

AEG-1 expression in invasive ductal and lobular breast cancers and its relationship with prognostic parameters

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Received: 12.03.2012 • Accepted: 05.07.2012 • Published Online: 18.01.2013 • Printed: 18.02.2013

Aim: The astrocyte-elevated gene-1 (AEG-1, also known as metadherin) is associated with various aspects of tumour malignancy; however, little knowledge is available related to the role of AEG-1 in ductal and lobular carcinomas. The aim of this study was to investigate the relationship of AEG-1 with the prognostic parameters in invasive ductal and lobular carcinomas.

Materials and methods: This study was conducted on a total of 72 paraffin-embedded breast tumour samples. They consisted of 61 ductal and 11 lobular carcinomas. Breast tumour samples were stained for AEG-1. The prognostic parameters were compared with the results of AEG-1 stains.

Results: We observed that more ductal carcinoma types than lobular carcinoma types resulted in high AEG-1 staining, whereas low AEG-1 staining occurred more in lobular carcinomas than in ductal carcinomas ($P = 0.05$). A significant negative correlation was also found between HER-2 ($r = -0.30$, $P = 0.019$) and AEG-1 ($r = -0.804$, $P = 0.003$) in lobular carcinomas. A significant relationship was found between increasing numbers of positive lymph nodes and AEG-1 in ductal carcinoma cases ($P = 0.05$).

Conclusion: These results are consistent with previous reports of the role of AEG-1 in tumour progression. AEG-1 could be a useful marker for the development of new treatments and resistant hormonal therapy.

Key words: Ductal carcinomas, lobular carcinomas, AEG-1, prognostic parameters

1. Introduction

Breast cancer is the most commonly diagnosed type of cancer, and the leading cause of death from cancer, among women in the United States (1). There were 192,370 new cases of breast cancer and 40,170 people died from this disease in 2009 (1). Moreover, in more than 40% of the women diagnosed with breast cancer, it had developed to metastasis (2). Breast cancer survival is linked to early detection, genetic predisposition, and timely appropriate treatment.

More recently, human epidermal growth factor receptor 2 (HER-2/neu), oestrogen (ER), and progesterone (PR) receptors have, with increasing importance, influenced the management of the malignancy. Prognosis is related to a variety of clinical, pathological, and molecular features, which include classic prognostic factors, histological type, grade, tumour size, and lymph node metastases (3).

With an established positive correlation of ER and PR with the degree of tumour differentiation, the determination of ER and PR status on biopsy specimens prior to therapeutic intervention is advocated as standard practice (4).

HER-2 status and hormone receptors are the most important predictive markers in breast cancer. Their assessment is generally performed on resected primary tumours in order to select patients eligible for hormone and HER-2 directed therapies (5–10).

Breast cancer is a heterogeneous disease with a diverse biology and natural history, for which there are a variety of treatment options. Therefore, it is important for the prevention of breast cancer to find reliable biomarkers that may be used to individualise patient prognosis.

The astrocyte-elevated gene-1 (AEG-1), also known as metadherin (MTDH), was initially identified as a human

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immunodeficiency virus (HIV-1) and tumour necrosis factor (TNF), an inducible gene in primary human foetal astrocytes (11,12). Partly due to the phenomenon by which chromosome 8q22 gains functions independently in poor prognosis in breast cancer, the human AEG-1 gene is located at 8q22 (11,13). Its genomic amplification has also been found in diverse cancers, including breast cancer, in comparison with their normal counterparts (14,15).

Recent studies have demonstrated that AEG-1 increases the invasiveness of malignant cells, favouring tumour genesis, metastasis, and neovascularisation, and that up-regulation in epithelial cells inhibits apoptosis (16). In addition, AEG-1 is frequently overexpressed in highly proliferative breast cancer and high-grade lesions (17,18).

In this study, we have shown there is a relationship between AEG-1 and prognostic parameters and tumour types.

2. Materials and methods

This study was conducted on a total of 72 paraffin-embedded breast tumour samples, which were histopathologically diagnosed at the Department of Pathology of the Düzce University Hospital between 2005 and 2010. All the diagnoses were made by 2 pathologists, following the pathology of the World Health Organization Classification of Tumours. The samples consisted of 61 ductal and 11 lobular carcinomas. The age distribution of the patients ranged from 31 to 83 years (mean: 53.85 ± 1.573). The tumour size distribution was as follows: less than 2 cm in diameter in 28 patients, from 2 to 5 cm in 36 patients, and more than 5 cm in 8 patients.

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 World Health Organization (WHO) classification.

The tumour grade of invasive carcinoma was classified according to the Scarff–Bloom–Richardson system (19). 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 stains.

2.1. Immunohistochemistry

Among the haematoxylin and eosin (H&E)-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 and scored independently by 2 observers, based on both the proportion of positively stained tumour cells

and the intensity of staining. The proportion of tumour cells was scored as follows: 0 (no positive tumour cells), 1 (<10% positive tumour cells), 2 (10%–50% positive tumour cells), and 3 (>50% positive tumour 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 tumour cells. Using this method of assessment, we evaluated the expression of AEG-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. A staining index score of ≥ 4 was used to define tumours as having high AEG-1 expression and ≤ 3 as having low expression of AEG-1 (16) (Figures 1 and 2).

2.2. Statistical analysis

The likelihood chi-square and Mann–Whitney U tests were used when comparing associations of AEG-1, ER, PR, and HER-2 immunoreactivity with tumour subtype. In addition, Spearman rank correlation analysis, the likelihood chi-square test, or Mann–Whitney U analysis (whichever was deemed appropriate in each case) was used to evaluate the relationship 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 package PASW (version 18).

3. Results

We observed that more ductal carcinoma types than lobular carcinoma types resulted in high AEG-1 staining, whereas low AEG-1 staining occurred more in lobular carcinomas than in ductal carcinomas ($P = 0.05$) (Table 1). No significant relationship was found between the subtype and ER, PR, or HER-2 ($P = 0.343, 0.532, \text{ and } 0.639$, respectively) (Table 2). The relationships among ER, PR, HER-2, and prognostic parameters were evaluated in cases of ductal carcinomas and showed a significant correlation between increasing ER and PR ($r = 0.551, P = 0.0001$). There was a significant negative correlation between ER and the histological grade ($r = -0.30, P = 0.019$). No significant correlation was found between AEG-1 and the intraductal component ($P = 0.398$) (Table 3). The relationships among ER, PR, HER-2, and prognostic parameters were evaluated in cases of lobular carcinomas and showed a significant negative correlation between HER-2 ($r = -0.30, P = 0.019$) and AEG-1 ($r = -0.804, P = 0.003$) using the Spearman rank correlation analysis. There was no significant correlation between AEG-1 and lymphovascular (LVS) invasion in ductal carcinoma cases ($P = 0.354$). However, there was a significant relationship between increasing number of positive lymph nodes (PLNs) and AEG-1 in ductal carcinoma cases ($P = 0.05$) (Table 4). No significant

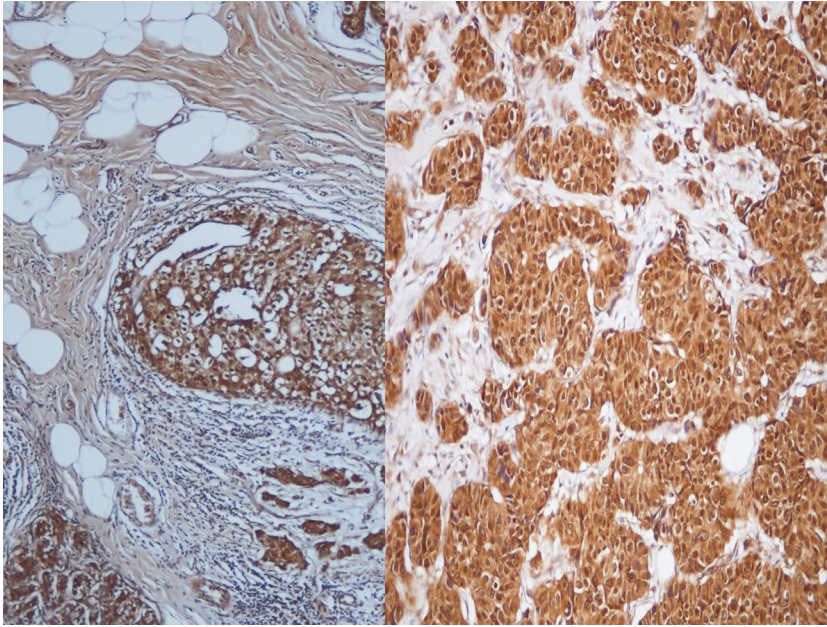


Figure 1. AEG-1 stain in ductal carcinomas, low and high stain (right side), 200×.

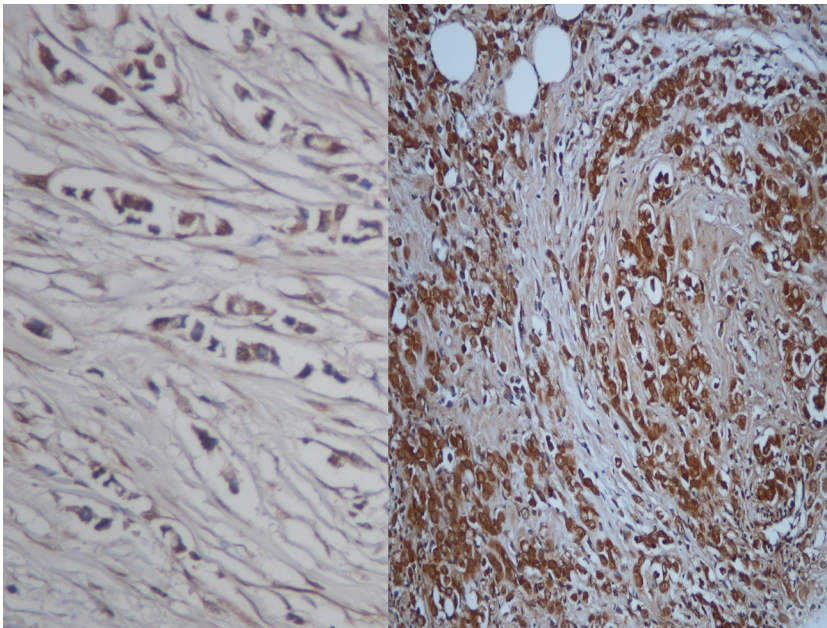


Figure 2. AEG-1 stain in lobular carcinomas, low and high stain (right side), 200×.

Table 1. Distribution of AEG-1 stain and subtypes.

	Low		High		Total
	n	%	n	%	
Ductal	26	2.6	35	7.4	61 (100%)
Lobular	8	72.7	3	27.3	11 (100%)
Total	34	47.2	38	52.8	72 (100%)

Table 2. Distribution of subtype and ER, PR, and HER-2.

	Type	n = 72	Mean (%)	Std. deviation	Std. error mean
ER	Ductal	61	48.11	35.498	4.545
	Lobular	11	35.91	36.319	10.951
PR	Ductal	61	50.18	35.121	4.497
	Lobular	11	41.82	37.435	11.287
HER-2	Ductal	61	1.62	1.128	0.144
	Lobular	11	1.45	1.128	0.340

Table 3. Distribution of AEG-1 and intraductal component.

Intraductal component	Low		High		Total
	n	%	n	%	
Negative	5	55.6	4	44.4	9 (100%)
Positive	21	40.4	31	59.6	52 (100%)
Total	26	42.6	35	57.4	61 (100%)

Table 4. Distribution of PLN and AEG-1.

Ductal carcinomas	Low		High		Total
	n	%	n	%	
Negative	18	52.9	16	47.1	34 (100%)
Positive	8	29.6	19	70.4	27 (100%)
Total	26	42.6	35	57.4	61 (100%)

correlation was found between AEG-1 and LVS invasion ($P = 0.385$) and PLNs ($P = 0.205$) in lobular carcinoma cases. There was no significant correlation between the intraductal component and ER ($P = 0.383$) and HER-2 ($P = 0.088$) in ductal carcinomas, whereas there was a significant negative correlation with PR ($P = 0.035$).

4. Discussion

The development of target therapy has changed the prognosis of patients, both in adjuvant and metastatic settings (18,20). Although new therapies have significantly improved the survival rate of cancer patients, a large number of patients still succumb to this disease, mostly due to metastasis and chemoresistance. In view of this fact, the discovery of novel targets and strategies for the

development of effective anticancer therapies is urgently required.

MTDH/AEG-1 is expressed in low levels or is absent in most normal human breast tissue, but was found to be frequently overexpressed in breast cancer cell lines or breast tumours (12,20–23). In this study, AEG-1 staining found more expression in ductal carcinomas than in lobular carcinomas.

Using breast tumour samples collected in the United States and in China, 2 independent analyses revealed strikingly similar patterns of MTDH/AEG-1 expression and clinical association (24,25). MTDH/AEG-1 was abundantly expressed in 44%–47% of the primary tumours and significantly correlated with the clinical stage, tumour size, lymph node spread, distant metastasis, and poor survival (23–26).

Dağlar et al. found that tumour histological grade was an independent factor in cancer-specific survival and disease-free survival (27).

Tokatlı et al. found that axillary nodal status and HER-2/neu were the most important determining factors for prediction of disease-free survival in breast cancer patients (28).

MTDH/AEG-1 expression was not correlated with other common clinicopathological parameters, including age, oestrogen receptor, progesterone receptor, HER-2, and p53 status. No significant difference in MTDH/AEG-1 expression was observed in basal or luminal subtypes of breast tumours (23). Multivariate analysis suggested that MTDH/AEG-1 expression is an independent prognostic indicator for the survival of patients with breast cancer (29–31).

In this study, AEG-1 staining found a significant negative correlation between HER-2 (in lobular carcinoma cases) and a significant relationship between increasing positive LN (in ductal carcinomas cases). No significant correlation was found between AEG-1 and lymphovascular invasion, age, ER, PR, or size.

Li et al. demonstrated the lack of influence of hormone therapy and chemotherapy on ER and PR status (18), while Idirisinghe et al. showed a correlation between ER and PR loss and hormone therapy (8). Sequential breast cancer biopsies have shown that ER levels are reduced slightly with intervening endocrine therapy, while PR levels decrease more dramatically, with up to half of the tumours completely losing PR expression, when resistance develops (25). Few studies have shown a shorter survival rate among women with ER-negative metastatic and locally recurrent tumours, regardless of the primary tumour ER status (32,33). Whether, and how, the loss of PR affects the clinical course of ER-positive/PR-negative metastatic tumours remains to be clarified (32). However, as with

earlier studies, there seems to be no correlation between receptor status and histological type of the tumours (14,16,32–34).

This study has found significant correlation between AEG-1 staining and subtype (ductal and lobular). There was no significant correlation between AEG-1 and ER or PR, but a significant relationship was observed between increasing positive LN and AEG-1 in ductal carcinoma cases. For this reason, AEG-1 may be considered to be high in cases of decreased ER and PR.

Statistical analysis of the relationship between AEG-1 staining and the clinical characteristics of patients presented a significant correlation of AEG-1 expression with clinical stage, lymph node metastasis, and LVS invasion, further supporting a potential role of AEG-1 in tumour angiogenesis (19). The reason may be that AEG-1 is commonly overexpressed in highly proliferative lesions of breast cancer (12,13). This could be related to other significant independent factors such as tumour size and lymph-node status. This study found a significant relationship between increasing positive LN and AEG-1 in ductal carcinoma cases. In addition, we observed a significant negative correlation between HER-2 and AEG-1 in lobular carcinomas. Whereas high AEG-1 staining occurred more in ductal carcinomas than in lobular carcinomas, low AEG-1 staining was more frequent in lobular carcinomas than in ductal carcinomas.

We feel there is a need for further studies of a larger scope in order to shed more light on the cause of these findings.

In conclusion, AEG-1 staining could be important in demonstrating the role of AEG-1 in histological subtype and tumour progression. In addition, AEG-1 could be a useful marker for the development of new treatments and resistant hormonal therapy.

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