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Tumor expression of Nectin-4 as a prognostic marker in breast cancer

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ABSTRACT

Background: Identification of new molecular tumor associated biomarkers is the most important current challenge in cancer research. Nectin-4 is one of the Nectin glycoproteins, which are cell adhesion molecules have been involved in tumor biology.

Objectives: The objective was to evaluate Nectin-4 expression by immunohistochemistry (IHC) as a prognostic tumor marker in breast cancer (BC).

Patients and Methods: This study was carried out on 100 female patients with BC. Their ages ranged between 29 and 67 years, with a mean of 41.3 years. Fifty other age-matched patients, subjected to reduction mammoplasty, served as controls. Data collected prospectively included patient demographics and tumor characteristics, including histopathological type and grade, IHC for Nectin-4 expression, estrogen receptors (ER), progesterone receptors (PR), and human epidermal growth factor receptor 2 (HER2-Neu). Patients were regularly followed-up for 2 years, recording loco-regional recurrence, distant metastasis, and mortality.

Results: Nectin-4 expression by IHC was detected in 62% of BC patients, but in none of the tumor-free controls (P = 0.0001). Nectin-4 expression showed a statistically significant positive correlation with higher tumor grade (P = 0.003) and axillary lymph node involvement (P = 0.0001), but not with increasing tumor size (P = 0.273). It had a significant inverse correlation with ER and PR, and a significant positive correlation with HER2-Neu (P = 0.0001). Furthermore, there was a significant correlation between Nectin-4 expression and the development of distant metastases (P = 0.014), local recurrence (P = 0.046), and mortality (P = 0.049).

Conclusions: Nectin-4 is a highly recommended biomarker for predicting progression and prognosis of BC.

Key words: *Breast cancer, prognosis, tumor markers, Nectin-4, immunohistochemistry*

Introduction

Breast cancer (BC) is a morphologically, clinically, and genetically heterogeneous disease. The current treatment modalities are efficient in only 70% of cases. Predicting the right treatment response according to the clinical and pathology features and their likely evolution is a difficult task [1]. Current practice in oncology emphasizes the importance of screening, early diagnosis, and proper prognostication of cancer in asymptomatic subjects. Recently, important clinical decisions have become increasingly dependent on the results of tumor markers [2-4].

There is a broad spectrum of tumor markers that include surface and oncofetal antigens, cytoplasmic pro-

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teins, enzymes, hormones, receptors, and oncogenes [5]. Any genetic alteration in a tumor cell may affect its gene expression pattern or that of the surrounding tissue, forming the molecular basis of tumor markers [6,7], which can be detected in the tissues (tissue tumor markers) or in body fluids (serological tumor markers) [8,9]. Clinical application of tumor markers includes screening, confirmation of diagnosis, prediction of therapeutic response and prognosis, as well as monitoring disease progression and detection of recurrence [10,11].

Many trials for upgrading treatment methods in BC have been tried using molecular profiling. These include status testing of estrogen receptors (ER), progesterone receptors (PR), and Human epidermal growth factor receptor 2 (HER2-Neu) receptors, as well as Gene profile testing using microarray assay or reverse transcription-polymerase chain reaction. Recently, Nectins, which are cell adhesion molecules homologous to PVR/CD155, have been discovered. The Nectin family comprises five transmembrane glycoproteins called Nectin-1 CD111, Nectin-2 CD112, Nectin-3 PVRL3, Nectin-4, and Nectin-5 [12,13].

The Nectin family of glycoproteins is part of the immunoglobulin superfamily, which includes members that are structurally related and exhibit three conserved immunoglobulin-like domain (V, C, and C types) in their extracellular regions. Nectins are both homophilic and heterophilic cell adhesion molecules that help organizing the epithelial and endothelial junctions, and function as receptors for herpes simplex virus entry. They are components of E-cadherin-based adheren junctions in epithelial cells. Nectins and E-cadherin are both connected to F-actin through AF-6/Afadin and catenins, respectively. The Nectin/Afadin systems, which are known to be involved in tumor biology, interact with the E-cadherin/catenins systems through Afadin and α-catenin [14,15].

Detection of Nectin-4 has been restricted to the placenta in human tissues as it belongs to the class of carcino-embryonic antigens. Recently, Nectin-4 has been reported to be present in BC cell lines, and claimed to be highly associated with metastatic breast cancer (MBC) and poor prognosis. Furthermore, the soluble form of Nectin-4, shed from the surface of the tumor cell in breast tumor cell lines by proteolytic cleavage of the metalloproteinase ADAM17/TACE, is suggested to represent a sensitive, reliable, and complementary serum marker for the diagnosis and follow-up of patients with MBC [16,17].

Patients and Methods

Study Population

The present study was carried out on 100 female consecutive patients with breast carcinoma (study group) and 50 age-matched female controls subjected to reduction mammoplasty, admitted to Alexandria Main University Hospital, Egypt between January 1, 2009 and December 31, 2011. Their ages ranged between 28 and 67 years, with a mean of 40.5 ± 9.1 years. The protocol of the study was approved by the Ethical Committee of the hospital. An informed written consent was obtained from all patients before enrollment in the study. Patients who refused to participate in the study, were unfit for general anesthesia, or were lost during follow-up were all excluded from the study.

Pre-operative Assessment

All patients were subjected to a detailed history-taking and complete physical examination including both breasts and axillae. Laboratory investigations for all patients included complete blood picture, coagulation profile, liver function tests, and the determination of the levels of blood glucose, serum urea and creatinine. Plain chest X-ray, breast mammo-sonography, and pelvi-abdominal ultrasound were also performed for all patients. Computed tomography, bone scan, magnetic resonance imaging, and positron emission tomography were performed in selected cases as indicated. Pre-operative histological diagnosis of BC was achieved using fine-needle aspiration cytology or Trucut needle biopsy.

Operative Treatment

Patients underwent either simple mastectomy (SM) or modified radical mastectomy (MRM) or conservative breast surgery (CBS), with conventional axillary clearance or sentinel lymph node (LN) biopsy according to the hospital management protocol.

Post-operative Evaluation and Follow-up

All surgical specimens were subjected to histopathological examination with histoprognostic grading using the Scarff, Bloom, and Richardson (SBR) grading

system [18]. An immunohistochemistry (IHC) study was performed on the prepared slides for estimation of Nectin-4 (monoclonal anti human clone 337506, R&D systems), ER (DAKO clone 1D5), PR (DAKO clone Pg R636), HER2-Neu (Human ErbB2 Herstatin Isoform MAb clone 416711, R&D systems). Patients were followed-up at regular 3-month intervals for a total of 24 months using clinical and imaging assessment as indicated, for detection of local recurrence and/or distant metastasis, and for recording mortality.

Statistical Analysis

The SPSS/PC version 21 computer software (Prentice-Hall; Chicago, IL, USA) was used for statistical analysis. The Student's t-test was used to compare the mean values. The Chi-square ($\chi 2$) test with Yate's correction was used for comparison between categorical (qualitative) values, and the Fisher's exact test was used to compare recurrences. The 5% level was set as the level of significance.

Results

The mean age of BC patients was 41.3 ± 12.8 years (range, 29-67 years) and that of the control group 38.3 ± 7.4 years (range, 28-59 years). There was no statistically significant difference between both groups regarding age, menopausal status, or body mass index. All patients in the study group presented with a breast lump, except for three in whom the tumors were detected by annual mammography for high-risk patients. The mean duration of symptoms was 5.1 ± 4.6 months (range 1-9 months). Approximately half of the tumors (52%) were located in the upper outer quadrant of the breast, and the mean size of the lump was 4.2 ± 2.9 cm (range 1-8 cm). As seen in Table 1, about two-thirds of the tumors (68%) were T2, 48% were N0 and 8% were M1. Distant metastases were detected at presentation in the lung (n = 3), liver (n = 3), lungs and bone (n = 2). Forty-three patients (43%) underwent MRM, 11 patients (11%) underwent SM together with sentinel LN biopsy (SLNB), and the remaining 46% underwent CBS, which was accompanied with SLNB in 31 patients.

As seen in Table 1, histopathological examination showed that 73% of the studied tumors were of the ductal type and 27% were lobular carcinomas. More than half of the tumors (57%) were SBR Grade II and 52% had positive axillary LNs. Regarding the receptor status, 57% of tumors were ER positive, 54% were PR positive, and 39% were HER2-Neu positive. During the 24-month follow-up, distant metastases developed in nine patients and local recurrence in four. Mortality was encountered in seven patients (7%) owing to distant metastases in six and cardiac disease in one.

Nectin-4 expression by IHC was detected in 62 patients with BC (62%) as compared to none among controls ($\chi 2 = 52.8$, P = 0.000). It was expressed significantly more among patients with ductal carcinoma (74%, 54/73) than those with lobular carcinoma (29.6%, 8/27) (P = 0.0005). Although Nectin-4 expression was found to have no significant correlation with tumor size (P = 0.273), it had a significant positive correla-

Table 1. Relation between tumor characteristics and Nectin-4 expression by IHC.

Tumor characteristics	IHC Nectin-4+ve, N=62 n (%)	IHC Nectin-4−ve, N=38 n (%)	χ^2 (P value)
Tumor size			
0-2 cm	10 (47.6)	11 (52.4)	0.98 (0.273)
2-5 cm	44 (64.7)	24 (35.3)	
>5 cm	8 (72.7)	3 (27.3)	
Tumor type			
Ductal	54 (74)	19 (26)	11.98 (0.0005)*
Lobular	8 (29.6)	19 (70.4)	
Tumor grade (SBR)			
Grade I	4 (26.7)	11 (73.3)	8.65 (0.003)*
Grade II	36 (63.2)	21 (36.8)	
Grade III	22 (78.6)	6 (21.4)	
Axillary LN involvement			
Negative	13 (27.1)	35 (72.9)	12.65 (0.0001)*
Positive	49 (94.2)	3 (5.8)	
ER			
Negative	36 (83.7)	7 (16.3)	13.88 (0.0001)*
Positive	26 (45.6)	31 (54.4)	
PR			
Negative	37 (80.4)	9 (19.6)	12.01 (0.0005)*
Positive	25 (46.3)	29 (53.7)	
HER2-Neu			
0-1	25 (41)	36 (59)	14.6 (0.0001)*
2-3	37 (94.9)	2 (5.1)	

*Statistically significant (P<0.05). **IHC:** Immunohistochemistry, **SBR:** Scarff-Bloom-Richardson system, **ER:** Estrogen receptors, **PR:** Progesterone receptors, **HER2-Neu:** Human epidermal growth factor receptor 2.

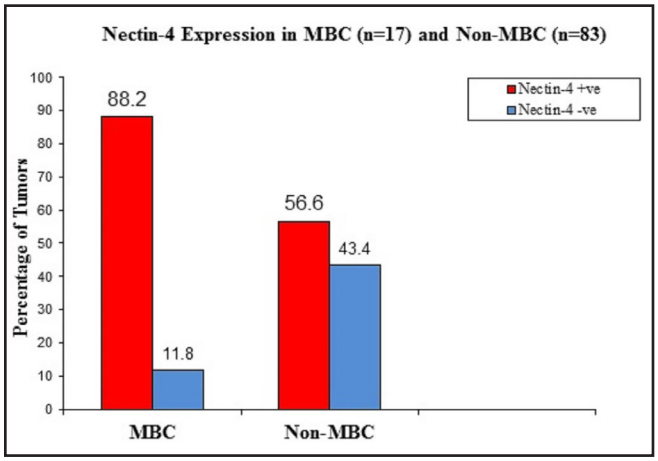

Figure 1. Nectin-4 expression by immunohistochemistry among patients with metastatic breast cancer (MBC) and non-MBC.

tion with tumor grade (P = 0.003), as it was positive in 78.6% (22/28) of Grade III tumors, 63.2% (36/57) of Grade II and 26.7% (4/15) of Grade I tumors. Moreover, Nectin-4 detection by IHC was found to be positive in significantly more patients with LN involvement (94.2%, 49/52) than those without (5.8%, 3/52)(P = 0.0001), and in those with negative ER and negative PR than in those with positive ER and PR receptors (P = 0.0001 and P = 0.00045, respectively). Regarding the HER2-Neu status, Nectin-4 expression by IHC was found to be positive in 94.9% (37/39) of patients with HER2-Neu 2-3, as compared to 41% (25/61) in those with HER2-Neu 0-1 (P = 0.0001) (Table 1).

A total of 17 patients had distant metastases (8 at presentation and 9 during follow-up) of whom 15 (88.2%) were positive for Nectin-4 as compared to 56.6% (47/83) among patients with non-metastatic disease (P = 0.0115) (Figure 1). Local recurrences that were all positive for Nectin-4 were encountered in four patients. Of the seven patients who passed away during the 2-year follow-up period, 5 (71.4%) were positive for Nectin-4 and only 2 (28.6%) were negative (P = 0.049).

Figure 2 shows the Nectin-4 expression by IHC for different histopathological types of BC. Figure 2a shows a case of ductal carcinoma in-situ (comedo-type) with severe positive brown continuous membranous staining in all the neoplastic cells of the duct (score 3) Nectin-4, ×400. Figure 2b shows a case of infiltrating duct carcinoma (IDC) with complete negative staining for all the tumor cells (score 0), Nectin-4, ×400, while Figure 2c shows a case of IDC with moderate positive brown continuous membranous staining of 90% of tumor cells (score 3), Nectin-4, ×400, and Figure 2d shows another case of IDC with severe brown cytoplasmic and membranous staining in 70% of tumor cells (score 3), Nectin-4, ×400. A case of infiltrating lobular carcinoma (ILC) is shown in Figure 2e exhibiting positive brown continuous membranous

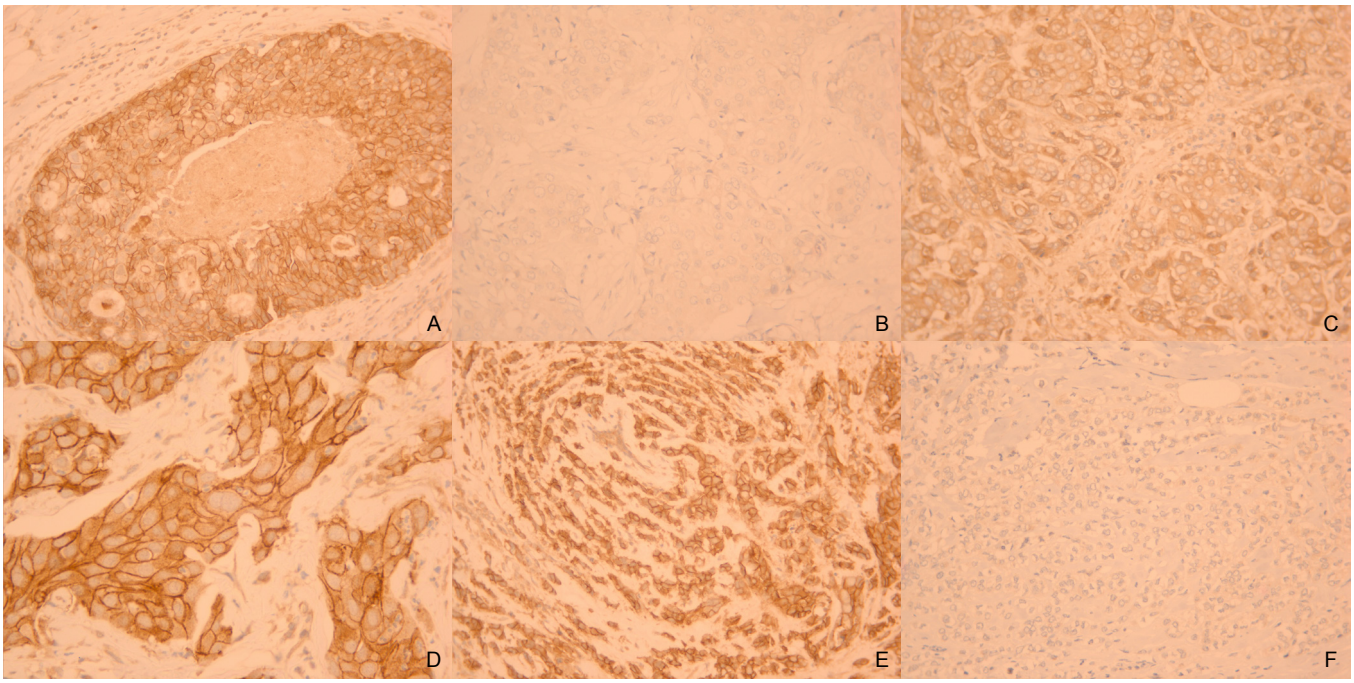

Figure 2. (A-F) Nectin-4 expression in different histopathological types of breast cancer. **(A)** Ductal carcinoma in-situ (comedo-type) positive Nectin-4. **(B)** Infiltrating duct carcinoma (IDC) negative Nectin-4. **(C)** IDC positive Nectin (membranous staining). **(D)** IDC positive Nectin (cytoplasmic staining). **(E)** Infiltrating lobular carcinoma (ILC) positive Nectin-4. **(F)** ILC negative Nectin-4.

staining in 80% of the tumor cell (score 3), Nectin-4, ×400, while Figure 2f shows a case of ILC with complete negative staining for all the tumor cells (score 0), Nectin-4, ×400.

Discussion

A molecular taxonomy of BC has been proposed that it may be related to the differentiation model of breast epithelium. This taxonomy has been well-established by gene expression profiling studies [19]. Studies attempting to define BC subtypes by protein expression profiling showed the necessity to have new reliable immunohistochemical markers [20,21]. Nectin constitutes a family that belongs to the immunoglobulin (Ig) superfamily that shares a common structural homology. In general, Nectins are important in the formation and maintenance of adherens junctions and tight junctions [22,23]. Nectin-4 expression is restricted to the placenta in normal human tissues. It is not expressed in normal cells, including endothelial, epithelial, and hematopoietic cells (bone marrow progenitors, monocytes, PMN, T and B lymphocytes, mast cells). However, Nectin-4 was also detected in the mouse brain, lung, and testis and in half of ovarian tumor cell lines 4 [24].

In the present study, Nectin-4 was not detected in any of the normal breast tissue of the control group, suggesting that it can represent a new tumor-associated antigen. Nectin-4 was detected by IHC in 74% and 29.6% of the studied tumors having ductal and lobular carcinoma, respectively, and almost equally in both non-invasive and invasive ductal carcinomas denoting that it strongly correlates with histological type. Our results showed also that Nectin-4 expression correlates positively with SBR grade and lymph node involvement, but not with tumor size.

Similar to our results, Fabre-Lafay et al. [25], in 2007 showed that Nectin-4 expression by IHC had no statistically significant correlation with increased tumor size, however, contrary to our findings; they reported no significant correlation with the SBR grade system, and axillary LN involvement. More recently, in 2011, Athanassiadou et al. [26] showed that positive Nectin-4 expression by IHC was not only significantly associated with increased grade and axillary LN involvement, but also with increased tumor size, which is in accordance with our findings.

The present study documented that Nectin-4 expression was more frequently detected in ER and PR negative tumors than in receptor positive ones. It also showed a significant positive correlation with HER2-Neu. Furthermore, Nectin-4 expression by IHC was significantly increased with distant metastasis, local recurrence, and mortality. Since disruption of adherens junctions is one of the hallmarks of cancer cells exhibiting malignant transformation, Nectins and Afadin have been involved in tumor biology, carcinogenesis and metastasis by causing cell depolarization, loss of contact-dependent inhibition of proliferation, and increased motility and invasiveness. In 2011, Athanassiadou et al. [26], evaluated the association between Nectin-4 expression and overall survival. They reported that positive staining for Nectin-4 was associated with lower 5-year survival rates compared to negative staining, respectively, 3.3% versus 58.0% ($P < 0.0001$). The limitations of the present study are the relatively small population size and the admittedly short follow-up reported here-in so far.

From the data presented, it can be concluded that (1) Nectin-4 is not expressed in normal breast tissue, but is expressed by IHC in approximately two-thirds of patients with BC, and (2) Nectin-4 expression indicates a poor prognosis of BC as it is strongly correlated with higher tumor grade, LN involvement and negative ER and PR receptors, in addition to its significant correlation with the development of local recurrence, distant metastasis and mortality. Nectin-4 is thus a highly suggested biomarker for predicting BC progression and its prognosis. Further studies are recommended for Nectin-4 as a strong target for developing new therapies, based on nucleic acid drugs, monoclonal antibodies, and cancer vaccines.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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