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Immunohistochemical Expression of Fn14 and GP88 as Prognostic Biological Markers in Invasive Human Breast Cancer

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Abstract **Original Research Article**

Fibroblast growth factor-inducible 14 (Fn14), is a member of the tumor necrosis factor receptor super family that normally expressed in healthy tissues, but its expression is increased in injured tissue where it thought to play role in tissue remodeling. Progranulin or acrogranin is an 88-kDa glycoprotein identified by a biological screen for protein targets associated with high tumorigenicity. The aim of the present work was to investigate the expression of Fn14 and GP88 as novel prognostic biomarkers in human invasive ductal carcinoma (IDC) versus benign tumors and normal breast tissues as well as their correlation with different pathological and histological parameters. Immunohistochemical technique was used to examine the expression of Fn14 and GP88 in normal, benign as well as in IDC. Present results showed higher expression of Fn14 and GP88 in IDC comparing to normal and benign breast tissues.

Keywords: Fn14 and GP88 prognostic marker.

INTRODUCTION

Breast cancer is the most frequently diagnosed cancer and a second leading cause of cancer death in women worldwide [1]. Breast cancer represents a major scientific, clinical and societal problem. It is the most common malignancy and the second leading cause of cancer death in females following lung cancer [2] with more than 1,000,000 new cases and 370,000 deaths yearly worldwide [3]. In many developing countries, the incidence of breast cancer is now rising sharply due to changes in reproductive factors, lifestyle, and increased life expectancy [4].

Fibroblast growth factor-inducible-14 (Fn14) is the smallest member of the tumor necrosis factor (TNF) super family of receptors identified so far [5]. It is a type I trans-membrane receptor of a tumor necrosis factor likes weak inducer of apoptosis (TWEAK). Fn14 binding to its ligand (TWEAK) induces receptor trimerization, tumor necrosis factor receptorassociated factors (TRAF) association with the cytoplasmic tail and activation of intracellular signaling cascades such MAPK, and NF-κB which seems to be a universal cellular response [6].

Progranulin is 88-kDa glycoprotein and known as GP88, PC-cell derived growth factor or acrogranin, GP88 gene is located on the 21q portion of chromosome 17, while the mouse gene was found on chromosome 11 [7]. The autocrine growth factor GP88 is abundantly expressed in epithelial cells, immune cells, neurons, and chondrocytes [7].

In the present study, expression of Fn14 and GP88 in an invasive ductal carcinoma (IDC) were investigated using immunohistochemical technique and the intensity of immunostaining was quantitatively estimated using image optical density (IOD) analyzer.

MATERIAL AND METHODS

Tissue samples were obtained from patients diagnosed with breast tumors in the Department of

Pathology, Medical Research Institute, Alexandria University, Egypt. Formalin-fixed and paraffin embedded tissue specimens from 60 patients diagnosed with IDC, 30 patients diagnosed with benign breast tumor and 10 were taken from normal breast tissue adjacent to the tumors were included. All the cases were asked to freely volunteer to the study and informed written consents were gathered prior to their inclusion in the study. Hematoxlin and eosin (H&E) stained slides for each patient were reviewed by two pathologists. Diagnosis of the specimens was made according to the WHO classification of the Tumors. Clinical parameters included patients' age, tumor size, lymph node metastasis (LNM).

Immunohistochemical investigation of Fn14, GP88

Immunohistochemical method was utilized to study the expression of Fn14 in 60 paraffin-embedded breast tissues. In brief, paraffin-embedded specimens were cut into 5µm thick sections. The sections were deparaffinized using 2 changes of xylen and rehydrated. The sections were submerged in an antigen retrieval (citrate buffer saline pH 6) in an oven at 95°C for 20 minutes and then left at room temperature for 20 minutes to cool. The sections were treated with 3% H_2O_2 in PBS to quench the endogenous peroxidase activity, and then incubated with serum blocking reagent for 30 minutes to block nonspecific binding. The sections were incubated with primary antibody for Fn14 and GP88 (Biorbyt Company, London, UK) at 4ºC overnight. Sections were treated with conjugated 2^{nd} antibody (ABC-HRP reagent) for 30 minutes, stained with diaminobenzedine (DAB) and counter stained with hematoxylin. For negative controls, antibody was replaced with PBS. Each step was followed by PBS washing. Evaluation of Fn14 and GP88 immunohistochemical results was arbitrarily graded as negative (0), weak (+1), moderate (+2) and strong (+3).

Statistical Analysis

Data were normally distributed according to the Kolmogorov-Smirnov (K-S) normality test, and then analyzed using statistical software package SPSS 20. P values≤ 0.05 were considered statistically significant.

RESULTS

A-Histopathological results:

a. Haematoxylin and Eosin (H&E) staining of control, benign and malignant breast tissues:

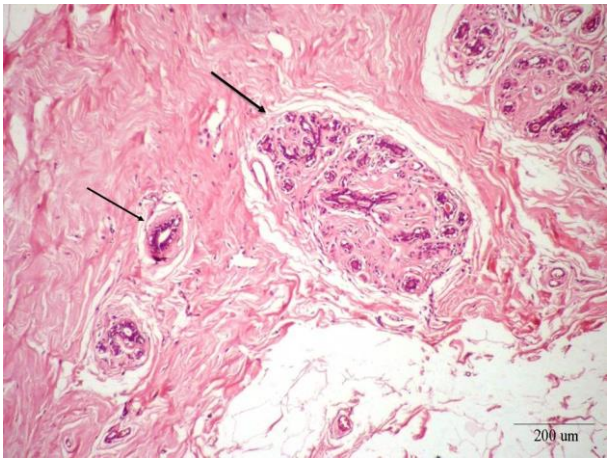

Figure 1: A section of control breast tissue showing normal ducts (thin arrow) and lobules (thick arrow) (H & E. Bar =200 µm)

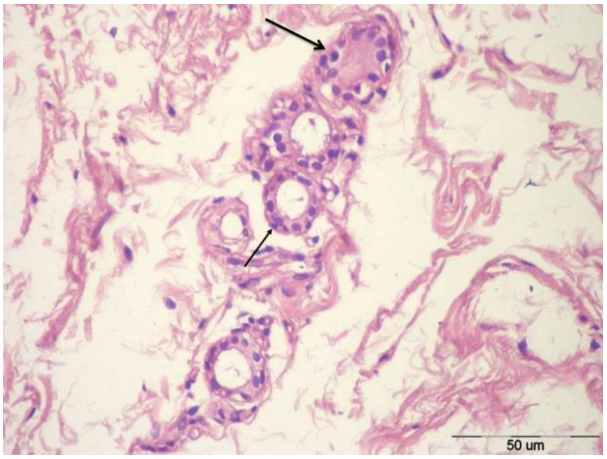

Figure 2: A view of the acini present in a normal lobule. The acini are lined by cuboidal epithelium (thick arrow) with underlying myoepithelial cells having clear cytoplasm (thin arrow) (H & E. Bar =50 µm)

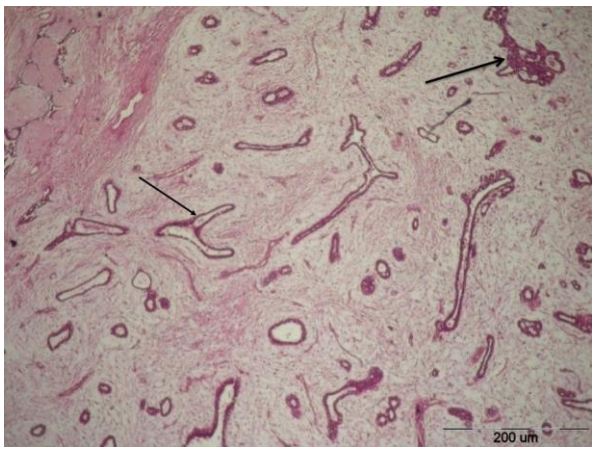

Figure 3: A section of fibroadenoma showing both periductal (thin arrow) and intraductal (thick arrow) patterns (H &E. Bar =200 µm)

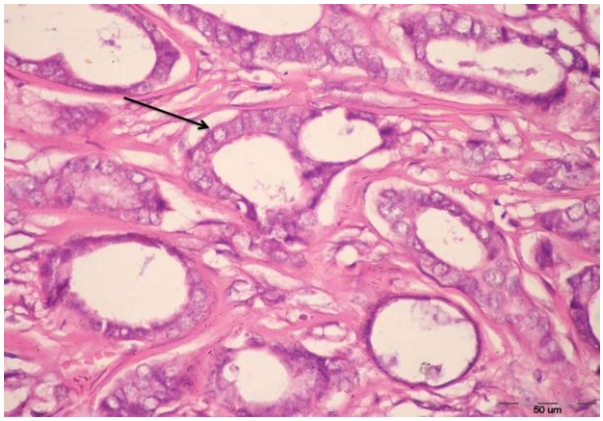

Figure 4: A section of IDC grade I showing well-defined ducts lined by cuboidal epithelial cells with vesicular nuclei (H&E. Bar =50 µm)

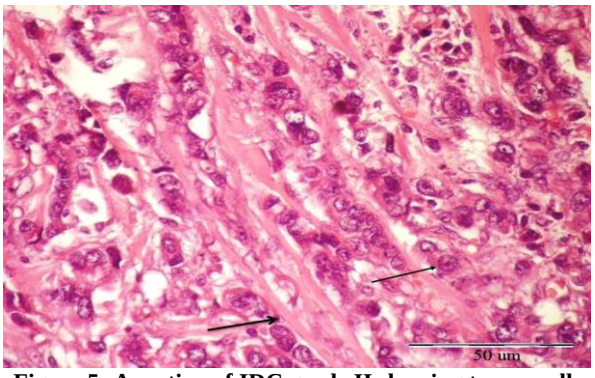

Figure 5: A section of IDC grade II showing tumor cells with abundant eosinophlic cytoplasm and pleomorphic round to ovoid vesicular nuclei (thin arraow). The cells which are arranged in cords infiltrate the desmoplastic stroma (thick arrow) (H&E. Bar =50 µm)

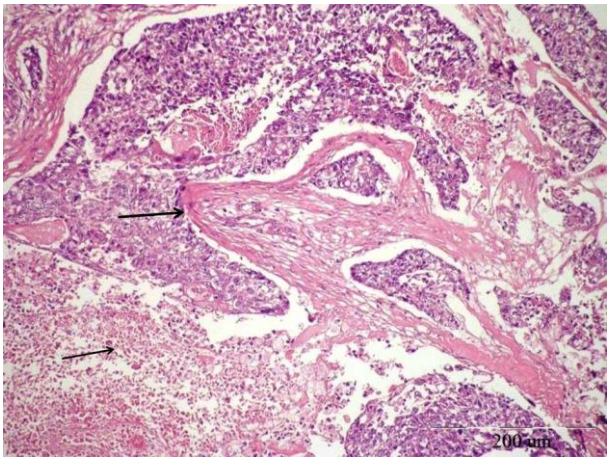

Figure 6: A section of IDC grade III (Atypical medullary variant) showing sheets of malignant ductal cells with wide areas of necrosis (thin arrow) and lymphocytes infiltration separated by fibrous tissue septa (thick arrow) (H&E. Bar=200 µm)

2-Immunohistochemical Results:

I-Fn14:

a. Immunohistochemical reactivity of Fn14:

- Immunostaining reactivity of Fn14 was detected as granular brown stain observed in the cytoplasm and cytomembrane of the epithelial cells of the studied groups.
- Fn14 immunostaining reactivity was negative (-ve) in 70% (7/10) of control breast tissues, weak +ve (1+) in 70% (21/30) of benign group, while it was moderate +ve (2+) in 78% (35/45) of IDC grade II and strong +ve (3+) in 77% (10/13) of IDC grade III.

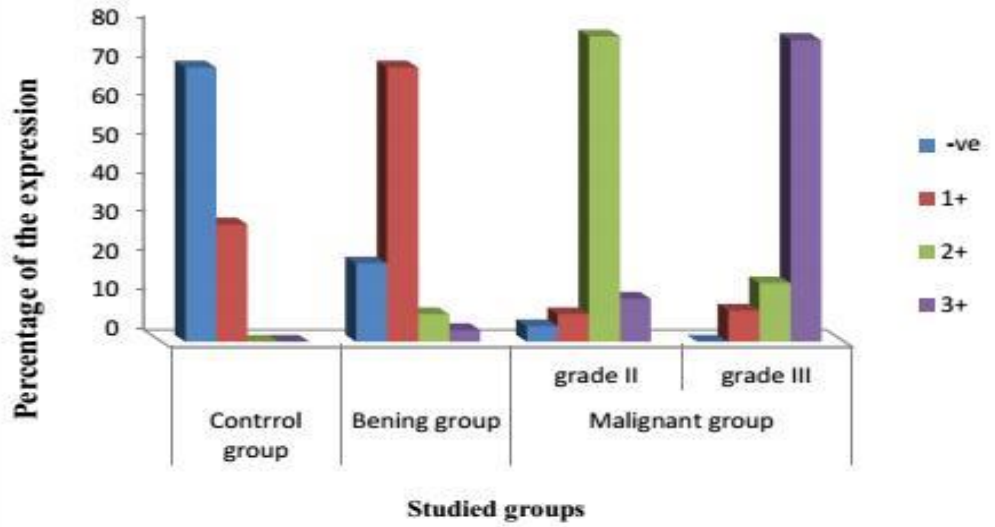

Figure 7: Immunostaining reactivity of Fn14 in the different studied groups. Note the overexpression of Fn14 in malignant group versus control and benign groups

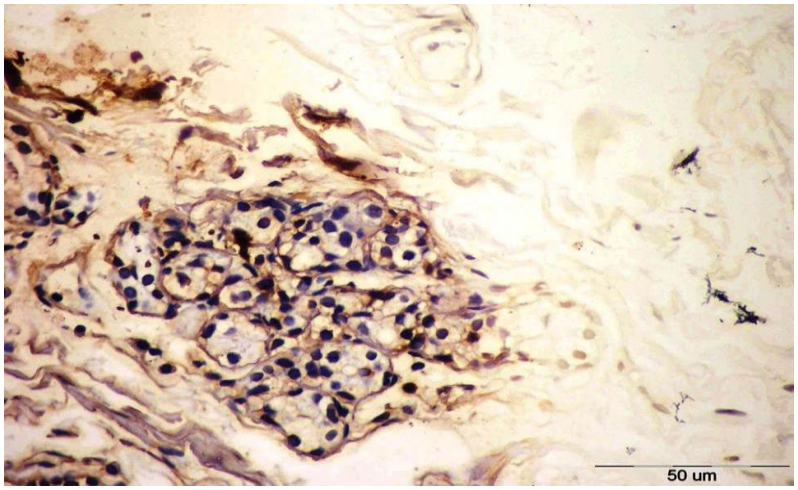

Figure 8: A control breast tissue showing negative (-ve) expression of Fn14 in the cytoplasm of the ductal epithelial cells (Bar =50 µm)

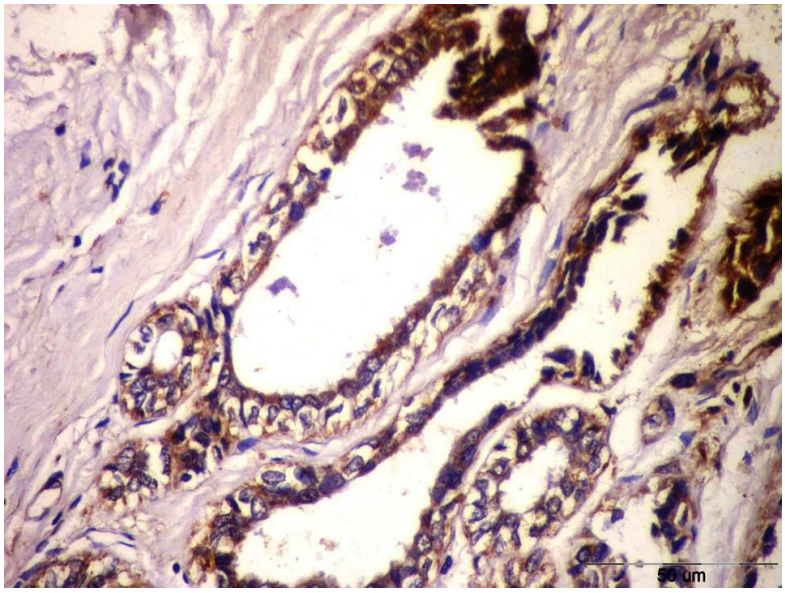

Figure 9: A benign breast tissue showing a weak (1+), granulated and membranous expression of Fn14 in the myoepithelial and ductal cells (Bar =50 µm)

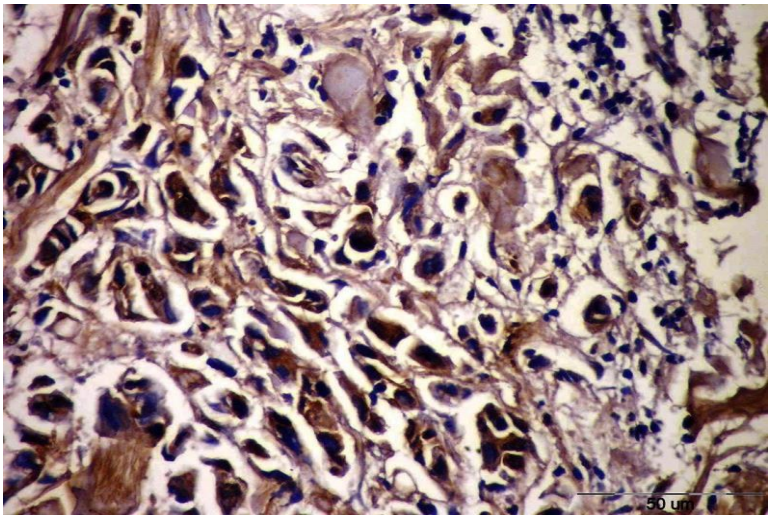

Figure 10: An IDC grade II breast tissue showing moderate (2+) expression of Fn14 in the cytoplasm of the ductal epithelial cells (Bar =50 µm)

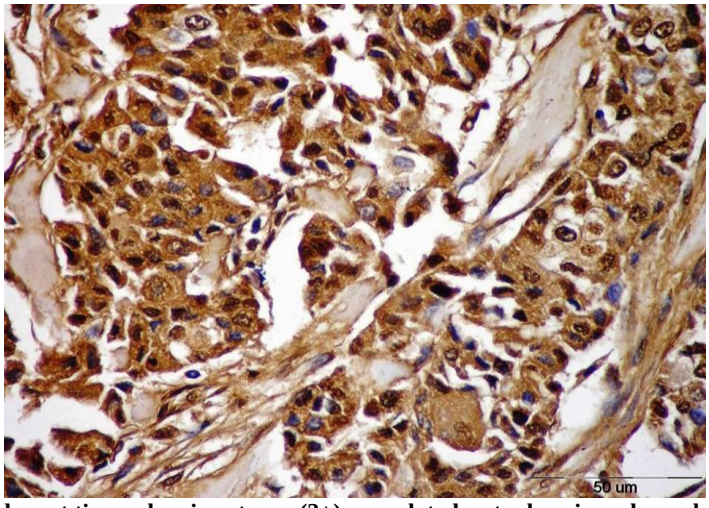

Figure 11: An IDC grade III breast tissue showing strong (3+) granulated, cytoplasmic and membranous expression of Fn14 in the ductal epithelial cells (Bar =50 µm)

II-GP88:

a. Immunohistochemical reactivity of GP88:

- Immunostaining reactivity of GP88 was detected as diffuse, homogenous brown color detected in the cytoplasm and membrane of the ductal epithelial cells of the studied groups.
- GP88 immunostaining reactivity was negative (-ve) in 80% (8/10) of control group, and 57% (17/30) of benign group, while it was moderate (2+) in 71% (32/45) of grade II IDC and strong (3+) in 77% (10/13) of grade III IDC as illustrated in Figures (12).

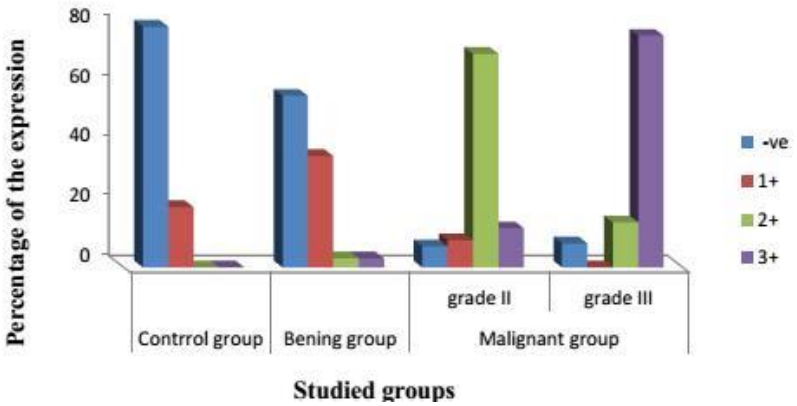

Figure 12: Immunostaining reactivity of GP88 in the different studied Groups. GP88 was overexpressed in malignant group versus control and benign groups

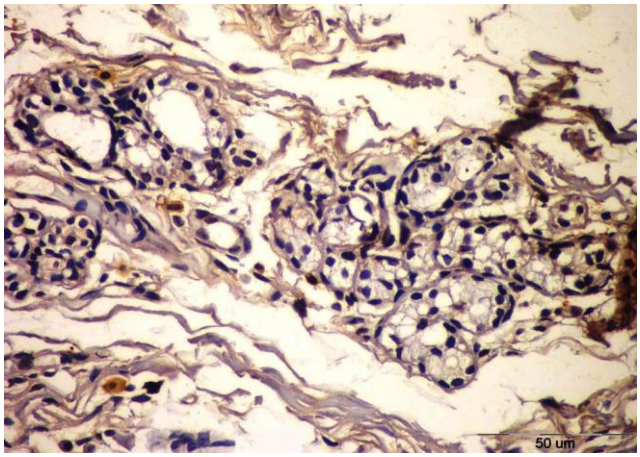

Figure 13: Immunohistochemical staining of control breast tissue showing negative expression of GP88 in the cytoplasm of the ductal epithelial cell (Bar =50 µm)

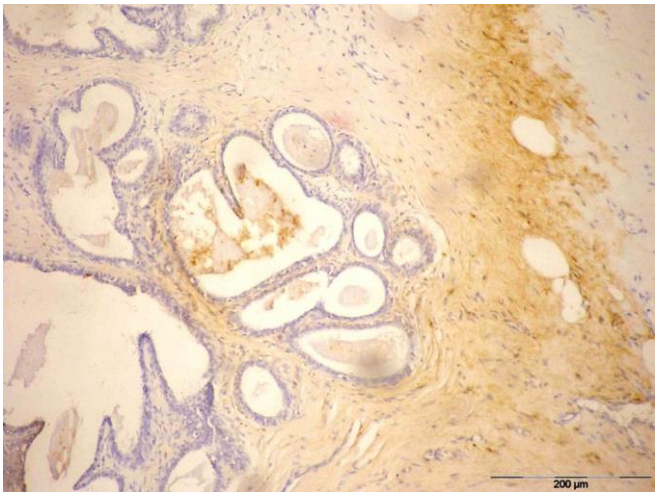

Figure 14: A benign breast tissue showing negative (-ve) expression of GP88 in the cytoplasm of the ductal epithelial cells (Bar =50 µm)

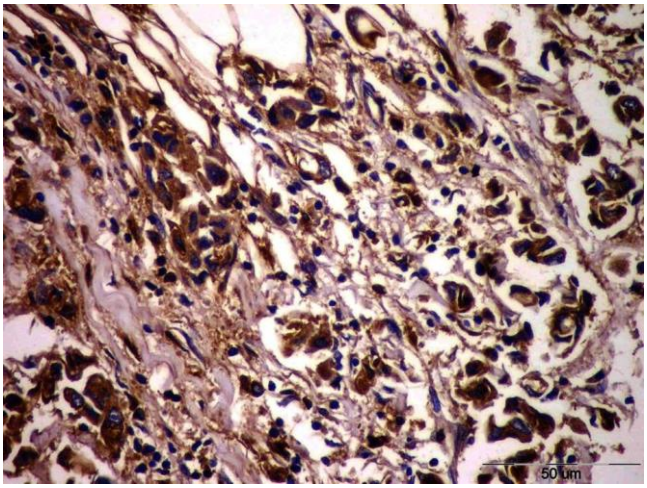

Figure 15: An IDC grade II breast tissue showing moderate expression (2+) of GP88 in the cytoplasm of the ductal epithelial cells (Bar=50 µm)

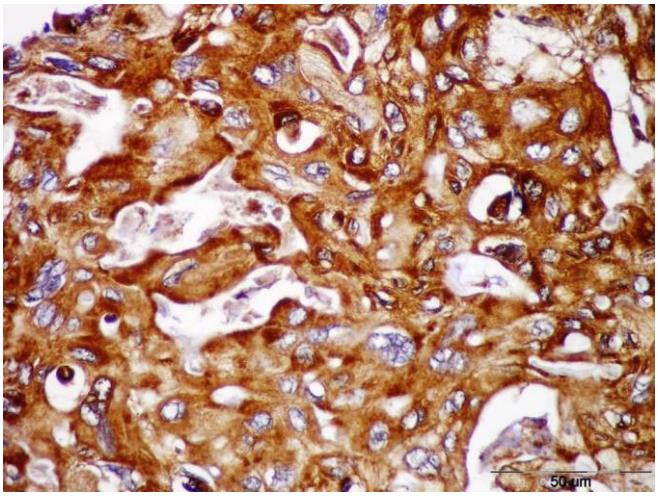

Figure 16: An IDC grade III breast tissue showing strong expression (3+) of GP88 in the cytoplasm of the ductal epithelial cell (Bar=50 µm)

DISCUSSION

Breast cancer remains a major scientific, clinical and societal challenge. It is the most common malignancy and the second leading cause of cancer death in females worldwide following lung cancer. In many developing countries, the incidence of breast cancer is now rising sharply due to changes in

reproductive factors, lifestyle, and increased life expectancy [8].

The results of the present study showed that 98% of the breast cancer cases were invasive ductal carcinoma (IDC), most of which were allocated to the age range of (>35-55) years. This results is consistent with several previous studies reported that IDC is the most common histological type of invasive breast cancer, and in the developing world it characterized by an early peak age of onset [9].

In the current study, the majority (75%) of the studied cases was grade II, followed by grade III (22%). This result is supported by previous studies claimed that most of the breast cancer cases undergoing surgical resection are at grade II and III [10].

Concerning tumor size, most of the studied breast cancer cases in the present work were allocated to the tumor size T2 (>2-5) cm and lymph node involvement was present in 73% of the studied cases. These results were in accordance with Sofi *et al*., (2012) [11].

Systemic outgrowth and spread of malignant cells is the main cause of deaths in cancer patients. Acquisition of cell-invasive behavior includes the ability to adhere to and migrate through a variety of barriers. One of the most difficult barriers to navigate through is the basement membrane (BM). BM invasion is an essential process that occurs during normal development, immune system surveillance, and spread of metastatic cancer [12].

Transition from normal breast epithelium to malignant breast cancer is a complex multistep process resulting from the uncoupling of the interactive systems controlling cell proliferation and differentiation, thus leading to extensive cellular growth [16]. Numerous molecular markers such as cytokines, cell-cycle regulators, cell-adhesion proteins and growth factors have recently been investigated in relation to cancer progression. Among these, the overexpression of cytokines and growth factors has been reported to play an important role in the process of cancer progression, invasion and metastasis [13].

Immunohistochemical technique is an effective method for clinical determination of antibody proteins expression owing to specific targeting of tumor cells, nowadays; it is used in the investigation of a broad range of disease processes with applications in diagnosis, prognostication and therapeutic decisions [14].

The present study was undertaken to assess the immunohistochemical expression of Fn14, GP88 in human breast invasive carcinoma versus normal control and benign breast tumors, as well as to investigate the correlation of their immunohistochemical expression with clinicopathological parameters.

Fibroblast growth factor–inducible 14-kDa protein (Fn14) is the cell surface receptor for the cytokine TNF-like weak (TWEAK) inducer of apoptosis. Fn14 receptor is a regulator of breast cancer cell invasive capacity in multiple biological contexts because of its strong overexpression in many types of solid tumors and the intrinsic tumor cell killing capacity of the TWEAK-Fn14 pathway and thus a negative prognostic indicator and potential therapeutic target for breast cancer [15].

The present study showed a statistical significant increase in the immunohistochemical expression of Fn14 in the malignant group versus normal and benign groups ($p < 0.05$). This finding is in agreement with those reported by other previous studies [16].

In the largest survey by Culp *et al*., (2010) [17] examining 1,655 tumor samples across 22 solid tumor subtypes by immunohistochemistry, Fn14 expression was detected in the majority of tumor types, including pancreatic cancer (60%), non-small cell lung cancer (55%), bone metastases (54%) and liver metastases in colorectal cancer (50%).

The fact that Fn14 expression is elevated in malignant tumors as compared with normal tissues suggests that it may be a potential tumor antigen and therefore, on the basis of expression alone, a valuable therapeutic target [18].

The results of the current study showed no statistical significant correlation between the immunohistochemical expression of Fn14 and clinical parameters such as patients' age ($r = -.03$, $p = .82$) and tumor size ($r = .246$, $P = .06$). These findings are consistent with those reported by Wang, *et al*., (2013) [19].

A statistical significant correlation was noticed in the present study between Fn14's immunohistochemical expression and LNM ($r = 0.28$*, $P = 0.03$). This is in agreement with previous studies showed that expression of Fn14 and its ligand TWEAK were both associated with metastasis and with four or more positive lymph nodes [20].

In the current study, Fn14's immunohistochemical expression has shown to be highly correlated with tumor histological grade ($r = 0.76$**, $P < .001$). This finding is contrasted with Wang, *et al*., (2013) [21], however the relationship between Fn14's expression and higher tumor grade that was noticed in the present study is going in accordance with Whitsett, *et al*., (2014) [22] and Li, *et al*., (2013) [23] who stated that Fn14 expression was significantly

correlated with more advanced grade and poorer prognosis.

Overexpression of Fn14 and higher tumor grade and/or poor prognosis has been noticed in brain, esophageal, prostate, gastric and bladder cancers.

The ability of cancer cells to produce and respond to their own (autocrine) growth factors is important in the proliferation and progression of the cancer cells [24].

Progranulin or GP88 is an autocrine growth factor and pleiotropic regulatory protein that has been shown to play role in tumorigenesis, including proliferation, survival, migration, angiogenesis invasion and matrix metallo-protease activity, in addition to its role in wound healing and in inflammation in normal tissues [25].

The results of the present study showed a statistical significant increase in the immunohistochemical expression of GP88 in IDC, versus, normal tissues and benign tumors ($p < .001$). This result is in alignment with previous finding reported high level of GP88 expression in breast cancer biopsies versus benign lesions and normal mammary epithelial tissues [26]. In addition, pathological studies with 203 formalin-fixed paraffin-embedded human breast cancer tissue biopsies indicated that GP88 was preferentially expressed in ductal carcinoma with little expression in lobular carcinoma while benign lesions and normal mammary epithelial tissues were negative [27].

Highly statistical significant correlation was observed in the current study between GP88's immunohistochemial expression and tumor histological grade ($r = .353^{**}$, $P = .006$). This result is contrasted with Serrero *et al.,* (2012) [28], but consistent previous studies reported overexpression of GP88 in 80% invasive ductal carcinoma, where it correlated with the clinical variables of poor prognosis such as tumor grade, p53 expression and Ki67 index [29].

Immunohistochemial expression of GP88 in the present study exhibited a highly statistical significant correlation with LNM of the studied breast cancer cases ($r = .493^{**}$, $P = .000$). This finding is in variance with Serrero *et al.,* (2012) [30]; however, previous study found that high levels of GP88 expression were closely correlated with clinicopathological parameters such as lymph node metastasis [31].

It has been reported that level of GP88 expression was a major determinant of the intrinsic activity of the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3'-kinase (PI3K) in cell line studies suggesting that the mitogen-activated protein kinase and phosphatidylinositol 3'-kinase signaling pathways may be involved in the promotion of tumor invasion and migration by GP88 [32].

Large tumor size, high tumor grade and positive lymph nodes are all signs of poor prognosis, and elevated GP88 expression has shown to be associated with tumorigenesis and poor prognosis in several cancer types including ovarian, renal prostate, liver, esophageal and breast cancers [33].

The present study showed no statistical significant correlation between the immunohistochemical expression of GP88 and ER ($r = -.09$, $P = .50$) and PR ($r = .06$, $P = .65$) status of the studied breast cancer cases. These results are conflicted with Song *et al*. (2009) [34] who reported that GP88 positive staining was more common in ER/PR negative samples than that in ER/PR positive tumors, however, the current results are going in accordance with Tkaczuk *et al*., (2011) [35] who observed no statistical correlation between GP88 expression and ER and PR levels. Moreover, screening of GP88 expression in human breast cancer cell lines indicated that GP88 was highly expressed in both ER positive and ER negative human breast carcinomas [36].

CONCLUSION

From the results of the present study, it could be concluded that:

- The marked immunohistochemical expression of Fn14 and GP88 in malignant group versus control and benign groups indicates that these biomarkers might be potential tumor antigens and therefore, valuable therapeutic targets in breast cancer.
- The strong statistical correlation between the immunohistochemical expression of GP88 with the large tumor size, high tumor grade and LNM, and the lack of correlation between its expression and ER and HER2/neu indicates that GP88 might be an independent prognostic marker for poor prognosis in breast cancer.
- The lack of correlation between the immunohistochemical expression of these markers with patients' age indicates that they might be assessed in both menopausal and post-menopausal women.

Competing Interests: Authors declare that they have no competing interests; financials or others.

REFERENCES

1. Khan, H. M. R., Saxena, A., Vera, V., Abdool-Ghany, F., Gabbidon, K., Perea, N. P., ... & Ramamoorthy, V. R. (2014). Black hispanic and black nonhispanic breast cancer survival data analysis with half-normal model application. *Asian Pac J Cancer Prev*, 15, 9453-9458.

2. Breast Cancer Association Consortium. (2021). Breast cancer risk genes—association analysis in more than 113,000 women. *New England Journal of Medicine*, *384*(5), 428-439.
3. Lehner, J., Stoetzer, O. J., Fersching, D., Nagel, D., & Holdenrieder, S. (2013). Circulating plasma DNA and DNA integrity in breast cancer patients undergoing neoadjuvant chemotherapy. *Clinica chimica acta*, *425*, 206-211.
4. Shulman, L. N., Willett, W., Sievers, A., & Knaul, F. M. (2010). Breast cancer in developing countries: opportunities for improved survival. *Journal of oncology*, *2010*.
5. Wiley, S. R., Cassiano, L., Lofton, T., Davis-Smith, T., Winkles, J. A., Lindner, V., ... & Fanslow, W. C. (2001). A novel TNF receptor family member binds TWEAK and is implicated in angiogenesis. *Immunity*, *15*(5), 837-846.
6. Winkles, J. A. (2008). The TWEAK–Fn14 cytokine–receptor axis: discovery, biology and therapeutic targeting. *Nature reviews Drug discovery*, *7*(5), 411-425.
7. Liu, C. J., & Bosch, X. (2012). Progranulin: a growth factor, a novel TNFR ligand and a drug target. *Pharmacology & therapeutics*, *133*(1), 124-132.
8. Shulman, L. N., Willett, W., Sievers, A., & Knaul, F. M. (2010). Breast cancer in developing countries: opportunities for improved survival. *Journal of oncology*, *2010*. doi:10.1155/2010/595167.
9. American Cancer Society, Global Cancer Facts & Figures. 2015. Atlanta Ga, USA.
10. Hüsemann, Y., Geigl, J. B., Schubert, F., Musiani, P., Meyer, M., Burghart, E., ... & Klein, C. A. (2008). Systemic spread is an early step in breast cancer. *Cancer cell*, *13*(1), 58-68.
11. Sofi, G. N., Sofi, J. N., Nadeem, R., Shiekh, R. Y., Khan, F. A., Sofi, A. A., ... & Bhat, R. A. (2012). Estrogen receptor and progesterone receptor status in breast cancer in relation to age, histological grade, size of lesion and lymph node involvement. *Asian pacific journal of cancer prevention*, *13*(10), 5047-5052.
12. Matus, D. Q., Li, X. Y., Durbin, S., Agarwal, D., Chi, Q., Weiss, S. J., & Sherwood, D. R. (2010). In vivo identification of regulators of cell invasion across basement membranes. *Science signaling*, *3*(120), ra35-ra35. doi: 10.1126/scisignal.2000654.
13. Jiao, F., Jin, Z., Wang, L., & Wang, L. (2013). Research and clinical applications of molecular biomarkers in gastrointestinal carcinoma. *Biomedical Reports*, *1*(6), 819-827.
14. Ramos-Vara, J. A., & Miller, M. A. (2014). When tissue antigens and antibodies get along: revisiting the technical aspects of immunohistochemistry—the red, brown, and blue technique. *Veterinary pathology*, *51*(1), 42-87.
15. Michaelson, J. S., Amatucci, A., Kelly, R., Su, L., Garber, E., Day, E. S., ... & Joseph, I. B. (2011, July). Development of an Fn14 agonistic antibody as an anti-tumor agent. In *MAbs* (Vol. 3, No. 4, pp. 362-375). Taylor & Francis.
16. Chao, D. T., Su, M., Tanlimco, S., Sho, M., Choi, D., Fox, M., ... & Culp, P. A. (2013). Expression of TweakR in breast cancer and preclinical activity of enavatuzumab, a humanized anti-TweakR mAb. *Journal of cancer research and clinical oncology*, *139*(2), 315-325.
17. Culp, P. A., Choi, D., Zhang, Y., Yin, J., Seto, P., Ybarra, S. E., ... & Dubridge, R. (2010). Antibodies to TWEAK Receptor Inhibit Human Tumor Growth through Dual MechanismsAntitumor Activity of TweakR Antibodies. *Clinical Cancer Research*, *16*(2), 497-508.
18. Zhou, H., Ekmekcioglu, S., Marks, J. W., Mohamedali, K. A., Asrani, K., Phillips, K. K., ... & Rosenblum, M. G. (2013). The TWEAK receptor Fn14 is a therapeutic target in melanoma: immunotoxins targeting Fn14 receptor for malignant melanoma treatment. *Journal of Investigative Dermatology*, *133*(4), 1052-1062.
19. Wang, J., Liu, Y., Wei, X. Y., & Wang, E. H. (2013). Clinical correlations and prognostic relevance of Fn14 expression in breast carcinoma. *Histol Histopathol*, 28, 859-864.
20. Willis, A. L., Tran, N. L., Chatigny, J. M., Charlton, N., Vu, H., Brown, S. A., ... & Cunliffe, H. E. (2008). The fibroblast growth factor–inducible 14 receptor is highly expressed in HER2-positive breast tumors and regulates breast cancer cell invasive capacity. *Molecular Cancer Research*, *6*(5), 725-734.
21. Wang, J., Liu, Y., Wei, X. Y., & Wang, E. H. (2013). Clinical correlations and prognostic relevance of Fn14 expression in breast carcinoma. *Histol Histopathol*, 28, 859-864.
22. Whitsett, T. G., Mathews, I. T., Cardone, M. H., Lena, R. J., Pierceall, W. E., Bittner, M., ... & Tran, N. L. (2014). Mcl-1 Mediates TWEAK/Fn14-Induced Non–Small Cell Lung Cancer Survival and Therapeutic ResponseMcl-1 and TWEAK in NSCLC survival. *Molecular Cancer Research*, *12*(4), 550-559.
23. Li, N., Hu, W. J., Shi, J., Xue, J., Guo, W. X., Zhang, Y., ... & Cheng, S. Q. (2013). Roles of fibroblast growth factor-inducible 14 in hepatocellular carcinoma. *Asian Pacific Journal of Cancer Prevention*, *14*(6), 3509-3514.
24. Fiaschi, T., & Chiarugi, P. (2012). Oxidative stress, tumor microenvironment, and metabolic reprogramming: a diabolic liaison. *International journal of cell biology*, *2012*. doi: 10.1155/2012/762825.
25. Tkaczuk, K. R., Yue, B., Zhan, M., Tait, N., Yarlagadda, L., Dai, H., & Serrero, G. (2011). Increased circulating level of the survival factor GP88 (progranulin) in the serum of breast cancer

patients when compared to healthy subjects. *Breast cancer: basic and clinical research*, *5*, BCBCR-S7224.

26. Serrero, G., Hawkins, D. M., Yue, B., Ioffe, O., Bejarano, P., Phillips, J. T., ... & Kim, W. E. (2012). Progranulin (GP88) tumor tissue expression is associated with increased risk of recurrence in breast cancer patients diagnosed with estrogen receptor positive invasive ductal carcinoma. *Breast Cancer Research*, *14*(1), 1-12.
27. Serrero, G., & Ioffe, O. B. (2003). Expression of PC-cell-derived growth factor in benign and malignant human breast epithelium. *Human pathology*, *34*(11), 1148-1154.
28. Serrero, G., Hawkins, D. M., Yue, B., Ioffe, O., Bejarano, P., Phillips, J. T., ... & Kim, W. E. (2012). Progranulin (GP88) tumor tissue expression is associated with increased risk of recurrence in breast cancer patients diagnosed with estrogen receptor positive invasive ductal carcinoma. *Breast Cancer Research*, *14*(1), 1-12.
29. Serrero, G., & Ioffe, O. B. (2003). Expression of PC-cell-derived growth factor in benign and malignant human breast epithelium. *Human pathology*, *34*(11), 1148-1154.
30. Serrero, G., Hawkins, D. M., Yue, B., Ioffe, O., Bejarano, P., Phillips, J. T., ... & Kim, W. E. (2012). Progranulin (GP88) tumor tissue expression is associated with increased risk of recurrence in breast cancer patients diagnosed with estrogen receptor positive invasive ductal carcinoma. *Breast Cancer Research*, *14*(1), 1-12.
31. Li, L. Q., Min, L. S., Jiang, Q., Ping, J. L., Li, J., & Dai, L. C. (2012). Progranulin expression in breast cancer with different intrinsic subtypes. *Pathology-Research and Practice*, *208*(4), 210-216.
32. Liu, Y., Xi, L., Liao, G., Wang, W., Tian, X., Wang, B., ... & Ma, D. (2007). Inhibition of PC cell-derived growth factor (PCDGF)/granulin-epithelin precursor (GEP) decreased cell proliferation and invasion through downregulation of cyclin D and CDK 4 and inactivation of MMP-2. *BMC cancer*, *7*(1), 1-9. doi:10.1186/1471-2407-7-22
33. Koo, D. H., Park, C. Y., Lee, E. S., Ro, J., & Oh, S. W. (2012). Progranulin as a prognostic biomarker for breast cancer recurrence in patients who had hormone receptor-positive tumors: a cohort study. *PloS one*, *7*(6), e39880. doi: 10.1371/journal.pone.0039880.
34. Song, H., Shi, L., Liu, C., & Huang, T. (2009). Expression of PC-cell-derived growth factor in breast cancer. *Frontiers of Medicine in China*, *3*(4), 426-430.
35. Tkaczuk, K. R., Yue, B., Zhan, M., Tait, N., Yarlagadda, L., Dai, H., & Serrero, G. (2011). Increased circulating level of the survival factor GP88 (progranulin) in the serum of breast cancer patients when compared to healthy subjects. *Breast cancer: basic and clinical research*, *5*, BCBCR-S7224.
36. Lu, R., & Serrero, G. (2000). Inhibition of PC cell-derived growth factor (PCDGF, epithelin/granulin precursor) expression by antisense PCDGF cDNA transfection inhibits tumorigenicity of the human breast carcinoma cell line MDA-MB-468. *Proceedings of the National Academy of Sciences*, *97*(8), 3993-3998.