

Original Research Paper

Impact of ADAM17 expression as a potential prognostic biomarker and target for therapy in node-negative Breast Carcinoma

ABSTRACT

Aims: To evaluate the prognostic impact of the tissue-based protein expression of ADAM17 in node-negative breast cancer and investigate its association with clinical and pathological features to aid in differentiating indolent node-negative breast cancer from aggressive node-negative breast

Study design: Cross sectional.

Place and Duration of Study: Department of Pathology and Molecular Immunology, Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, between December 2012 to June 2013.

Methodology: 50 cases of breast cancer patients (40 node-negative and 10 node-positive) with ages ranging from 24 - 89 and with tumor size ranging from 0.6 cm to 4.7 cm were used. The ADAM17 protein expression was confirmed by immunohistochemistry analysis, which was performed on formalin-fixed, paraffin-embedded, 3 micro meters thick tissue sections using the avidin-biotin-peroxidase complex method. In addition, the presence of HER2, Ki67, Progesterone and Estrogen receptors were also determined

Results: Both node-negative and node-positive breast cancer showed ADAM17 expression with more expression in node-negative cases. The anti-ADAM17 antibodies showed immunostaining with 19 strong expression (38% - 19/50) and 31 weak expression (62% - 31/50). In addition to ADAM17, other markers included such as ER, PR, HER2 and Ki67 were also expressed. In analyzing the relationship between ADAM17 expression and clinicopathological parameters, the expression level was independent of all the conventional prognostic factors with P value >0.05 except histological type with P value of .009 and .01.

Conclusion: Our study provides further evidence that ADAM17 protein is involved in human breast cancer initiation, progression and distant metastasis and its prognostic impact could be independent of conventional prognostic factors. In grouping high expression against low, we had 19 high risk and 31 low risk. Upon further confirmation, ADAM17 could be used to determine individuals who may need adjuvant chemotherapy and those who could be spared from the toxic effect of chemotherapy.

Keywords: ADAM17, node-negative, node-positive, Carcinoma, adjuvant chemotherapy

1. INTRODUCTION

Breast cancer is the most frequently diagnosed and the second most common cause of cancer related mortality in women worldwide. The disease accounts for 23% (1.38 million) of the total new cancer cases and 14% (458,400) of the total cancer deaths in 2008 [1]. The

22 most common form of breast cancer is node-negative and in places where there is
23 widespread of screening and awareness among women, its rate is more likely to fall within
24 65%–70%. Patients therefore have a good prognosis since the disease is at its earliest
25 stage. [2].

26 Currently, giving adjuvant chemotherapy to patients in addition to loco-regional therapy
27 requires proper patient selection. Unlike node-positive which is associated with an overall
28 mortality of 20%, clinicians/oncologist hesitate before indicating chemotherapy in node-
29 negative patients due to the uncertainty in determining whether patients with node-negative
30 disease actually benefit from chemotherapy [2]. Prognostic and predictive factors that
31 influence adjuvant chemotherapy in patients with early stage breast cancer include:
32 histological type, nodal status, tumor size, tumor stage and tumor grade. Other factors
33 include hormone receptor status, Ki-67 status, HER2 amplification and patient age.
34 However, by using these traditional histomorphologic and clinical factors alone, about 60-
35 90% of node-negative patients may be candidate for adjuvant chemotherapy [3, 4]. Although
36 patients can be cured by surgery alone, about 30% of them will relapse and thus need
37 adjuvant chemotherapy [5]. It is therefore likely that detection of early additional markers is
38 paramount to predict individual risk that may or may not benefit from adjuvant
39 chemotherapy. This will avoid unnecessary exposure of women to the potential toxicity and
40 side-effects of such treatment, and also to reduce the overall cost of breast cancer treatment
41 and management.

42 Tumor invasion and metastasis involving the interaction between tumor cells and
43 extracellular matrix are carried out by certain proteinases including ADAMs. The ADAMs (A
44 Disintegrin And Metalloproteases) family, are modular type I transmembrane proteins which
45 belongs to the zinc protease superfamily. Although forty gene members have been identified
46 so far, it is believed that about 21 is functional in humans [6]. They have been reported to
47 support both proteolytic activity and cell adhesion, making them candidates to mediate both
48 the remodeling of the extracellular matrix (ECM) and the changes in cell adhesion that
49 characterize certain pathological processes such as tumor development [7]. Furthermore, a
50 number of selective ADAM inhibitors, especially against ADAM10 and ADAM17, have been
51 shown to have anti-cancer effects which led to the proposal of these metalloproteases as
52 putative targets of anti-tumor therapy.

53 One of the first ADAMs shown to have diagnostic potential was ADAM12 in breast cancer.
54 ADAM- 12, an apoptosis-modulating gene accelerated the development of tumor by delaying
55 tumor cell apoptosis [8, 9]. There has been an enhanced levels in the urine of breast cancer
56 patients indicating a potentially important non-invasive biomarker in breast cancer [10].

57 ADAM17 is also named as tumor necrosis factor-alpha-converting enzyme, (TACE). In
58 addition to the signal peptide, it contains four functionally distinct extracellular domains
59 followed by a transmembrane region and a cytoplasmic tail [11,12]. It is the most extensively
60 studied ADAMs to have a role in malignancy due to its persistent cleavage abilities with
61 respect to the large variety of substrates it is able to cut. Certain growth factors and
62 receptors can be activated and inactivated respectively by ADAM17 by shedding their
63 extracellular domain from the cell membrane. Besides, it can detach cells by cleaving cell
64 adhesion molecules. As a primary sheddase for multiple EGFR pro-ligands, it can transform
65 growth factor-alpha (TGF-a), heparin-binding epidermal growth factor (HB-EGF), and
66 amphiregulin and all these ligands have been implicated in cancer development and
67 progression [13]. Through EGFR/PI3K/ AKT pathway, ADAM17 has been reported to be
68 overexpressed in tumors such as the breast cancer, ovarian cancer, colon cancer etc. [14].
69 In a study conducted by McGowan et al, the overexpression of ADAM- 17 in breast cancer

70 cells increased invasion and proliferation [15]. In another study, it was shown that the human
71 breast cancer cell lines, MCF-7 and MDA-MB453, strongly express ADAM17 [16].

72 However, there are few reports of ADAM17 and its association especially in node-negative
73 breast cancer. Hence the aims of this study were to evaluate the prognostic impact of the
74 tissue-based protein expression of ADAM17 in node-negative breast cancer and investigate
75 its association with clinical and pathological features to aid in differentiating indolent node-
76 negative breast cancer from aggressive node-negative breast cancer .This will help to
77 differentiate those patients who may benefit from adjuvant chemotherapy and those who
78 may be spared from the toxic effect due to their low risk of the disease recurrence.

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81 **2. MATERIAL AND METHODS**

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84 **2.1 Patients and tissue sample**

85 All samples were obtained with institutional board approval from St. Anthony Hospital, Porto,
86 Portugal. 50 Cases of cancer patients who have undergone modified radical/partial
87 mastectomy with no prior treatment, with ages ranging from 24 - 89 were obtained from
88 Saint Anthony General Hospital. Patients involved were pathologically confirmed of the
89 following node-negative and node-positive breast cancer: invasive ductal carcinoma, in situ
90 ductal carcinoma and invasive lobular carcinoma with histological grades 1 to 3. There were
91 40 node-negative cases and 10 node-positive cases with tumor size ranging from 0.6 cm to
92 4.7 cm. In addition to ADAM17, HER2, Ki67, Progesterone and Estrogen receptors
93 expression were also determined. The ADAM17 protein expression in breast cancer tissue
94 samples were confirmed by immunohistochemistry analysis, which was performed on
95 formalin-fixed, paraffin-embedded, 3 micro meters thick tissue sections using the avidin-
96 biotin-peroxidase complex method.

97 **2. 2 Immunohistochemistry analysis**

98 The procedure for immunohistochemical analysis was the Avidin-Biotin-Peroxidase Complex
99 method. However, in our work we used water bath to increase the antigenicity by using 50ml
100 of 10% Dako target retrieval solution. Also we used a dilution factor of 1:150 for ER, PR and
101 HER2, 1:100 for Ki67 and 1:300 for ADAM17.

102 Following a Hematoxylin counterstaining, HER-2/neu was considered negative when scores
103 of 0 and +1, and positive when scores of +2 and +3 were recorded. To be considered as +2,
104 +3, the cellular membrane should be completely stained in more than 10% of the tumor
105 cells. In the cases where a score 2 was found by IHC, it was further studied by FISH and
106 classified as positive or negative according FISH results. Cells without staining, or with weak
107 staining in part of the cell membrane and in less than 10% of the tumor cells were
108 considered negative. In the case of ER and PR they were graded by considering 10% of
109 tumor cells stained in the nucleus as negative and more than 10% of stained tumor cells as
110 positive. With Ki67, scores were obtained by counting at least 100 cells (excluding mitotic
111 cells) and taken the percentage of stained cells in the nucleus. The cells were counted at
112 places where there was high proliferation of cells.

113 ADAM17 levels of expression were determined by using staining intensity. The staining
114 intensity was evaluated using three-tier grading system (0- negative; 1-weak; and 2-strong).

To delineate between low and high levels of ADAM17 expression, the tumors with strong ADAM17 expression were grouped against those with none to weak expression.

2. 3 Statistical Analysis of Results

Statistical significance was calculated using Fisher's exact test to assess the relationships between ADAM17 protein expression and different clinical parameters through IBM SPSS Statists 20.0 software. This is due to the smaller sample size and the fact that some of the cells had values less than five. Significance was accepted at $p < 0.05$.

3. RESULTS

3.1 ADAM17 expression in node-negative and positive breast cancer

The expression was predominantly expressed in the cytoplasm and less common at the cell membrane. Both node-negative and node-positive breast cancer showed ADAM17 expression with more expression in node-negative cases.(Table 1). The anti-ADAM17 antibodies showed immunostaining with 19 strong expression, 38% -19 out of 50, (Fig. 3) and 31 weak expression 62% -31 out of 50, (Fig. 4) leading to 19 high risk and 31 low risk. There was also expression in cancerous cases compared to their adjacent non-cancerous tissues (Fig. 2).

In addition to ADAM17, other markers expressed were ER, PR, HER2 and Ki67. From the results obtained, we realised that some cases with high Ki67 expression and positive ER, PR and HER2 expression corresponded to weak ADAM17 expression and vice versa.(Table 1). There was also 6 triple negative cases from our studies but two of these corresponded to high ADAM17 expression and four corresponded to low ADAM17 expression. We were also able to differentiate among grade 2 patients into low and high risk based on the level of ADAM17 expressed (Table 1).

3.2 Association of ADAM17 protein expression with standard prognostic factors

The association between ADAM17 expression and standard prognostic factors of breast cancer was further analyzed. The results obtained showed ADAM17 was independent of all the conventional prognostic factors with P value $> .05$ except histological type with P value of .009 and .01 (Table 1).

Table 1. ADAM17 protein expression and standard prognostic factors

Age (years)	No. of cases	ADAM17 expression (n, %)	
		Low expression	High expression
≤ 50	22	14(63.63)	8 (36.37)
> 50	28	17(60.71)	11 (39.29)
Tumor size (cm)			
≤ 2	30	19 (63.33)	11 (36.67)
> 2	20	12 (60.00)	8 (40.00)
Nodal status			

Negative	40	24 (60.00)	16 (40.00)
Positive	10	7 (70.00)	3 (30.00)
Histological Grade			
Grade 1	9	6(66.67)	3 (33.33)
Grade 2	23	17 (73.91)	6 (26.09)
Grade 3	18	8 (44.44)	10 (55.56)
ER expression			
Negative	6	3 (50.00)	3 (50.00)
Positive	44	28 (63.64)	16 (36.36)
PR expression			
Negative	9	6 (66.67)	3 (33.33)
Positive	41	25 (60.98)	16 (39.02)
HER2 expression			
Negative (0)	45	28 (62.22)	17 (37.78)
Negative (+1)	3	2 (66.67)	1 (33.33)
Positive (+2)	2	1 (50.00)	1 (50.00)
Histological Type			
IDC	32	14 (43.75)	18 (56.25)
DCIS	7	7(100.00)	0 (0.00)
ILC	11	10 (90.91)	1 (09.09)
Ki67 expression (%)			
≤ 10	13	6 (53.85)	6 (46.15)
> 10	37	24 (64.87)	13 (35.13)

IDC*DCIS $P = 0.009$ IDC*ILC $P = 0.011$

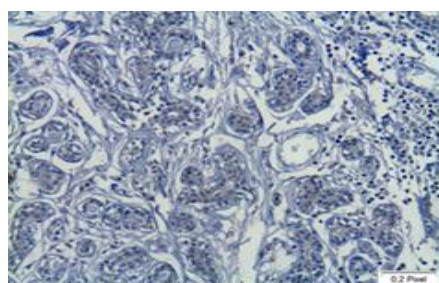


Fig. 1. Immunohistochemical staining of negative ADAM17 expression in node-negative breast tissue. (IHC-Mayer's Hematoxylin, 100x), where IHC stands for immunohistochemistry and Mayer's Hematoxylin is the counterstain.

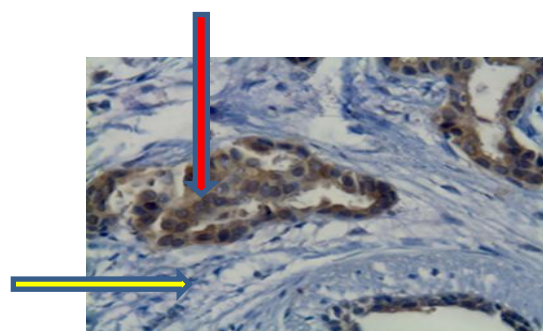


Fig. 2. Positive immunohistochemical staining of ADAM17 expression of node negative breast cancer (red arrow) and its corresponding negative expression (yellow arrow) in non-cancerous breast tissue in node-negative invasive ductal carcinoma. (IHC-Mayer's Hematoxylin, 100x), where IHC stands for immunohistochemistry and Mayer's Hematoxylin is the counter stain

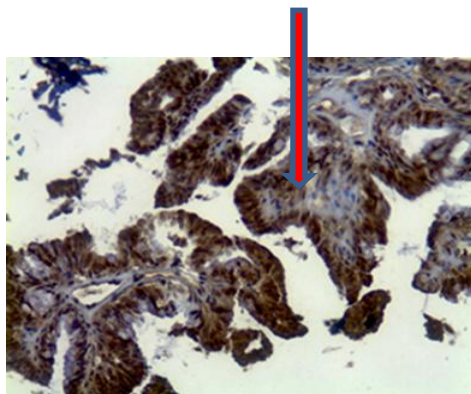


Fig. 3. Immunohistochemical staining of strong ADAM17 expression (red arrow) in node negative invasive ductal carcinoma. (IHC-Mayer's Hematoxylin, 100x), where IHC stands for immunohistochemistry and Mayer's Hematoxylin is the counterstain

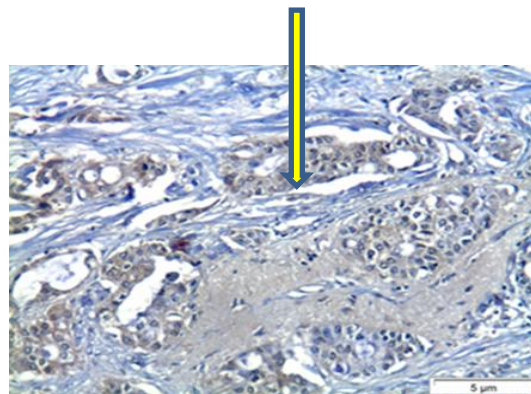


Fig. 4. Immunohistochemical staining of weak ADAM17 expression (yellow arrow) in node positive invasive ductal carcinoma. (IHC-Mayer's Hematoxylin, 100x), where IHC stands for immunohistochemistry and Mayer's Hematoxylin is the counterstain

4. DISCUSSION

In this study, our main focus was on the expression of ADAM17 in node-negative breast cancer and its ability to identify individuals who may be classified as high or low risk. We investigated its impact on other clinicopathological features of breast cancer and in that essence we engaged other prognostic markers such ER, PR, HER2 and Ki67 expression. ADM17 is the most extensively studied ADAMs known to have role in malignancy and it carries out its role by shedding a variety of important cell surface molecules, which includes cytokines, growth factors, and adhesion molecules [13]. For example, It has been shown that ADAM17-mediated EGFR ligand cleavage enhances the proliferation and survival of squamous cell carcinoma cells as well as lung cancer cells [17]. ADAM-17 was also overexpressed in pancreatic ductal adenocarcinoma (PDAC) and pancreatic cancer cell lines. [18]. Brain tumor cell lines cultured under hypoxic conditions demonstrated an upregulation of ADAM-17 expression levels, and its activity correlated with increased tumor cell invasion [19].

A number of selective ADAM17 inhibitors have been reported, suggesting their role in targeted therapy in cancer [20, 21]. Examples are INCB3619, INCB7839, WAY-022 and GW280264X. In a study by Witters et al, combining INCB7839 with lapatinib completely prevented growth of human breast cancer xenografts in mice [22]. WAY-022, which is a selective inhibitor of ADAM-17, was found to decrease DNA replication and cell growth in colorectal cancer cells [23]. In preclinical models, INCB3619 synergized with paclitaxel inhibited the growth of breast cancer in a xenograft model [24].

In our study, there was expression of ADAM17 protein in both node-negative and positive breast cancer, (Fig. 2-4) which was verified by immunohistochemistry analysis. There was high and low expression of ADAM17 in both cases. However in general, we obtained higher number of low expression as compared to high ADAM17 expression. This could be due to the fact that our study involves higher number of node-negative as compared to node-positive cases. Most works that showed higher frequency of ADAM17 expression was carried out in advance or node-positive cases in contrary to our study with a focus on node-

negative breast cancer. We found significantly higher concentration of ADAM17 expression in grade 3 which confirms its expression in advanced cases. The high expression we observed are also in accordance with other published reports, [25, 16].

In addition, some cancerous cases showed the presence of ADAM17 protein expression but was absent in their adjacent non-cancerous tissues. (Fig 2). This supports previous works on ADAM17. For instance in a study performed by Lendeckel U et al., [16] on 24 breast cancer specimen and corresponding non-cancerous tissue, mRNA expression of ADAMs 9, 12 and 17 were increased in cancerous tissue compared to normal adjacent tissues. Again, it was reported that, in a study on ADAM expression in breast cancer tissue, there was an elevated expression ADAM 17 [26] in breast carcinomas compared with non-neoplastic or adjacent healthy tissue. The evidences of low expression in some of the node-negative cases through to high expression in some of the node-positive cases, and its absence in non-cancerous cases, may indicate that ADAM17 expression may be involved in processes leading to the initiation of tumor, its progression and distribution of tumor cells to lymph nodes and lead to distant metastasis in breast cancer.

We again observed weak ADAM17 expression in some high Ki67 expression, positive ER, PR and HER2 expression and vice versa. Following current guidelines, patients in this category may be recommended for adjuvant chemotherapy but from our current studies using ADAM17, they may be spared from adjuvant chemotherapy if confirmed by further studies. Ductal and lobular are the two common breast cancer type, with ductal being the most common. From our results, there was more expression in ductal than lobular, confirming a work done by McGowan PM et al which suggest a different role played by ADAM17 in ductal and lobular carcinoma formation [25].

In analyzing the relationship between ADAM17 expression and clinicopathological parameters of breast cancer, our results obtained in agreement with a study published by McGowan et al [25] showed that expression level was independent of all the conventional prognostic factors, except histological type. This suggests ADAM17 may be an independent prognostic factor.

In determining the high and low risk category, we grouped strong ADAM17 expression against none to weak expression, since we were not able to carry out a quantitative analysis that will enable us set up a cut off point by which we can confidently group cases into high and low risk patients.

By doing so, we obtained 19 high risk and 31 low risk. Another limitation of our study was the small sample size. Due to that we were not able to carry out a multivariate analysis. Nevertheless, this is one of the few works already established to assess the prognostic impact of ADAM17 expression, specifically in node-negative breast cancer. Our results further confirm the importance of this kind of studies and the need for more of such studies to help in treatment selection especially among node-negative breast cancer patients.

5. CONCLUSION

In conclusion, our study provides further evidence that ADAM17 protein is involved in human breast cancer initiation, progression and distant metastasis and its prognostic impact could be independent of conventional prognostic factors. Though we were not able to prove ADAM17 prognostic impact on individual survivorship and patient outcome, relating our data with other studies where its levels of expression were significantly associated with uPA, and its high level significantly associated with poor outcome in patients with breast cancer, [15, 25] it could be confirmed that, ADAM17 may be a risk assessment biomarker, capable of determining individuals who may be at high risk and may benefit from adjuvant

chemotherapy and those who may be spared from the toxic effect of adjuvant chemotherapy due to their low risk of recurrence. Lastly, if our work is confirmed by carrying out further studies, ADAM-17 inhibitors or blocking ADAM17 could be a target for breast cancer treatment and management.

COMPETING INTERESTS

No competing interest

CONSENT

Not applicable

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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