investigate the expression of Fn14 and GP88 in FFPE sections of breast tissues, using a universal kit (ABC-HRP reagent) and DAB stain from Thermo Fisher [30, 31]. In brief, the paraffin-embedded samples were sectioned into slices with a thickness of 5 μm and picked up on coated slides. The sections underwent deparaffinization with two applications of xylene and were subsequently rehydrated. The sections were subjected to antigen retrieval by incubation in citrate buffer with a pH of 6.0 at 60°C for 40 min, followed by cooling at room temperature for 20 min. The sections were treated with 3% H_2O_2 /PBS for 5 min to quench the endogenous peroxidase activity and incubated with serum blocking reagent for 30 min to block non-specific binding. The sections were then exposed to primary antibodies for Fn14 and GP88 (Biorbyt Company) and incubated at 4°C overnight. On the second day, the sections were washed and treated with conjugated secondary antibody (ABC-HRP reagent) for 30 min. The sections stained with the chromogen DAB were washed, rinsed with distilled water, and then stained with hematoxylin as a counterstain for the nuclei. For the negative control section, the antibody was substituted with PBS. PBS washing was conducted after each step. The semi-qualitative immunohistochemical evaluation of Fn14 and GP88 was performed arbitrarily according to the NordiQC guidelines (via the NordiQC website), staining scored negative (-ve) and positive (+ve), with the positive staining scoring weak (+ve +1), moderate (+ve +2), and strong (+ve +3) based on their stain density.

Image analysis

The analysis of individual images of control, benign, and malignant groups was performed to calculate the relative integrated optical density (IOD) of the immunostaining density at each image of both markers (FN14 and GP88) using a digital image analyzer. The slides were photographed and documented using an Olympus microscope equipped with a Spot digital camera, and the resulting images were processed using the ImageJ software. It was completed at the

Histochemistry and Cell Biology Department, Medical Research Institute, Alexandria University. The maximum and minimum gray levels of IOD were assessed based on 10 fields for each case. The mean values of IOD for both markers were based on the pixel numbers [32]. In the digitized images, the probability of gray-level transition was from dark to light, ranging from 250 down to 0.

Statistical analysis

All distributed data were collected and analyzed using the statistical software package SPSS 20. $p \leq 0.05$ values were considered statistically significant.

RESULTS

Histopathological examination

The pathological diagnosis of the current cases showed that among the 30 benign cases, most were differentiated between the fibroadenomas, while fibrocystic cases account for 17% of the benign cases. Among the 60 malignant cases of IDC, 75% were diagnosed as grade II IDC and 25% were diagnosed as grade III IDC according to Bloom-Richardson histological grading system. The photomicrograph of the paraffin section showed the breast tissue characterized by ducts and acini lined by a layer of epithelial cells with minimal width lumen, an outer layer of myoepithelial cells, and a basement membrane that is enveloped by stroma (Figure 1a). The benign lesions of fibroadenoma cases showed both pericanalicular and intracanalicular patterns. The epithelial cells within the ducts were compressed and lined by bland-looking ductal epithelial cells, which were located on an intact layer of myoepithelial cells. The stroma appeared myxoid (Figure 1b). Microphotograph of the malignant cases showed the IDC, with tumor cells possessing eosinophilic cytoplasm and featuring large, pleomorphic, round, and vesicular nuclei. The proliferation of cells was invasive in ducts, arranged in cords, and infiltrated into the desmoplastic stroma in grade II IDC (Figure 1c). In addition, the neoplastic proliferating cells formed nests, characterized by hyperchromatic nuclei, large vesicular nuclei, and residual ones. In grade III IDC, numerous pyknotic cells and mitotic activity of ductal epithelial cells were observed (Figure 1d).

Clinical pathological parameters

The patients' barcode in the pathological department computer archive of the selected patients in our study showed that the age ranges of benign cases were

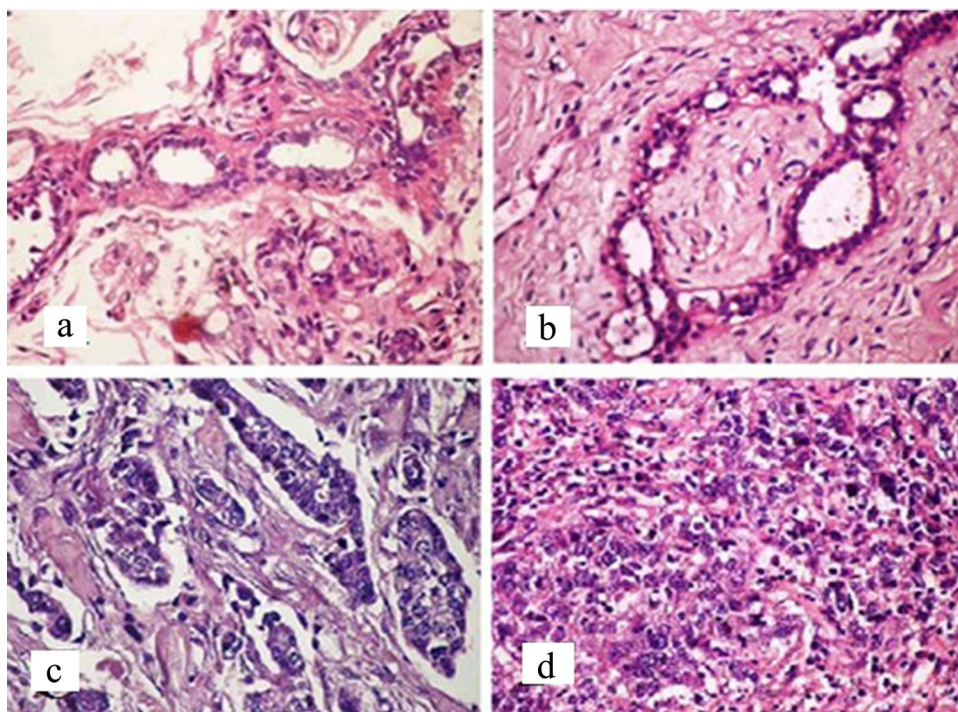


FIGURE 1 Paraffin section microphotograph of human breast tissue stained by H&E under the $\times 400$ magnification. (a) Normal acini lined by cuboidal epithelium with underlying myoepithelial cells having clear cytoplasm. (b) Benign lesion of fibroadenoma case. Both pericanalicular and intracanalicular patterns are shown, with compressed ducts lined by bland-looking epithelial ductal cells, which are located on an intact layer of myoepithelial cells. The stroma is myxoid. (c) Malignant case of grade II IDC. Tumor cells with pleomorphic round to ovoid vesicular nuclei are shown. The cells are invasive and arranged in cords to infiltrate the desmoplastic stroma. (d) Malignant case of grade III IDC. Numerous mitotic figures, large vesicular nuclei, many residual nuclei, as well as other tumor cells with large pleomorphic nuclei and pyknotic nuclei with vacuolated cytoplasm are shown.

25–64 years and malignant cases were 35–74 years with a mean \pm SD of 36 ± 11 and 52 ± 10 years, respectively. The statistical data shows that in the age group of 35–45 years, 57% of the cases are benign, while in the age group of 45–55 years, malignant cases account for 31.5% (Figure 2a). The results demonstrated that most benign cases were less than 45 years, whereas the malignant cases were more than 45 years. According to the tumor, node, metastasis (TNM) staging system for breast lesions, the tumor size was assessed. The results showed that the majority of benign cases were T2 (2–5 cm), and most malignant cases were also within the same size range (Figure 2b). The positive rate of vascular invasion (VI) in malignant cases was 100%. In grade II and grade III IDC, the majority of positive cases for LNM were 78% and 73%, respectively, while the cases free of LNM were 22% and 27%, respectively (Figure 2c).

Immunohistochemical investigations

The semiquantitative evaluation

The immunostaining of Fn14 protein was performed using DAB chromogen, and the immunorexpression of

Fn14 was observed as brown granules in the cytoplasm and cell membrane of ductal epithelial cells, as well as in the stroma tissue (Figure 3). The qualitative evaluation of Fn14 immunorexpression showed negative expression (–ve) in about 70% of control healthy tissues. Weak (+ve +1) positive immunostaining was observed in 70% of the benign group, and the positivity of Fn14 immunostaining increased in the IDC malignant group. There was 78% moderate immunostaining (+ve +2) in grade II IDC and 77% strong immunostaining (+ve +3) in grade III IDC (Figure 2d).

Immunostaining of GP88 protein was observed under an optical microscope after staining with DAB chromogen. The immunohistochemical staining of GP88 showed a diffuse, homogenous brown color, which was localized in the cytoplasm and cell membrane of ductal epithelial cells in breast lesions (Figure 4). The qualitative positive evaluation of GP88 immunostaining showed a negative expression (–ve) in about 80% of the control group and 57% of the benign group. However, the positivity of GP88 immunostaining was observed in IDC malignant lesions. The moderate immunostaining (+ve +2) of the GP88 protein was observed in 71% of grade II IDC, and strong positivity immunostaining (+ve +3) was seen in 80% of grade III IDC (Figure 2d).

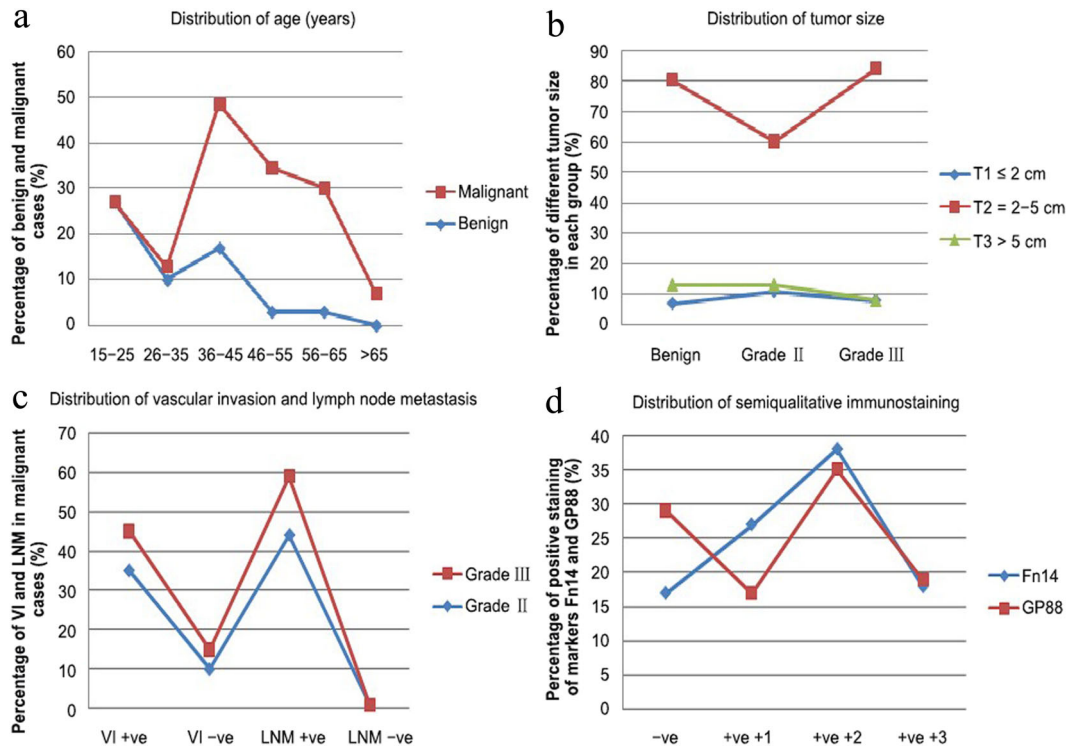


FIGURE 2 Line charts illustrating the clinical parameters evaluated for different patient groups (a–c) and the percentage of immunostaining positivity distributed in different groups (d).

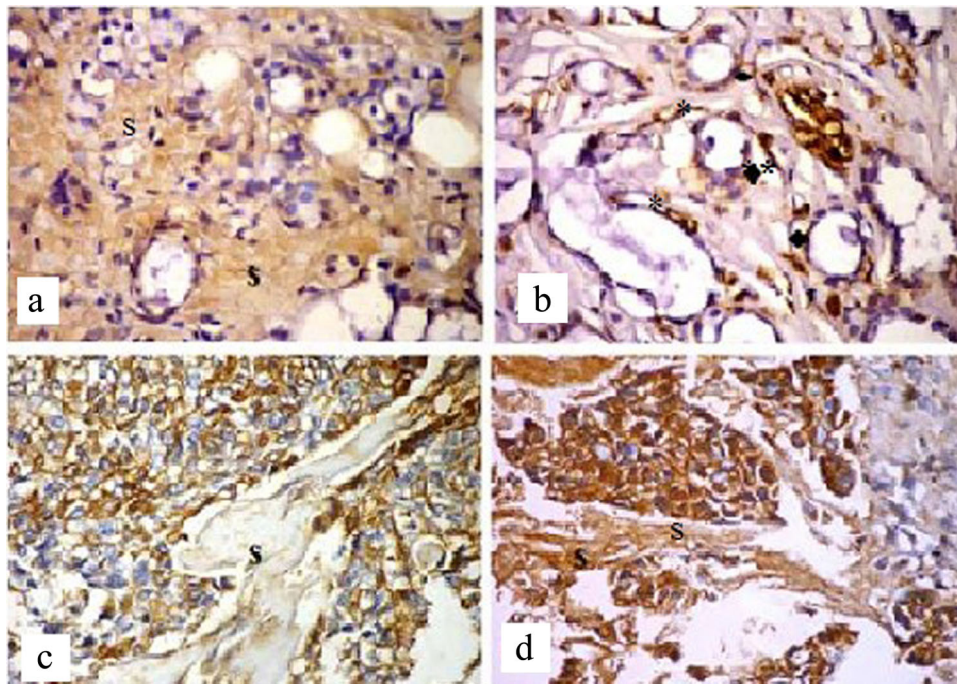


FIGURE 3 Paraffin section micrograph of human breast stained by ABC immunostaining protocol (Fn14 protein, DAB stain, x400 magnification). (a) A negative immunoreactivity of Fn14 shown in cytoplasm of ductal epithelial cells (–ve) and weak diffused immunoreactivity (+ve +1) in stroma (s) in control breast tissue. (b) Benign breast tissue showing a weak (+ve +1) staining as a brown granulated expression of Fn14 in the myoepithelial and ductal cells distributed in cytoplasm and membrane(*). (c) IDC grade II showing moderate (+ve +2) expression of Fn14 in the cytoplasm of most ductal epithelial cells. (d) Grade III IDC showing increased immunostaining for Fn14 in the cytoplasm and membrane of invasive ductal epithelial cells and weak (+ve +1) staining in the stroma (S).

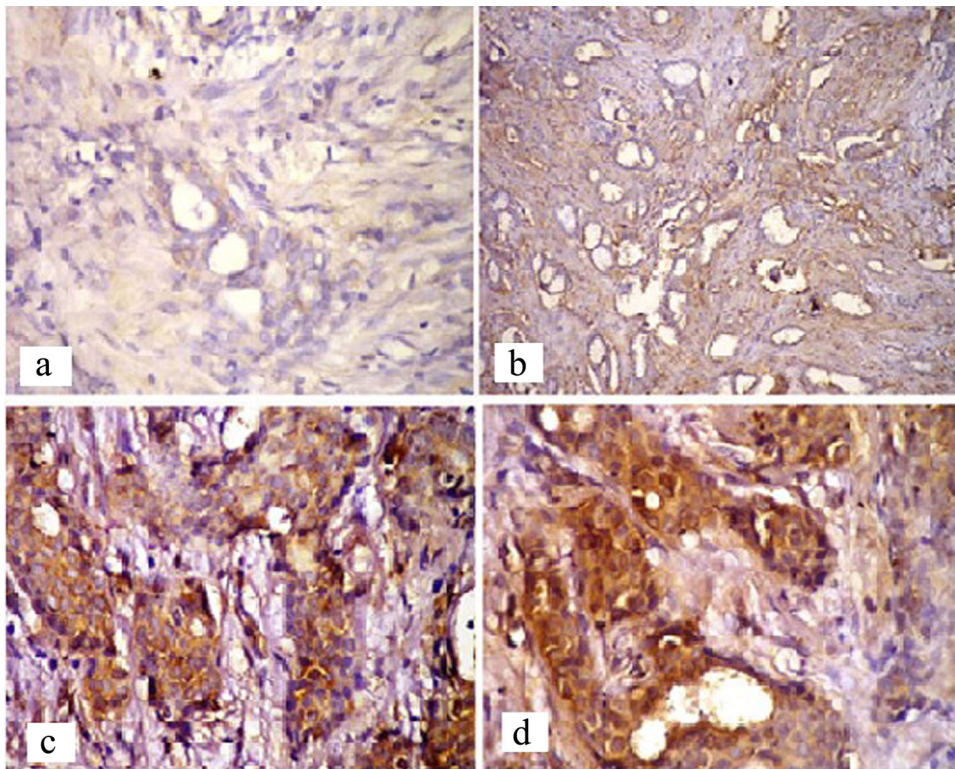


FIGURE 4 Paraffin section micrograph of human breast stained by ABC immunostaining protocol (GP88 protein, DAB stain, $\times 400$ magnification in a, c, d, $\times 100$ magnification in b). (a) Control breast tissue with negative GP88 immuno-expression in the duct and stroma (-ve). (b) Benign case with a negative GP88 immunostaining in the cytoplasm of ductal epithelial cells and weak stain in stroma (+ve +1). (c) IDC grade II case with a positive GP88 (+ve +3) immunoexpression was increased in the cytoplasm and stroma of the ductal epithelial cells. (d) IDC grade III case with a strong GP88 (+ve +3) immunostaining in the cytoplasm of invasive ductal cells. Diffuse activity in stroma was seen.

The quantitative immunostaining analysis

The IOD values were obtained through digital image analysis of the immunostaining color density, with the gray value of the color emission calibrated by the pixel numbers. The mean IOD value of Fn14 in control group was 27 ± 3 , and that in benign group was 32 ± 4 . In malignant group, the values were 136 ± 6 in grade II IDC and 156 ± 4 in grade III IDC cases. A statistically significant increase in Fn14 IOD was observed in grade III IDC compared to grade II IDC ($p < 0.001$). In addition, a considerable increase in the IOD immunostaining coefficient of Fn14 was observed in malignant cases compared to normal and benign groups. The mean IOD values of GP88 expression were 35 ± 3 for the control group, 41 ± 4 for the benign group, 140 ± 8 in IDC grade II malignant cases, 162 ± 7 in IDC grade III cases. Compared with grade II IDC, the coefficient of GP88 IOD in malignant cases of grade III IDC increased significantly ($p < 0.001$). A considerable increase in the coefficient was observed in malignant cases of IDC compared to the normal and benign cases, but there was no significant difference in GP88 IOD between the normal and benign groups ($p = 0.1$). In the comparison of IOD between Fn14 and GP88, the mean IOD values of Fn14 and GP88 were 141 ± 10 and 161 ± 9 , respectively, revealing insignificant differences ($p = 0.06$) in

the IOD expression between Fn14 and GP88 in different cases (Figure 5a).

Correlation of IOD between Fn14 and GP88 expression

Table 1 illustrates the correlation between the IOD evaluation of both markers and some clinical parameters. There was an insignificant statistical correlation between the IOD of Fn14 and the patient's age ($p = 0.8$), and between Fn14 IOD and tumor size ($p = 0.06$) of the studied cases, while the IOD of GP88 showed a highly statistically significant correlation with tumor size ($p = 0.006$). The IOD expression of both markers showed a highly statistically significant correlation with the LNM status ($p = 0.000$) and tumor grade. Also, the correlation between the IOD evaluation of Fn14 and GP88 immunoexpression revealed a highly statistical difference ($p < 0.001$) (Figure 5b).

DISCUSSION

The improvement in lifestyle and changes in life expectancy have led to an increase in the incidence of breast cancer lesions. Globally, breast cancer ranks as

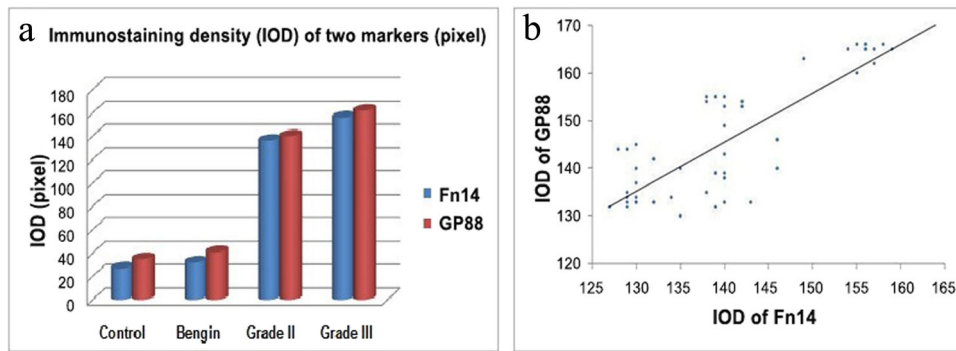


FIGURE 5 The IOD values of Fn14 and GP88 expression (a) and the correlation IOD between Fn14 and GP88 expression (b).

TABLE 1 Correlation between Fn14 and GP88 IOD and clinical parameters.

Pathological parameter	Fn14	GP88
Age	$r = -0.03$ $p = 0.82$	$r = -0.14$ $p = 0.28$
Tumor size	$r_s = 0.246$ $p = 0.058$	$r_s = 0.354^{**}$ $p = 0.006$
Grade	$r_s = 0.755^{**}$ $p = 0.000$	$r_s = 0.353^{**}$ $p = 0.006$
LNM	$r_s = 0.283^*$ $p = 0.030$	$r_s = 0.493^*$ $p = 0.030$
GP88	$r = 0.802^{**}$ $p = 0.000$	

Note: r : Pearson coefficient, r_s : Spearman coefficient.

*Correlation is significant at the 0.05 level.

**Correlation is significant at the 0.01 level.

the most prevalent malignancy among women and is the second-highest cause of cancer-related fatalities, surpassed only by lung cancer [33]. The IDC is the most common histological type, with the majority undergoing surgical resection of grade II and grade III [34]. The histopathological staining results in the present study revealed that in the IDC group, the separation of the myoepithelial cell layer from the basement membrane and the invasion of the malignant cells to the adjacent microenvironment are characteristic features. The focal disruption of the myoepithelial cell layer may be stimulated by invasive behavior through cues released from the degraded basement membrane to the interstitial extracellular matrix or the stromal tissue [35]. In cases of IDC, the tumor cells exhibit copious eosinophilic cytoplasm and display pleomorphic nuclei, arranged in cords and infiltrating in desmoplastic stroma, which characterized the grade II IDC. The nests were formed of the neoplastic proliferating cells that had hyperchromatic nuclei, large vesicular nuclei, and residual ones.

The grade III cases represented many pyknotic nuclei, and mitotic activities were seen in ductal epithelial cells. The present statistical studies revealed that most IDC cases were grade II (45/60), and a quarter of cases were grade III (15/60). This result is supported by a study of 691 retrospective samples of non-special type invasive ductal breast cancer, which showed that the largest percentage of tumors (53.96%) was grade II and 28.98% was grade III [36]. The present study of IDC cases revealed that most breast lesions were distributed among the age range of 35–55 years for grade II cases and 35–65 years for grade III cases. This finding is corroborated by a report indicating that the prevalent histological type of invasive breast cancer is distinguished by an early peak age of onset [36]. Regarding the tumor size, the research indicated that the typical tumor size was T2 (2–5 cm), and lymph node involvement was observed in 73% of the cases studied. This finding aligns with the results reported by Sofi et al. [37]. Other clinical pathological variables recorded showed that the high accuracy of an increased LNM number of more than three was due to the tumor size, tumor stages, and lymphovascular invasion [38]. This statement was consistent with the present results. There was a statistically significant increase ($p < 0.001$) in the association between the number of nodal metastases and the tumor size of 2–5 cm, as well as lymph node involvement. Another study reported that breast cancer patients with tumors larger than 2 cm had a high risk of nodal metastasis, which revealed that larger tumor size is associated with nodal metastasis and poor prognosis [39, 40].

The systemic proliferation and dissemination of malignant cells is a complex, multi-step process that leads to extensive cellular growth and results in the death of cancer patients; thus, the development of invasive cellular behavior is a process associated with normal physiological processes and immune system impairments, and is completed during the spread of metastatic cancer [41]. Research into various molecular markers, including cytokines, cell-cycle regulators, cell-adhesion proteins, and growth factors, has

revealed their association with the progression of cancer. The excessive production of cytokines and growth factors significantly contributes to cancer progression, invasion, and metastasis [42]. The clinical assessment of antibody protein expression, facilitated by the immunohistochemistry technique targeting specific tumor tissues, has been proven to be an effective approach. This method offers a wide scope for disease investigation, diagnosis, prognosis, and therapeutic decision-making [43]. Therefore, the current study was carried out to correlate the quantitative immunohistochemical expression of Fn14 and GP88 with human IDC, which led to a comparison between the cytokine expression and growth factors. Fn14 is a type I single transmembrane receptor, and it has been identified as a transcriptional target of the TNFR family. Cytokines have emerged as modern therapeutic targets. They possess the capability to block their receptors or signaling pathways, as well as to stimulate the immune response or hematopoiesis [43]. The Fn14 receptor serves as a modulator of breast cancer cell invasiveness across various biological settings. This is attributed to its pronounced overexpression in numerous solid tumor types and the inherent tumor cell-killing ability of the TWEAK-Fn14 pathway. Consequently, it acts as a negative prognostic marker and a promising therapeutic target for breast cancer [44]. The immunohistochemical study of 22 solid tumor subtypes showed that the expression of Fn14 was detected in most tumor types, including pancreatic cancer (60%), non-small cell lung cancer (55%), bone metastases (54%), and liver metastases of colorectal cancer (50%) [6]. In the present study, the immunorexpression of Fn14 protein was assessed by staining with DAB chromogen and appeared as brown color granules deposited in the cytoplasm of the ductal epithelial cells. Weak expression was observed in benign cases (70% of cases) as well as in stromal tissue. In contrast, high expression of Fn14 was evident in the IDC malignant case groups. The evaluation of the immunohistochemical expression of the Fn14 protein showed moderate positive staining in 78% of grade II IDC and strong immunostaining in 80% of grade III IDC. Also, the quantitative immunostaining density (IOD) results demonstrated that the expression of Fn14 in the IDC malignant group showed a statistically significant elevation when compared to the normal and benign groups ($p < 0.05$). This finding aligns with numerous studies that indicate that Fn14 expression is increased in malignant tumors relative to normal tissues. These studies propose that Fn14 may serve as a potential tumor antigen and a promising therapeutic target [45, 46]. In addition, the current study showed that there was no statistically significant association between Fn14 IOD and clinical parameters such as patients' age ($p = 0.82$) and tumor size ($p = 0.06$), which is consistent with the findings of

Wang et al. [30]. However, a statistically significant and highly positive correlation was found between Fn14 IOD and LNM ($p = 0.03$), as well as between Fn14 IOD and tumor grade ($p < 0.001$). This result revealed that high expression of Fn14 is associated with malignant metastasis and positive lymph nodes with more than four nodes. This finding was consistent with the observation that the expression levels of Fn14 and its ligand TWEAK are linked to metastasis and the presence of four or more positive lymph nodes [47]. Many scholars have studied the relationship between Fn14 expression and higher tumor grade, and pointed out that Fn14 expression is significantly correlated with more advanced grades and poorer prognosis [35, 48, 49]. Therefore, the high expression of Fn14 in IDC cases is associated with high tumor grade and increased LNM numbers. It can be concluded that Fn14 plays a role in cancer progression and cancer prognosis, and serves as a therapeutic marker in IDC patients.

On the other hand, the ability of cancer cells to produce and respond to regulatory growth factors mainly lies in the proliferation and progression of the cancer cells [50]. GP88 serves as an autocrine growth factor and a pleiotropic regulatory protein, playing a significant role in tumor genesis. This includes processes such as cell proliferation, survival, migration, angiogenesis, and invasion, and the activity of matrix metalloproteinases [32, 51]. In this study, the immunostaining of GP88 was observed in the cytoplasm and cell membrane of the ductal epithelial cells in breast lesions. Immunohistochemical staining evaluation showed that 80% of the control group and 57% of the benign group were negative for GP88 expression. In contrast, the positivity of GP88 immunostaining was observed in the malignant group (IDC) and increased with the histological grade. Most grade II cases (71%) showed moderate immunohistochemical expression, while most grade III cases (80%) exhibited strong positive immunostaining. These findings underscore the role of GP88 expression in promoting cell division and invasion in tumor cells. This conclusion was corroborated by researchers who reported that GP88 stimulates cell division, invasion, and survival [52]. Moreover, the quantitative assessment of the IOD of GP88 in this study demonstrated a highly significant difference in IOD of GP88 expression between grade II and grade III IDC compared to both normal and benign groups ($p < 0.001$). Thus, the quantitative analysis indicated that GP88 expression is correlated with elevated tumor grades in IDC cases and elucidated its involvement in the proliferation and invasion of tumor cells within breast lesions. Our findings corroborated that GP88 plays a regulatory role in multiple processes or stages of carcinoma progression [53]. Nevertheless, no statistically significant correlation was observed between the immunohistochemical expression of GP88 and patients' age ($p = 0.3$). This result aligns with several studies that

have reported on the clinical and tumor characteristics of patients aged ≤ 50 years compared to those over 50 years [52, 54]. The quantitative analysis showed that the IOD of GP88 expression had a highly statistically significant correlation with LNM of breast cancer cases ($p = 0.000$), tumor size ($p = 0.006$), and tumor histological grade of malignant breast cases ($p = 0.006$). These findings are consistent with the results by some investigators who found that the GP88 immunoeexpression level was associated with clinical pathological parameters such as LNM [54, 55]. It is well known that signs of poor prognosis in breast cancer include large tumor size, high tumor grade, and increased LNM. The upregulation of GP88 expression has been linked to tumor development and adverse outcomes in various types of cancer, such as breast, ovarian, renal, prostate, liver, and esophageal cancers [52]. GP88 is implicated in the processes of cellular invasiveness and modulates the progression of tumor cells due to its capacity to activate the MAPK pathway. It can serve as both a biomarker and a therapeutic target [56]. Therefore, the involvement of GP88 in regulating the progression of tumor cells makes it one of the biomarker proteins associated with therapeutic agents.

CONCLUSION

There was a significant statistical difference in the IOD of Fn14 and GP88 expression in different cases of breast lesions and patient age, which suggests that these two markers might be potential tumor antigens. The correlation between the two markers and some clinical pathological parameters showed that there was a significant correlation between LNM numbers and Fn14 expression, which was increased significantly with GP88 expression. The current results indicate that these two biomarkers may be used as prognostic markers of poor prognosis in breast cancer. Therefore, further clinical studies on Fn14 and GP88 in breast lesions will be required in the future, so as to use them as diagnostic and therapeutic agents for breast cancer.

AUTHOR CONTRIBUTIONS

Mona A. H. Yehia was responsible for conceptualization, work design, interpretation of the immunohistochemical data, software utilization, manuscript preparation, and substantive revision of the work. Sabah A. Al-Qadasi was responsible for the acquisition, analysis, interpretation of immunohistochemical and clinical data, statistical software utilization, and draft writing. Amel S. Al-Sedfy designed the work, interpreted the diagnosis and clinical data of patients, wrote the draft, and reviewed the manuscript. Noura A. K. Matar was responsible for the acquisition and analysis of the data, practical work of histopathology and immunohistochemistry, operating software used in the

work and statistical software, and writing the draft of the investigation.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. Supplemental Digital software (ImageJ bundled with 64-bit Java 8) is available in image analyzer instrument at the Histochemistry and Cell Biology Department, Medical Research Institute, Alexandria University. It was provided in the ImageJ website.

ETHICS STATEMENT

All the cases were asked to freely volunteer to the study and informed written consents were gathered before their inclusion in the study according to the guide ethics of the Medical Research Institute, Alexandria University (IORG#: IORG0008812).

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