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Expression of Galectin-1, Galectin-3 and T-Antigen in Breast Carcinoma Tissues and its Significance in Axillary Lymph Node Infiltration

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Abstract

Galectins are a family of low molecular weight galactoside specific endogenous lectins with functions in cell growth, cell activation, cell–cell, cell–matrix adhesion including binding to carcinoembryonic antigen, laminin and metalloprotenase. Gal-3 by interacting with T- antigen has been found to enhance metastatic potential of human tumors in a number of experimental studies. We correlated the endogenous expression patterns of Gal-1, Gal-3 along with their respective binding sites and T- antigen expression of breast cancer tissues of lymph node positive cases with that of lymph node negative cases. A lower cytoplasmic expression of Gal-3 and a higher expression of T- antigen were found to differentiate lymph node positive cases from node negative cases, suggesting that Gal-3 and T- antigen expression analysis can have a role to predict lymph node metastasis in breast cancer patients. Further detailed analysis to explore the significance of these markers in breast cancer is warranted.

Key Words: Galectin-3, Galectin-1, T- antigen, Predictor lymph node breast cancer

Introduction

The clinical course of breast cancer varies considerably from patient to patient; some women have recurrent cancer and die within a year of mastectomy, others are cured by mastectomy, and still others survive for ten or fifteen years after mastectomy with proven metastatic disease. Although it is not possible to predict with certainty how a particular breast cancer will behave in a particular patient, certain parameters are useful in predicting the chance of a woman's being cured or dying of the disease. Axillary lymph node status for the presence of metastasis is one of the main parameters among them. It has been shown through numerous studies that routine histological examination of dissected nodes may be inadequate depending on the thoroughness of examination [1,2]. Sapir and Amreman have reported that re-examination of multiple sections of 30 patients, whose lymph nodes have been reported as negative by standard histopathological methods revealed 10 patients (33%) to have metastatic disease. Working on lymph node specimens from 199 patients, it was found that 22% those free of disease on routine histopathological examination had occult metastasis on serial sectioning. The presence of such metastasis on serial sectioning has been shown to confer a significant adverse effect on recurrence and survival. The major disadvantages, however, are the cost and labor implications in processing axillary lymph node through serial sectioning to search for occult disease [3]. The consequences of missing axillary micro metastasis can directly influence treatment strategies. So, the axillary lymph node should be dissected as a unanimous policy in clinically axillary node positive patients. But, the need for immediate axillary dissection in clinically axillary node negative patients has been questioned [4]. The main reason for axillary dissection in clinically axillary node negative patients is for the staging of breast cancer. But, the chance that axillary node are involved is only 20-25% in those patients [5]. If other biological markers can accurately assess axillary and internal mammary metastasis, unwanted dissection of axillary and internal mammary lymph nodes can safely be avoided and at the same time the consequence of missing of the occult micro metastasis can be eliminated.

The endogenous lectins, galectin-1 (Gal-1) and galectin-3 (Gal-3) are a family of low molecular weight galactoside specific lectins with functions in cell growth, cell activation, cell–cell, cell–matrix adhesion including binding to carcinoembryonic antigen, laminin and metalloprotenase [6]. galectin-3 and galectin-1 have been found to enhance metastatic potential of human tumors by interacting with T-antigen in a number of experimental studies. A recent study on the implication of Gal-3 function during metastasis of breast carcinoma cells suggest that Gal-3 is a critical determinant for anchorage–independent and free–radical resistant cell survival during metastasis [7]. Down regulation of Gal-3 was reported to suppress the tumorigenicity human breast carcinoma cells [8]. The endogenous galectins and their respective binding

sites can be demonstrated by specific antibodies directed against them and by using biotinated galectins respectively.

Apart from the molecule for which a role in cell-cell and cell-extra cellular matrix interaction has been mentioned, other molecules may also be involved in the development, progression and metastasis of tumor. The Thomson Friedenreich (T) antigen, which is a protein linked galectose -1-3-N-acetylgalactosamine strain, is one among them. A significant role for T- antigen has been reported in the progression of breast cancer [9,10]. In a recent study on the role of T- antigen in adhesion of human breast and prostate cancer cells to endothelium has demonstrated that T- antigen plays a leading role in the docking of breast and prostate cancer cells onto endothelium by specifically interacting with endothelium expressed Gal-3. The T- antigen bearing glycoproteins are also capable of mobilizing Gal-3 to the surface of endothelial- cells, thus priming them for harboring metastatic cancer cells. The expression of T- antigen can be demonstrated by monoclonal anti T- antigen antibody.

The aim of the present study was to characterize the level of expression of Gal-1, Gal-3, their respective binding sites and T- antigen expression in primary breast cancer tissues of axillary lymph node negative and axillary lymph node positive breast cancer patients to know whether any of this markers can be used to predict occult micro metastasis in tissues of primary breast carcinoma.

Materials and Methods

Paraffin sections from archival blocks of 54 patients of primary breast carcinoma tissues removed during the year 2000-2001 by modified radical mastectomy in the division of surgical oncology, Regional Cancer Center, Trivandrum were used for the study. Representative blocks were selected by reviewing the H&E sections used for the initial diagnosis. Clinical details of the lymph node status were obtained from the case records of the patients and from histopathology records. All the procedures followed in the study were in accordance with the ethical standards of the center.

Antigalectin-1 and antigalectin-3antibodies and biotinylated galectin-1 and galectin-3 were kindly supplied by Gabius. H.J. Anti T antibody directed against Thomson Friedenriech antigen was purchased from DAKO (monoclonal mouse anti human Thomson Friedenriech antigen clone HB-T1). The standard avidine biotin method was followed with DAB as the chromogen. The sections were incubated with primary antibodies/biotinylated lectins at 4^{O}C for overnight. Anti- galectin antibodies were used in concentration of 0.04 g / mL and the T- antigen antibody in a concentration of 1/50 dilution. The biotynilated lectins were used in a final concentration of 0.05 g /mL. Negative controls were included for each batch. The sections were then counterstained with haematoxylene for two minutes, dehydrated in ascending grades of alcohol and mounted in DPX.

The slides were assessed in a semi quantitative method. The whole area of the sections were examined under10 x objective. The staining intensity and the percentage of cells with different grades of staining were assessed in each of the sections. Areas of sections with the highest percentage of positive stained cells were chosen to score. The grades of staining were assessed as (0), for negative, (+) for mild, (++) for moderate and (+++) for intense. Univariate statistics was performed to calculate the mean score for each of the markers in lymph node positive and negative samples. Significance (two tailed p) was assessed using students T test.

Results

The mean score values for the expression patterns of galectin-3, galectin-1, their respective binding sites and T-antigen in infiltrating duct cell-carcinoma of axillary lymph node positive and negative breast cancer tissues are shown in the table (I). Samples with lymph node metastasis showed predominance of mild grade of staining with a mean value of 51.03 for gal-3. Where as, in samples without axillary node metastasis, cells with moderate expression were higher with a mean value of 58.20.Intense expression of Gal-3 was confined to samples without metastasis only with a mean value of 8. Similarly a higher number of samples with metastasis showed predominance of mild grade of gal-1 expression. Moderate grade of expression was higher in samples without node metastasis; the difference was statistically significant (p=0.001). The difference between node positive and node negative samples with regard to grade of staining for galectin-1 expression was lesser than galectin-3. Intense grade of expression of Gal-3 were confined to node negative samples alone with a mean value of 8. Moderate grade of Gal-1 expression was higher for samples without metastasis. But the difference was not as much significant as that of Gal-3. No significant differences between node negative and positive samples were observed for either of the galectin binding sites. The mean values for Gal-3 biding site were 43.28(+), 16.21(++) and for Gal-1biding site 42.91(+), 8.79(++) for samples with lymph node metastasis and 39, 15 and 37and 11 for samples without lymph node metastasis. The expression patterns for both of the galectins and for the respective binding sites were uniform, regular and mainly cytoplasmic (Fig. 1-A,B,C,D)

Mild nuclear expression was found in two of the cases for galectin-3. Expression of galectin-3 as well as galectin-1 were seen in the stroma also.

The mean value for mild grade of T-antigen expression was 2.93 for samples with lymph node metastasis and 8 for samples with out metastasis. Moderate grade of expression was higher in node positive cases with a mean value of 37.07. Significant difference was observed in the moderate grade of expression of T- antigen between samples with lymph node metastasis and samples without metastasis (P=0.000). Intense grade of staining was confined to node positive cases only and the mean value was 5.52. The expression was uniform regular, and mainly membrane. However, cytoplasmic expression was also seen.in all of the samples. Fig.(1-E,F).

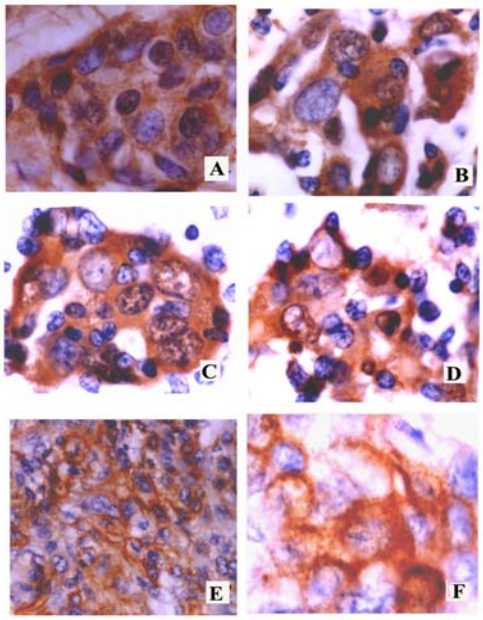

Fig I:

A. Intense expression of Galectin-1 in the cytoplasm of the malignant cells of infiltrating duct cell carcinoma.100x10
B. Intense expression of Galectin-3 in the cytoplasm of the malignant cells of infiltrating duct cell carcinoma.100x10
C. Intense expression of Galectin-1 binding site in the cytoplasm of the malignant cells of infiltrating duct cell carcinoma.100x10
D. Intense expression of Galectin-3 in the cytoplasm of the malignant cells of infiltrating duct cell carcinoma.100x10
E. Intense expression of T-antigen in malignant cells of infiltrating duct cell carcinoma.40x10
F. Intense expression of T-antigen in malignant cells of infiltrating duct cell carcinoma 100x10

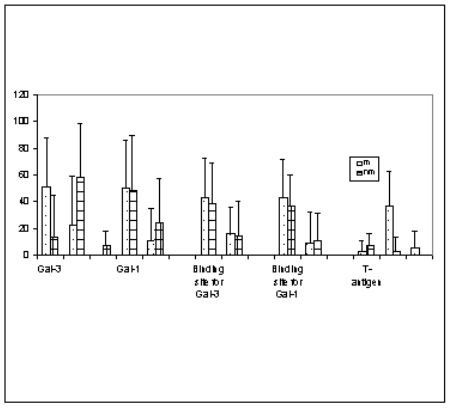

Fig 2: Bar graph showing the patterns of expression of Gal-3, Gal-1 and T-antigen.

Table I: Univariate statistics of the different grades of endogenous expression of Gal-3,Gal-1 and their respective binding sites and T antigen

Intensity of expressions in grades	Univariate statistics of different grades of expressions							
	Mean		Standard deviation		Standard error mean		T value	Significance Two tailed p
	M (n=29)	NM (n=25)	M (n=29)	NM (n=25)	M (n=29)	NM (n=25)		
Galectin-3								
+	51.03	14	36.92	31.52	6.86	6.30	3.929	0.000
++	22.59	58.20	37.07	39.58	6.88	7.92	3.412	0.001
+++	0	8	0	10.21	0	2.04	2.939	0.007
Galectin-1								

+	50	30.20	35.98	41.21	6.68	8.25	0.457	0.650
++	10.9	24.40	24.35	33.21	4.52	6.64	1.719	0.092
+++	0	0						
Binding site for Gal-3								
+	43.28	39	29.35	29.86	5.45	5.95	0.777	0.441
++	16.21	15	20.29	25	3.77	5	1.404	0.167
+++	0	0						
Binding site for Gal-1								
+	42.91	37	28.87	22.96	5.36	4.59	0.754	0.454
++	8.79	11	23.93	20.51	4.44	4.10	-.361	0.720
+++	0	0						
T- antigen								
+	2.93	8	7.85	8.42	1.46	1.68	2.288	0.026
++	37.07	3.20	26.03	10.19	4.83	2.04	6.456	0.000
+++	5.52	0	12.42	0	2.31	0	2.393	0.024

Discussion

The recent findings demonstrate that hematogeneous cancer metastasis originate from intra vascular growth of endothelium attached rather than extra vasated cancer cells emphasizing the key role of tumor endothelial cell interaction in the phenomenon of metastasis [11]. A broad array of adhesion molecules has been implicated in the adhesion of tumor cells to vascular endothelium. The different adhesion molecules are thought to participate at distinct stages of this multi step binding process. Galectin–3 has been proposed to participate in docking of cancer cells onto the capillary endothelium [12]. Studies on cellular distribution of Gal-3 during cancer cell adhesion to endothelium has been reported by Vladislave *et al*. [13]. They used laser scanning confocal microscopy to study the localization of Gal-3 in both cancer cells and endothelial cells upon their interaction and have found Gal-3 distributing during breast cancer cell adhesion to endothelial cells. They observed extra cellular expression of Gal-3 in cultured tumor cells than endothelial cells and suggested that Gal-3 of cancer cells does not actually participate in their adhesion to endothelium, in contrast, they observed the mobilization and clustering of Gal-3 endothelial cells towards the cancer endothelial cell contacts. They have also analyzed the interaction of T- antigen with Gal-3 as the terminal sugar of T-antigen is beta- galactoside and considering the fact that human Gal-3 exhibit a 200 fold higher specific activity towards Gal beta 1-3 Gal NAc disaccharide than Gal-1. It was also observed that both T- antigen masking and T- antigen mimicking compounds specifically inhibited the interaction of Gal-3 with T- antigen on ELISA experiments confirming the interaction. There are several reports correlating galectin expression and tumor metastasis. Galectin –3 has been reported as a useful tumor marker for gastric cancers with respects to tumor progression and potentiality of lymph node metastasis [14] Cindolo *et al.* [15] have reported a higher expression pattern of Gal-1 and Gal-3 correlating with the grade of tumor in transitional cell carcinoma. While a comparative analysis of galectin in primary and metastatic pancreatic cancer revealed no significant relation between galectin expression and tumor grade [16]. Galectin-3 has been reported to enhance the metastatic potential of BT 549 cells through resistance to the product of iNOS, possibly through its bcl-2-like ant apoptotic function [17]. Andre [18] has analyzed the expression of Gal-1 and Gal-3 and their binding sites in node negative and node positive breast cancer tissues and has reported that correlation of either increased Gal-1 binding and reduced gal-3 binding or reduced binding of both galectins with the occurrence of lymph node lesions. Idikio [19] also has reported a reduction of Gal-3 expression, which correlates with the higher grade of breast cancer that possibly reduced matrix binding and increased cancer cell motility.

The present study confirms the immuno-histochemical demonstration of galectins and their respective binding sites in formalin fixed tissues. Even though galectin expression were found in breast cancer tissues irrespective of the lymph node status, significantly higher expression of Gal-3 was observed in the breast cancer tissue samples of lymph node negative patients than node positive cases. Despite a higher percentage of cells with mild grade of expression was seen in samples with lymph node metastasis, moderate grade of expression was significantly higher in samples with out lymph node metastasis ($p=0.001$). Cells with intense grade of expression, even though lesser in number, were observed in samples without lymph node metastasis only. A slight higher expression for Gal-1 was also observed in node negative samples but it was not significant as observed for Gal-3. Thus, the present study confirms the observations of the previous reports of Andres and Idikio [18,19]. But, for the biding sites of Gal-1 as well as Gal-3, no significant difference were observed between lymph node positive and negative samples.

Springer and colleagues were the first to propose T-antigen as a tumor associated antigen in man. They have demonstrated T- antigen in breast tissues, gastrointestinal tract

and respiratory tract tissues [20]. T- antigen has been reported to play a major role in determining the invasive and metastatic properties of tumor cells [21]. T- antigen expression has been reported to correlate with tumor recurrence in transitional cell carcinoma of the bladder [22]. In another study the T- antigen expression has been correlated with nuclear volume in relation to recurrence and prognosis of cancer of the bladder [23]. They have found T- antigen expression in 54% of the patients who had invasive disease. Similarly a higher nuclear volume also suggested invasive disease, indicating that the combined use of T- antigen expression and mean nuclear volume is of potential clinical interest for determining patients who are at high risk of disease progression. Sata *et al*.[24] have reported the usefulness of gold-labeled amerantin for the detection of T-antigen and its cryptic form in normal, dysplastic and neoplastic colonic epithelium. They have found that T- antigen was expressed in normal and dysplastic and neoplastic colonic epithelium in the cryptic form only. In breast carcinoma, the relative proportion of T- antigen and Tn antigen has been reported to correlate with the tumor aggressiveness [25]. While Imai *et al.* [26] has analyzed the expression patterns of T- and Tn antigen in breast carcinoma and have reported that a significant inverse correlation has been found between the expression of T/Tn antigen expression and long term overall survival. Howard *etal*. [27] and Klein *et al.* [28] have suggested that the T antigen can be detected by PNA, the plant lectin binding and have a role in the modulation of host immune response to breast carcinoma. They also found that in benign breast tissues and in well-differentiated areas of neoplastic lesions T- antigen is located in the luminal membrane and they considered it as outside the body of cell or in an immunologically privileged site. T- antigen has been reported to be present in upto 91% of breast carcinomas and the high frequency of it has been exploited to identify micro metastasis on axillary lymph nodes by PNA reactivity [29]. As PNA reacts with ordinary D-galactopyranose residues, D-galactosamine, alpha linked D-glucosamine and even to D-fucose, this plant lectin is not specific for T- antigen and anti T- antigen antibody is more specific to demonstrate T antigen.

In the present study, T- antigen expression was observed in 48 samples of the total 54 samples studied. Among the T antigen positive samples, mild grade of expression were the predominant pattern in breast cancer tissues of the node negative patients. Whereas, moderate and intense expression patterns were observed in lymph node positive cases. None of the lymph node negative samples showed intense grade of expression. So the present study observed a positive correlation between T- antigen expression and axillary lymphnode metastasis. Here also the expression was mainly membrane as reported by Howard by using PNA. However, cytoplasmic expression was also seen in almost all the cases. They have further shown that T- antigen acts both as a major cell surface carbohydrate ligand for Gal-3 on breast cancer cells and as a factor causing mobilization of Gal-3 to the outer membrane on endothelial cells. A similar explanation can be offered for the lower level expression of Gal-3 and a higher expression of T- antigen in samples of lymph node positive cases than the samples of lymph node negative cases.

Thus, a higher T- antigen expression and a lower Gal-3 expression pattern appear to differentiate primary breast carcinoma with axillary lymph node metastasis form breast carcinoma without axillary lymph node metastasis. This preliminary report suggests further detailed evaluation of the potential of galectins and T- antigen in predicting occult micro metastasis in primary breast carcinoma tissues.

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