Correlation of astrocyte elevated gene-1, basic-fibroblast growth factor, beta-catenin, Ki-67, tumor necrosis factor-alfa with prognostic parameters in ductal carcinomas and ductal intraepithelial neoplasms

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Abstract

Background: Breast cancer is the second most frequent cancer in the world. Although it is widely accepted that the etiology of breast cancer includes both genetic and environmental factors, the molecular mechanism of its development and progression remains poorly understood, and thus far, no specific signature of breast cancer gene expression has been reported to allow for patient-tailored therapy strategies. Hence, it is of great clinical value to further understand the molecular mechanisms underlying the progression of breast cancer and to identify effective early markers for the diagnosis and prognosis of the disease as well as novel therapeutic targets.

Materials and Methods: This study was conducted on a total of 90 paraffin-embedded breast tumor samples. Immunohistochemical stains for astrocyte elevated gene-1 (AEG-1), basic-fibroblast growth factor (b-FGF), beta-catenin, Ki-67, tumor necrosis factor-α (TNF-α) were performed on tissue microarray using standard procedures. Each patient age, grade, estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER-2) status, size, lymphovascular invasion, metastasis of lymph node (LNM), nipple and ductal hyperplasia areas were assessed.

Results: We observed significant relationship between the age and LNM or FGF (P = 0.018, 0.035, respectively). The relationship between histological and nuclear grade, LNM, ER, PR, HER-2 and prognostic parameters was evaluated in cases of ductal carcinomas (DC). There was a significant positive correlation between TNF-α, size, LNM ($P \leq 0.0001$, 0.002, 0.005). We found that significant relationship between AEG-1 and TNF-α. There was a significant positive correlation between FGF and Ki-67 and negative correlation AEG-1. Although, FGF, TNF-α, AEG-1 staining in DC were observed higher than ductal intraepithelial neoplasms, this observation could not statistically ($P \geq 0.05$).

Conclusions: The present work aims to investigate the relationship between the expression of AEG-1, b-FGF, beta-catenin, Ki-67, TNF-α other prognostic parameters in DC and ductal intraepithelial neoplasm. We found a relationship between these factors.

Key words: Astrocyte elevated gene-1, beta-catenin, basic-fibroblast growth factor, ductal carcinomas, prognostic parameters, tumor necrosis factor-α

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Introduction

Breast cancer is the second most common malignancy in the world to date.[1] Classification of this cancer is based on a number of aspects such as pathology and tumor progression, estrogen receptor (ER), progesterone receptor (PR) status and human epidermal growth factor receptor 2 (HER-2) status. Prognosis is related to a variety of clinical, pathologic and molecular features, which include classical prognostic factors (such as histologic type, grade, tumor size and metastasis of lymph node [LNM]). All of these clinical parameters dictate the most suitable patient treatment.[2]

However, continued improvements in treatment will undoubtedly depend on our ability to identify novel targets contributing to this disease. Fibroblast growth factor receptors (FGFRs) as well as a number of fibroblast growth factor (FGF) ligands, play a critical role in regulating normal mammary gland development and tissue homeostasis. Such target, which has been implicated in the etiology of breast cancer, is the FGFR.[3]

In addition, high-throughput sequencing studies have shown activating FGF mutations to be highly common in multiple forms of human cancer, including breast cancer. There is also increasing evidence for the importance of FGF signaling in human cancers, including breast cancer.[4]

Almost 25% of cancers are reported to occur through chronic inflammation related processes.[5] The pro-inflammatory regulators such as tumor necrosis factor-α (TNF-α) and other cytokines and their receptor networks seem to play crucial functions in tumorigenesis.[6]

Beta-catenin is considered to be involved in the carcinogenesis of many tumors and is also thought to be effective in the development of breast cancer.[7] It is one of the proteins that E-cadherin, which is an adhesion molecule, has to interact with in order to carry out its mission, and it is also involved in intracellular signal transduction pathways.[8]

Recent studies have showed that astrocyte elevated gene-1 (AEG-1) increases the invasiveness of malignant cells, favoring tumor genesis, neovascularization, metastasis and that up-regulation in epithelial cells inhibits apoptosis.[9]

The possible involvement of AEG-1, basic-FGF (b-FGF), beta-catenin, TNF-α in tumorigenesis, angiogenesis and metastasis led us to examine the relationship between AEG-1, b-FGF, beta-catenin, Ki-67, TNF-α and prognostic parameters in 90 primary breast carcinomas samples.

Materials and Methods

This study was conducted on a total of 90 paraffin-embedded breast tumor (ductal carcinomas [DC]) samples, which were histopathologically diagnosed at the Department of Pathology between 2005 and 2012. All the diagnoses were made by two pathologists, following the pathology of World Health Organization (WHO) Classification of Tumors. The samples consisted of 90 (all cases) DC. Age distribution of the cases ranged from the 31 to 83 (56.4 ± 13.4). The tumor size distribution was as follows: Less than 2 cm in diameter in 9 (10%) patients, from 2 to 5 cm in 70 (77.8%) patients and more than 5 cm in 11 (12.2%) patients. Lymph node metastasis number distribution of the cases ranged from the 0 to 16 (3.32 ± 2.37). In addition, 47 (52.2%) patients in LNM.

In all cases, the histological diagnosis and prognostic parameters were confirmed by corresponding paraffin-embedded materials, and when necessary, immunohistochemical study panels were carried out according to the most recent WHO classification.

The tumor grade of invasive carcinoma was classified according to the Scarff–Bloom–Richardson system. Based on the frequency of cell mitosis, tubule formation, and nuclear pleomorphism, invasive carcinoma was graded as grade 1 (low), 2 (moderate) or 3 (high). The presence of lymph node metastases was reviewed for each patient. Prognostic parameters were compared with the results of AEG-1, b-FGF, beta-catenin, TNF-α, Ki-67 stains.

Immunohistochemistry

Among the Hematoxylin-Eosin stained slides, one suitable paraffin block was chosen. For AEG-1, Genetex brand 2F11C3 clone was used, and it was diluted at a ratio of 1:200. The degree of immunostaining was reviewed. They were scored independently by two observers, based on both the proportion of positively-stained tumor cells and the intensity of staining. The proportion of tumor cells was scored as follows: 0 (no positive tumor cells), 1 (<10% positive tumor cells), 2 (10–50% positive tumor cells), and 3 (>50% positive tumor cells). The intensity of staining was graded according to the following criteria: 0 (no staining), 1 (weak staining = light yellow), 2 (moderate staining = yellow brown), and 3 (strong staining = brown). The staining index was calculated from the staining intensity score and proportion of positive tumor cells. Using this method of assessment, we evaluated the expression of AEG-1 [Figure 1] in normal tubular epithelia and malignant lesions by determining the staining index, which was scored as 0, 1, 2, 3, 4, 6, and 9. The staining index score of ≥4 was used to define tumors as having high AEG-1 expression and ≤3 as having low expression of AEG-1.[9]

The paraffin-embedded tumor sections were deparaffinized for immunohistochemical detection of cells positively-stained for TNF-α. Endogenous peroxidase activity was blocked using 3% H_2O_2 for 10 min, followed by incubation with nonspecific staining blocking reagent (DakoCytomation) for 10 min. The sections were incubated with the optimal dilutions of anti-TNF-α (15 μg/mL), antibodies overnight at 4°C followed by 1 h with horse radish peroxidase-labeled secondary antibodies. Immune complexes were visualized with using the streptavidin-peroxidase method with 3,3'-diaminobenzidine (DAB) as chromogen. (Vector Laboratories Inc., Burlingame, California, USA).

Immunostaining was performed by the streptavidin-biotin-peroxidase method. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide. No antigen retrieval was used for b-FGF antibody. The sections were incubated at room temperature for 1 h reactive (b-FGF [1–24] [Polyclonal] concentration 0.1 ml [1:100], code: F3393, Sigma Aldrich). After washing in phosphate-buffered saline with Tween-20, the tissues were incubated with a biotin-conjugated secondary antibody and then with a biotin-streptavidin complex for 30 min at room temperature. Reactions were visualized with 3,3-diaminobenzidine tetrahydrochloride. Sections were counterstained with hematoxylin, rinsed, and mounted.

The avidin biotin complex immunoperoxidase method with primer antibody (catenin, beta [17C2] C.Lyo. 1 ml (1:100-200, code: B-CAT, Leica/Novocastra) and Ki-67 (BioGenex: Species mouse, clone Ki-88, isotype IgG1/kappa) were applied to the cases included in the study group [Figure 2]. Normal breast tissue surrounding the tumor was used as a positive control.

Immunoreactivity for b-FGF, beta-catenin, Ki-67, TNF-α was scored using a semi-quantitative scale for intensity of staining: 0 negative, no staining; 1 + weak positive; 2 + moderately positive; 3 + strongly positive [Figures 1-4].

Statistical analysis

Descriptive statistics were computed as count and percent frequency. Spearman rank correlation analyze was used for determination of the relations between prognostic factors and the degree of staining, and the relation of staining results to each other. The level of significance was determined to be 0.05 ($P = 0.05$). Analyses were performed using the statistical PASW SPSS version 18 (IBM, Chicago, USA).

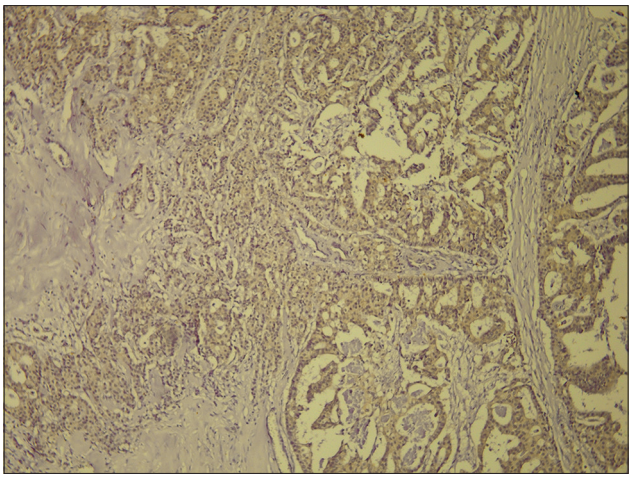

Figure 1: Astrocyte elevated gene-1 expression seen in malign glands (AEG-1 ×100)

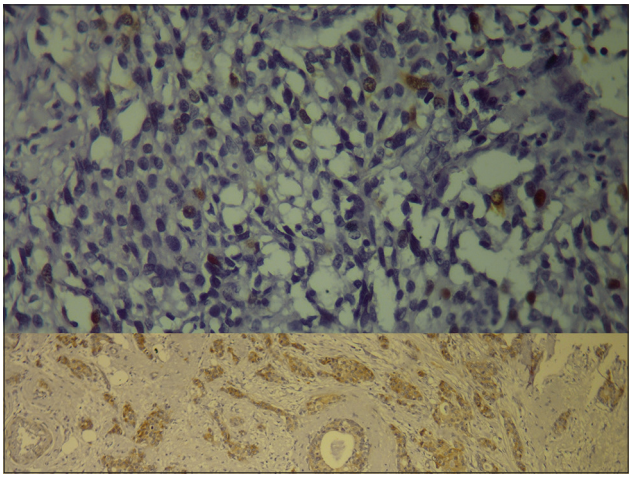

Figure 2: Beta-catenin expression seen in malign glands and intraductal areas (lower ×100) ki-67 staining of malign glands (upper x400)

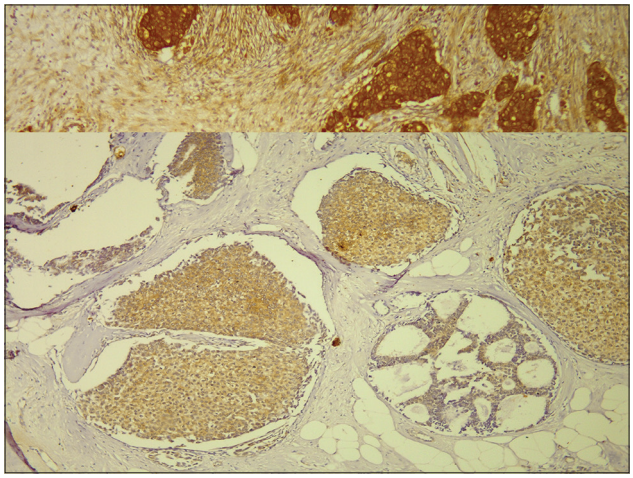

Figure 3: Tumor necrosis factor alpha expression seen in ductal carcinomas and intraductal areas (TNF alpha x100)

Results

We observed significant relationship between the age and LNM or FGF ($r = 0.250$ and $P = 0.018$; $r = 0.224$ and 0.035, respectively). The positive relationship between histological grade and nuclear grade, tumor size, LNM,

Table 1: The relationships among the variables (*n*=90)

	Histological grade (HG)	Nuclear grade (NG)	size	LNMt	ER	PR	cerbb2	TNF	FGF	B-catenin	Ki-67	AEG-1
age												
p	0.744	0.874	0.620	0.018	0.799	0.256	0.392	0.112	0.035	0.296	0.339	0.253
HG												
p		0.000	0.010	0.044	0.000	0.002	0.037	0.484	0.333	0.113	0.005	0.314
NG												
p			0.321	0.448	0.000	0.013	0.093	0.583	0.870	0.114	0.003	0.986
size												
p				0.000	0.098	0.272	0.977	0.004	0.960	0.583	0.366	0.882
LNM												
p					0.918	0.559	0.831	0.425	0.249	0.206	0.551	0.362
ER												
p						0.000	0.005	0.864	0.358	0.256	0.189	0.586
PR												
p							0.025	0.533	0.907	0.478	0.969	0.063
cerbb2												
p								0.362	0.172	0.231	0.675	0.466
TNF												
p									0.170	0.743	0.446	0.043
FGF												
p										0.060	0.000	0.002
B-catenin												
p											0.130	0.318
Ki-67												
p												0.758

HG=Histological grade; NG=Nuclear grade ER=Estrogen receptor; PR=Progesterone receptor; TNF=Tumor necrosis factor; FGF=Fibroblast growth factor; AEG-1=Astrocyte elevated gene-1

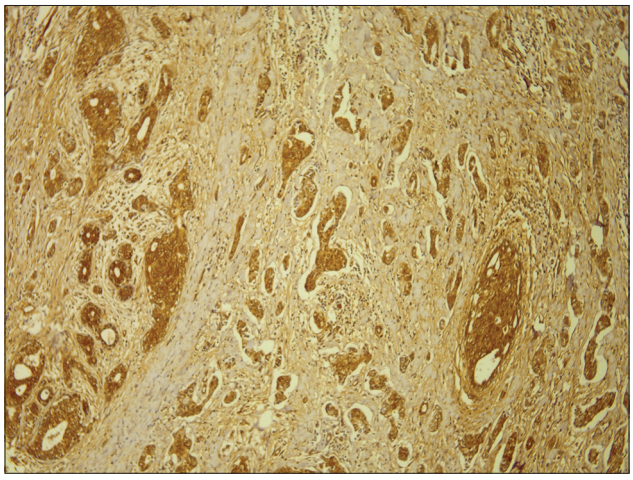

Figure 4: Fibroblast growth factor expression seen in ductal carcinomas (FGF x100)

HER-2 ($P \leq 0.0001$, 0.010, 0.044, <0.0001, 0.002, 0.037, respectively). However, the negative significant correlation were found between histological grade and ER, PR ($P \leq 0.0001$, 0.002). There was a significant positive correlation between TNF-α, size, LNM ($P \leq 0.0001$, 0.002, 0.005). We found that a significant relationship between AEG-1 and TNF-α [Table 1]. There was a significant positive correlation between FGF and Ki-67 and negative correlation AEG-1. No relationship was found beta-catenin and FGF, TNF-α and prognostic parameters [Table 1].

In addition to, FGF and TNF-α staining in DC were observed higher than ductal intraepithelial neoplasms [Figures 3 and 4]. However, this observation could not statistically ($P \geq 0.05$).

Discussion

Recently, anti-cancer research continues to rapidly. In this study, it was found a significant relationship between FGF, TNF-α, AEG-, Ki-67 and tumor prognosis.

Fibroblast growth factor is a potent angiogenic molecule that has been shown to promote tumor cell mitosis. Dow *et al*. found that FGF has been implicated in the differentiation of stromal and epithelial cells from a dormant to an invasive phenotype.[10]

Basic-fibroblast growth factor has been the focus of a plethora of studies into human tumor biology and has important

implications for cancer therapies and clinical outcomes. Presta *et al*. showed that interact with various vascular endothelial growth factors and cell surface receptors that are known to play a role in tumor growth and angiogenesis.[11]

The extent of metastatic disease and the correlation between angiogenesis has been widely demonstrated in a large and diverse range of human cancers.[12,13]

Abnormally high concentrations of b-FGF have been shown to correlate significantly with the extent of disease, clinical status and risk of future mortality. They have been found in the serum of patients with active metastatic cancers.[14]

In this study, FGF was found a significant relationship between the age and LNM, also, FGF ekspression was higher metastatic tumors than nonmetastatic tumors.

Tumor necrosis factor-α is highly expressed in tumors where it has been considered initially as a potent tumor cell killer and an anti-vascular cytokine at high doses.[15]

However, low chronic doses of the cytokine are thought to be pro-angiogenic.[16] In order to test the involvement of TNF-α activation/endothelial dysfunction of the breast tumor vasculature. Perrot-Applanat *et al*. further explored if the correlations identified in their *ex vivo* cellular model (endothelial cells) between the expression of TNF-α mRNA and the expression of putative TNF-α-inducible nuclear factor-κB (NF-κB) target genes were also observed *ex vivo* in human breast tumors.[17]

Two additional members of the TNF/TNF receptor superfamily involved in the immune system.[18] Such induction occurs through TNFR1 and TNFR2 and via NF-κB activation.

Tumor necrosis factor-α and its related NF-κB transcription factor are described as critical components of tumor progression.[19] NF-κB is deregulated in breast cancer patients and in breast cancer cells.[20]

Several studies have shown that tumor associated neovascularization is a precondition of rapid growth and metastasis.[21]

Different markers and many essential interactions have to be yet determined though great strides have been made in the elucidation of molecular mechanisms of tumor vasculature.[19-22]

In this study, TNF-α was found a significant relationship between the poor prognostic parameters (such as size and LNM). It was correlation between angiogenesis, also, TNF-α ekspression was metastatic tumors higher than nonmetastatic tumors, similarly FGF.

This study, beta-catenin staining in ductal intraepithelial neoplasms (DIN) was observed higher than DC [Figure 3], but this observation could not statistically ($P \geq 0.05$). For this reason, it could be small of DIN areas in slides.

A possible interaction partner of AEG-1 was found by Emdad *et al*. They reported that AEG-1 promotes anchorage-independent growth and invasion through IκBα degradation, NF-κB binding, and nuclear translocation.[22] Furthermore, several studies demonstrated a functional role of AEG-1 in several aspects of cancer development including angiogenesis, invasion, metastasis, apoptosis and chemo-resistance.[23-25]

In this study, was found a significant relationship between AEG-1 and TNF-α. This correlation could be due to NF-κB signaling pathway.

Cell-cell and cell-matrix interactions play a role in every stage of metastasis. Adhesion molecules enable these interactions.[26]

Interaction with catenins is essential for adhesive functions of E-cadherin, which is a conventional cadherin type involved in Ca+2 mediated cell adhesion in epithelial cells and this interaction largely depends on beta-catenin expression and function. The cadherin family should occur changes in cell-cell adhesions mediated for a cancer cell to break free from the primary tumor.[27] Many studies regarding the expression of adhesion molecules in breast carcinomas have been conducted to date.[28]

Beta-catenin expression was demonstrated to be decreased or completely lost in cultured human breast cancer cell lines in studies.[28-30]

Jonsson *et al*. have revealed that beta-catenin signals also play a role in human breast cancers by showing increased levels of cytosolic beta-catenin in 13% of primary breast tumors.[30]

In this study, no relationship was found beta-catenin and FGF, TNF-α and prognostic parameters.

Conclusions

The relationship between TNF and FGF, AEG-1 protocols may be useful new treatment.

Difference stain between DIN and DC in terms of beta-catenin FGF, TNF-α, AEG-1 expressions in our study shows that any change in beta-catenin FGF, TNF-α, AEG-1 expressions develop in early carcinogenesis. In addition to, in this study was found a significant relationship between AEG-1 and TNF-α. However, more comprehensive studies

should be performed in order to demonstrate at what stage the change in beta-catenin FGF, TNF-α, AEG-1 expression occurs.

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