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ORIGINAL ARTICLE

N-Acetylglucosaminyltransferase III (GnT-III) but not N-Acetylgalactosaminyltransferase-6 and 8 are Differentially Expressed in Invasive and In Situ Ductal Carcinoma of the Breast

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Abstract

Mammary carcinoma is the most common malignant tumor in women, and it is the leading cause of mortality. In tumor context, glycosylation promotes post translational modifications necessary for cell progression, emerging as a relevant tumor hallmarker. This study aimed to analyze the association between polypeptide N-acetylgalactosaminyltransferase-6 (ppGalNAc-T6), −T8, N-acetylglucosaminyltransferase III (GnT-III) expression, *Phaseolus vulgaris*-leucoagglutinin (PHA-L), wheat germ agglutinin (WGA) and peanut agglutinin (PNA) staining with clinic-histopathological factors from patients with pure ductal carcinoma in situ (DCIS) and DCIS with invasive ductal carcinoma (DCIS-IDC) of breast. Formalin-fixed and paraffin-embedded samples ($n = 109$) were analyzed. In pure DCIS samples GnT-III was over-expressed in comedo lesions ($p = 0.007$). In DCIS-IDC, GnT-III expression was associated with high nuclear grade tumors ($p = 0.039$) while the presence of PHA-L and WGA were inversely related to HER-2 expression ($p = 0.001$; $p = 0.036$, respectively). These findings pointed to possible involvement of GnT-III, ppGalNAc-T8, L-PHA and WGA as probes in prognostic evaluation of DCIS.

Keywords Glycosiltranferases · Glycocode · Lectins · In situ ductal carcinoma · Invasive ductal carcinoma

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Abbreviation

CEN 17	Centromere 17
CISH	Chromogenic in situ hybridization
DAB	Diaminobenzidine
DCIS	Ductal carcinoma in situ
DCIS-IDC	Ductal carcinoma in situ with invasive ductal carcinoma
ER	Estrogen receptor
GalNAc	N-acetylgalactosamine
GnT-III	N-acetylglucosaminyltransferase III
GnT-V	N-acetylglucosaminyltransferase V
HER-2	Human epidermal growth factor receptor-type 2
PHA-L	Phaseolus vulgaris-leucoagglutinin
PNA	Peanut agglutinin
PpGalNAc-T6	Polypeptide N-acetylgalactosaminyltransferase-6
PpGalNAc-T8	Polypeptide N-acetylgalactosaminyltransferase-8
PpGalNAc-Ts	Polypeptide N-acetylgalactosaminyltransferases
PR	Progesterone receptor
TMA	Tissue microarray
WGA	Wheat germ agglutinin

Introducion

Currently, post-translational modifications of proteins have been constant targets of research due to discovery of this influence on normal and pathological cell function [1, 2]. Various enzymes as kinases are involved in these changes however glycosylation of proteins is one of the most abundant modifications [3].

The study of glycobiologyc universe began by monomeric carbohydrate identifying and analysis of polysaccharide chains composition [4]. So scientific advances led to discovery of meaningful participation of N and O-glycans on cell biology aspects such as adhesion [5], signaling [6], migration [7] and endocytosis [8]. Glycans are ordered in sequence by glycosyltransferases which act as the anabolic component in glycobiology context, allowing the molecular synthesis of biomarkers potential [9].

Cancer is a pathologic condition in which various aspects of cell biology are altered, including the glycidic profile formation [10]. The increased or decreased activity of glycosyltransferases and glycosidases promotes the rearrangement of various glycoconjugates favoring tumor progression, including the breast cancer [11].

Breast cancer presents as the most incident malignant neoplasm in women around the world [12]. Its progression is influenced by risk factors such as age, family history, late menopause, or by tumor clinical features as nuclear grade, hormonal status and morphological subtyping [13, 14].

Ductal carcinomas of the breast are the most frequent morphological type and can occur as in situ (DCIS) and/or invasive (IDC) forms. Although the in situ tumor may be considered as an initial step for the invasive tumor

Table 1 Relationship between ppGalNAc-T6 and –T8 expression with clinicopathologic features and immunohistochemical markers in pure DCIS lesions

Clinico-histopathologic features	ppGalNAc-T6$^-$ n (%)	ppGalNAc-T6$^+$ n (%)	*P* value	ppGalNAc-T8$^-$ n (%)	ppGalNAc-T8$^+$ n (%)	*p* value
Age (years)						
<50	13 (54.2)	7 (50)	0.535[a]	3 (25)	17 (58.6)	0.052[a]
>50	11 (45.8)	7 (50)		9 (75)	12 (41.4)	
Menopausal status						
Pre-menopausal	14 (58.3)	7 (50)	0.435[a]	3 (25)	18 (62.1)	**0.034**[a]
Post-menopausal	10 (41.7)	7 (50)		9 (75)	11 (37.9)	
Tumor size (cm)						
<2.0	10 (41.7)	5 (35.7)	0.930[b]	5 (41.7)	12 (41.4)	0.284[b]
2.0–5.0	9 (37.5)	6 (42.9)		3 (25)	13 (44.8)	
>5.0	5 (20.8)	3 (21.4)		4 (33.3)	4 (13.8)	
Nuclear grade						
Low	4 (16.7)	5 (35.7)	0.206[b]	4 (33.3)	4 (13.8)	0.250[b]
Intermediate	3 (12.5)	0 (0)		2 (16.7)	3 (10.3)	
High	17 (70.8)	9 (64.3)		6 (50)	22 (75.9)	
Multifocal						
Yes	12 (50)	9 (64.3)	0.304[a]	7 (58.3)	17 (58.6)	0.626[a]
No	12 (50)	5 (35.7)		5 (41.7)	12 (41.4)	
Comedo lesions						
Yes	16 (66.6)	10 (71.4)	0.528[a]	7 (58.3)	21 (72.4)	0.300[a]
No	8 (33.3)	4 (28.6)		5 (41.7)	8 (27.6)	
ER						
Negative	7 (31.8)	5 (35.7)	0.544[a]	4 (36.4)	9 (32.1)	0.542[a]
Positive	15 (68.2)	9 (64.3)		7 (63.6)	19 (67.9)	
PR						
Negative	5 (27.8)	7 (53.8)	0.137[a]	2 (20)	10 (41.7)	0.211[a]
Positive	13 (72.2)	6 (46.2)		8 (80)	14 (58.3)	
HER-2						
Negative	3 (30)	1 (10)	0.291[a]	2 (33.3)	3 (18.8)	0.419[a]
Positive	7 (70)	9 (90)		4 (66.6)	13 (81.3)	

Bold indicates *p* value <0.05

[a] Fisher's exact test

[b] Chi-square test

development, both lesions represent distinct prognostic factors [15]. Patients early diagnosed with DCIS have a longer survival rate than those early IDC diagnosed [16].

From a molecular standpoint, these differences may be influenced by an altered glycosylation. The main type of human *O*-glycosylation is mediated by the UDP-GalNAc:polypeptide *N*-acetylgalactosaminyltransferases (ppGalNAc-Ts), responsible for catalysis the first addition of GalNAc to the hydroxyl groups of serine or threonine residues in protein structures. The catalytic products of this enzyme are involved in several physiological and pathological conditions [17]. Other important component of the glycobiology context is N-acetylglucosaminyltransferase III (GnT-III), as direct concurrent of GnT-V this enzyme is considered a key glycosyltransferase in *N*-glycan biosynthetic pathway [18].

These cancer glycocode has been investigated by lectin histochemistry, since these (glyco)proteins have saccharide-binding sites that can recognize and specifically bind their specifics glycol moiety in glycoconjugates [19].

There is a vast need for discovery of new biomarkers that can help in the prognosis and diagnosis, and consequently provide subsidies for therapy sorting of the different breast cancer forms [20]. Face to this context, this study aimed to identify the glycosiltranferases ppGalNAc-T6 and –T8, GnT-III as glycoprobes in breast cancer tissue. Moreover was performed the evaluation of WGA, PNA and L-PHA lectins, wicth can bind specific components of the sugar branch built by glycosiltranferases. Then, it was intended to find molecules that are significantly associated with progression of in situ and invasive ductal carcinoma.

Methods

Samples

Formalin-fixed paraffin embedded samples of pure DCIS ($n = 47$) and DCIS with IDC component ($n = 62$) diagnosed from 1994 to 2010 were randomly chosen from the Department of Pathology of Ribeirão Preto Medical School at São Paulo University (USP). The clinical data of these patients were retrieved from medical files, and included age, menopausal status, tumor size, hormone receptors status (ER and PR), nuclear grade and tumor multifocal status. Patients were selected based on their histopathologic diagnosis and for each case diagnosis slides (Hematoxylin and Eosin) were reviewed by an independent and experiment pathologist. None of the patients received any oncology treatment before the biopsy procedure. Protocols used in this study were in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the local Ethics Committee.

Tissue Microarray (TMA)

For the construction of TMAs blocks, core biopsies of 1-mm diameter were punched from the selected regions of each 109 donor paraffin blocks and arrayed into a new paraffin block using the *Manual Tissue Arrayer* I (Beecher Instruments, Silver Spring, USA). Thus were arrayed into three-micrometer-thick sections cut from the TMA paraffin block using a paraffin tape-transfer system (Instrumedics, Saint Louis, USA).

Some imperfections in the sample fixation and processing prevented a complete analysis of markers and clinical characteristics. In this way, the number of samples analyzed varies according to the marker, but these variations did not cause a relevant reduction on statistically significant of the cases amount.

One section of each was stained with hematoxylin and eosin to confirm the presence of the tumor by light

Table 2 Relationship between GnT-III expression with clinicopathologic features and immunohistochemical markers in pure DCIS lesions

Clinico-histopathologic features	GnT-III$^-$ n (%)	GnT-III$^+$ n (%)	*p* value
Age (years)			0.087[a]
<50	6 (37.5)	12 (66.7)	
>50	10 (62.5)	6 (33.3)	
Menopausal status			0.087[a]
Pre-menopausal	6 (37.5)	12 (66.7)	
Post-menopausal	10 (62.5)	6 (33.3)	
Tumor size (cm)			0.222[b]
<2.0	8 (50)	4 (22.2)	
2.0–5.0	5 (31.3)	10 (55.6)	
>5.0	3 (18.8)	4 (22.2)	
Nuclear grade			0.063[b]
Low	4 (25)	4 (22.2)	
Intermediate	4 (25)	0 (0)	
High	8 (50)	14 (77.8)	
Multifocal			0.262[a]
Yes	8 (50)	12 (66.7)	
No	8 (50)	6 (33.3)	
Comedo lesions			**0.007**[a]
Yes	7 (43.8)	16 (88.9)	
No	9 (56.3)	2 (11.1)	
ER			0.154[a]
Negative	3 (18.8)	7 (41.2)	
Positive	13 (81.3)	10 (58.8)	
PR			0.076[a]
Negative	2 (16.7)	8 (50)	
Positive	10 (83.3)	8 (50)	
HER-2			0.101[a]
Negative	3 (42.9)	1 (7.7)	
Positive	4 (57.1)	12 (92.3)	

Bold indicates *p* value <0.05

[a] Fisher's exact test

[b] Chi-square test

microscopy. TMA spots containing less than 50% of tumor were removed from the analysis.

Immunohistochemistry

TMAs sections (3 μm) were immune stained with the Mach 4 Universal Polymer Detection kit (Biocare Medical, CA, USA) according to Ribeiro-Silva et al. (2006) and dos-Santos et al. (2012) [21, 22]. The dilution and clone specification of the primary antibodies used in this study are in supplementary file. DCIS cases previously known to be positive for ER, PR, HER-2, ppGalNac-6, −T8 and GNTIII were used as positive controls for each reaction. The reaction was revealed with diaminobenzidine (DAB) followed by hematoxylin counterstaining. Negative controls were prepared omitting the primary antibody.

Lectin Histochemistry

Phaseolus vulgaris agglutinin (PHA-L; specific for β1,6-N-acetylglucosamine); Wheat germ agglutinin (WGA; specific for N-acetylglucosamine) and Peanut agglutinin lectin (PNA; specific for β-1-3-N-acetylgalactosamine) conjugated to biotin were used in this study. TMAs sections (3 μm) were deparaffinized in xylene, rehydrated in graded ethanol (100–70%), incubated with a 0.3% hydrogen peroxide solution for 30 min at 25 °C and treated with a 0.1% trypsin solution for 15 min at 37 °C for PNA analysis.

For PHA-L, after trypsin treatment, sections were also treated with a neuroaminidase solution (0.1 U/mL) from *Clostridium perfringens* (Sigma Aldrich, Missouri, USA), for 1 h at 37 °C. After enzymes treatment slices were incubated with 5% BSA (bovine serum albumin) solution and then incubated, separately, with the lectins solutions overnight at 4 °C (see dilution and manufactures in supplementary file). After that slices were incubated with streptavidin-peroxidase polymer (Sigma Aldrich, Missouri, USA) for 45 min at 25 °C. Phosphate buffer solution (PBS) 100 mM pH 7.4 was used for slice washes between each protocol step. Lectin staining was revealed with DAB followed by hematoxylin counterstaining. Negative controls were prepared replacing the lectin by PBS and the positive obtained from cases with previously known staining.

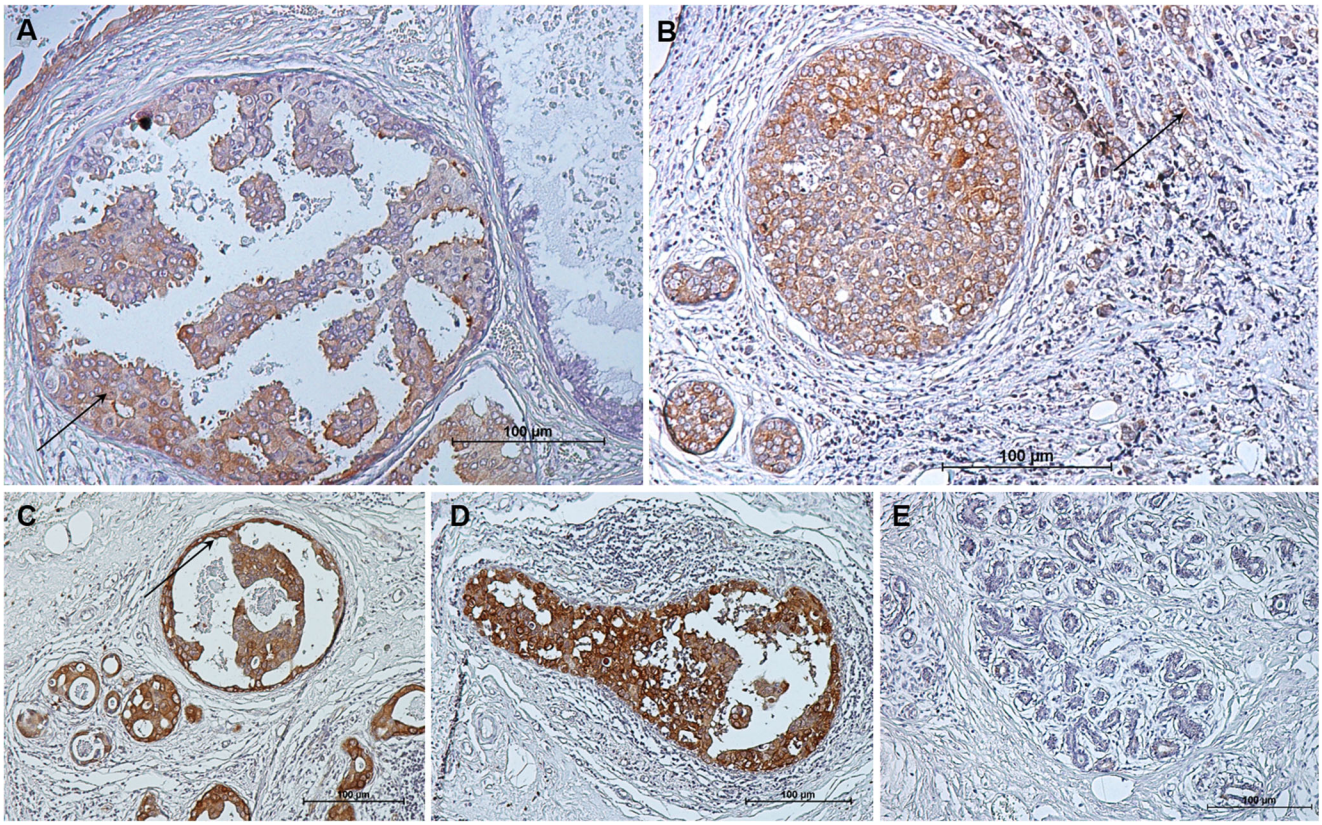

Fig. 1 Immunohistochemistry panel. Figure **a** and **b** presents the GnT-III staining in pure CDIS and CDIS-CDI samples, respectively. However, the fig. **a** shows a papillary-like lesion with heterogeneous citoplasmatic staining (black and white arrows) and Fig. **b** shows characteristic areas of stromal invasion. Figure **c** and **d** illustrates the ppGalNAc-8 staining in the same kind of samples, but although both have cytoplasmic staining, only the Fig. **c** exhibits membrane staining (black arrow). Figure **e** represents the normal control

Immuno and Histochemistry Evaluation

In immunohistochemistry Estrogen and Progesterone Receptors (ER and PR) were positive for >1% of cancer cells [23]. HER-2 status was evaluated according to the ASCO/CAP HER2 Guideline and cases as 2+ were submitted to chromogenic in situ hybridization (CISH) according to Oliveira-Costa et al. (2010) [24, 25]. Only HER-2 2+ cases in immunohistochemistry amplified on CISH were considered positive for statistical purposes. Lectin histochemistry were considered negative for <10%, moderate for 10% to 30% and intense for >30% of neoplastic cells according Dosaka-akita et al. (2004) [26]. PpGalNAc-T6 and T8 expressions were classified as positive when >10% of cancer cells were stained and negative when ≤10% [27]. GNT-III expression was classified as high or low (≥50% was positive and < 50% was negative) [26]. All staining score were evaluated using integrated analysis system EVOS® FL with an software and highly sensitive camera Sony ICX445 (1280 × 960 pixels).

Chromogenic In Situ Hybridization (CISH)

Positive (2+) immunohistochemistry HER-2 cases were also evaluated by chromogenic in situ hybridization (CISH). The ZytoDot 2C SPEC HER2/CEN 17 probe kit (Zytovision, Bremerhaven, Germany) was used for the detection of the human HER-2 gene and alpha-satellites of chromosome 17. All procedures were performed step by step according to the manufacturer's instructions. Using this kit, two green (HER-2) and two red (CEN 17) signals were expected in a normal interphase nucleus. HER-2 was considered amplified when the HER-2/CEN 17 ratio was ≥2 on average for 60 cells. Only cases scored as 2+ by immunohistochemistry in which the HER-2 was amplified on CISH analysis were considered positive.

Statistical Methods

Statistical analysis we carried out according to Santos et al. (2012) where the results were dichotomized as positive (strong positive staining 3+ and moderately staining 2+) or negative (weak positive 1+ and negative staining 0) for tumor cells [22]. Tests were performed positive with PASW Statistics 19.0 software (Chicago, IL, USA). The relationships among immunohistochemistry and lectin histochemistry findings and clinic-histopathologic features were tested with cross tables applying the χ^2 (three or more variables) or Fisher tests (2 variables). All tests were 2-tailed.

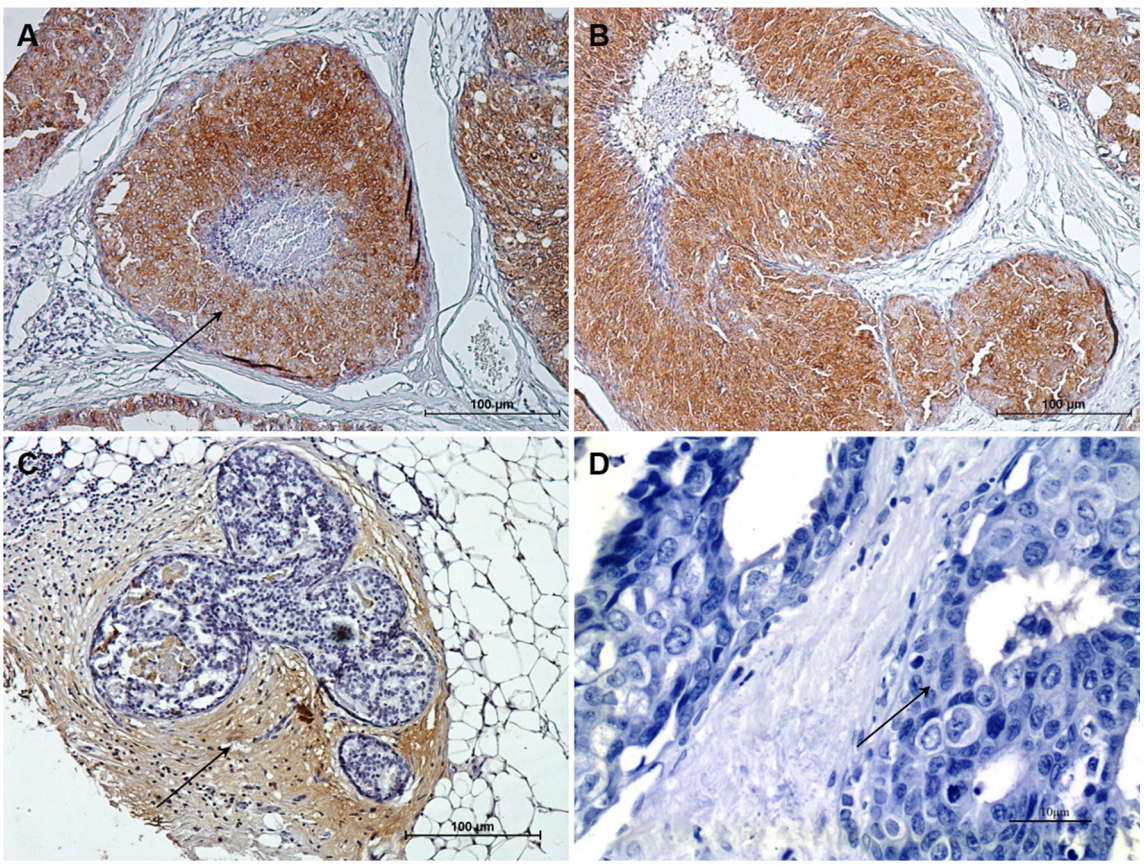

Fig. 2 Histochemistry panel. Figure **a** and **b** presents the L-PHA staining in CDIS-CDI samples. Both images show lesions with similar citoplasmatic staining (black arrow). Further, Fig. **c** shows WGA stromal staining. Fig. **d** is the (tumor) negative control

Results

Clinical parameters were associated with glycosyltransferases and lectins staining in both groups (DCIS and DCIS with IDC component).

Glycosyltranferases and Carbohydrates Expression and Their Relationship with Clinical Factors in Pure DCIS

The evaluation of glycosyltransferases and carbohydrate expression in pure DCIS (Tables 1 and 2) revealed a significant association between the expression of GNT-III and ppGALNAc-T8 with the comedo subtype presence and menopausal status, respectively, where 16 out of 18 GNT-III positive cases presented comedo subtype ($p = 0.007$). While 18 out of 29 ppGalNAc-T8 positive samples belonged to premenopausal patients ($p = 0.037$) (Fig. 1).

Contrary, ppGalNAc-T6 had no correlation with the markers used in this analysis. The same pattern was observed for PHA-L, WGA and PNA histochemistry that showed no significant association with clinic-histopathological factors (Fig. 2, Supplementary file).

Glycosyltranferases and Carbohydrates Expression and Their Relationship with Clinical Factors in DCIS with IDC Component

In this samples, GNT-III expression was associated with tumor nuclear grade, in 31 of 33 cases classified as high nuclear grade, showed positivity for GNT-III ($p = 0.039$; Table 3). However, ppGAlNAc-T6 and -T8 showed no correlation to nuclear grade (Supplementary file).

The presence of the target carbohydrate for PHA-L and WGA (specific for β1,6-N-acetylglucosamine and N-acetylglucosamine, respectively) was correlated to HER-2 expression (Table 4). For PHA-L the absence of staining is associated with HER-2 positive and vice-versa, where 17 of 28 PHA-L negative cases were positive for HER-2 ($p = 0.001$). This profile was also found for WGA where 14 WGA positives samples were HER-2 negative ($p = 0.036$) (Table 4). In addition, an increased expression of β1,6-N-acetylglucosamine residues (recognized by PHA-L) was found in premenopausal patients samples ($p = 0.047$).

Table 3 Relationship between GnT-III expression with clinicopathologic features and immunohistochemical markers in DCIS lesion with invasion

Clinicopathologic features	GnT-III$^-$ n (%)	GT-III$^+$ n (%)	*p* value
Age (years)			
<51	2 (50)	17 (43.6)	0.602[a]
>51	2 (50)	22 (56.4)	
Menopausal status			
Pre-menopausal	2 (50)	17 (43.6)	0.602[a]
Post-menopausal	2 (50)	22 (56.4)	
Size (cm)			
<2.0	2 (50)	13 (33.3)	0.696[b]
2.0–5.0	2 (50)	22 (56.4)	
>5.0	0 (0)	4 (10.3)	
Nuclear grade			
Low	2 (50)	3 (7.7)	**0.039**[b]
Intermediate	0 (0)	5 (12.8)	
High	2 (50)	31 (79.5)	
Multifocal			
Yes	3 (75)	24 (68.5)	0.521[a]
No	1 (25)	15 (38.5)	
Comedo lesion			
Yes	2 (50)	26 (66.7)	0.436[a]
No	2 (50)	13 (33.3)	
ER			
Negative	0 (0)	8 (21.1)	0.414[a]
Positive	4 (100)	30 (78.9)	
PR			
Negative	0 (0)	15 (38.5)	0.166[a]
Positive	4 (100)	24 (61.5)	
HER2			
Negative	4 (100)	19 (54.3)	0.108[a]
Positive	0 (0)	16 (45.7)	

Bold indicates *p* value <0.05

[a] Fisher's exact test

[b] Chi-square test

Discussion

Glycosylation is an important post-translational modification regulated indirectly through the gene expression [28]. Nevertheless, several evidences suggest that changes in cell glycobiology profile induced by oncogenic or suppressor genes transformation are one of the main factors involved in tumor progression to malignancy [29]. Therefore, the differential glycosylation can be defined as a cancer hallmark and leads to altered expression of enzymes including glycosyltransferases [30].

Inside the family of the six glucosyltransferases stands out N-acetylglucosaminiltransferase III for its association with lower malignancy tumors [7, 31]. Our results pointed the controversy association between GnT-III expression and the comedo subtype presence in pure DCIS samples and the high nuclear grade in mixed lesion. GnT-III is linked to a good prognostic through the synthesis and addition of binary residues of GlcNAc that alters the N-glycans composition and conformation, preventing the action of GnT-V commonly associated with metastasis [32, 33]. However, presence of necrosis, characteristic of comedo subtype, is often related with a more aggressive behavior with a higher rate of short-term local recurrence [34–37].

Table 4 Relationship between lectin staining with clinicopathologic features and immunohistochemical markers in DCIS lesion with invasion

Clinico pathologic features	L-PHA$^-$ n (%)	L-PHA$^+$ n (%)	*p* value	WGA$^-$ n (%)	WGA$^+$ n (%)	*p* value	PNA$^-$ n (%)	PNA$^+$ n (%)	*p* value
Age (years)									
<51	10 (35.7)	9 (69.2)	**0.047**	8 (36.4)	11 (57.9)	0.144	20 (46.5)	7 (63.6)	0.250
>51	18 (64.3)	4 (30.8)		14 (63.6)	8 (42.1)		23 (53.5)	4 (36.4)	
Menopausal status									
Pre-menopausal	12 (42.9)	7 (53.8)	0.374	10 (45.5)	9 (47.4)	0.576	17 (39.5)	8 (72.7)	0.576
Post-menopausal	16 (57.1)	6 (46.2)		12 (54.5)	10 (52.6)		26 (60.5)	3 (27.3)	
Size (cm)									
<2.0	10 (35.7)	5 (38.5)	0.351	8 (36.4)	7 (36.8)	0.653	15 (34.9)	5 (45.5)	0.809
2.0–5.0	14 (50)	8 (61.5)		11 (50)	11 (57.9)		23 (53.5)	5 (45.5)	
>5.0	4 (14.3)	0 (0)		3 (13.6)	1 (5.3)		5 (11.6)	1 (9.1)	
Nuclear grade									
Low	1 (3.6)	3 (23.1)	0.061	0 (0)	4 (21.1)	0.068	7 (16.3)	0 (0)	0.334
Intermediate	3 (10.7)	3 (23.1)		3 (13.6)	3 (15.8)		5 (11.6)	2 (18.2)	
High	24 (85.7)	7 (53.8)		19 (86.4)	12 (63.2)		31 (72.1)	9 (81.8)	
Multifocal									
Yes	16 (57.1)	9 (69.2)	0.350	13 (59.1)	12 (63.2)	0.522	28 (65.1)	8 (72.7)	0.463
No	12 (42.9)	4 (30.8)		9 (40.9)	7 (36.8)		15 (34.9)	3 (27.3)	
Comedo lesion									
Yes	19 (67.9)	7 (46.2)	0.300	14 (63.6)	12 (63.2)	0.614	24 (55.8)	8 (72.7)	0.253
No	9 (32.1)	6 (53.8)		8 (36.4)	7 (36.8)		19 (44.2)	3 (27.3)	
ER									
Negative	7 (25)	2 (15.4)	0.399	6 (27.3)	3 (15.8)	0.308	8 (19)	2 (18.2)	0.660
Positive	21 (75)	11 (84.6)		16 (72.7)	16 (84.2)		34 (81)	9 (81.8)	
PR									
Negative	8 (28.6)	4 (30.8)	0.581	6 (27.3)	6 (31.6)	0.581	13 (31)	3 (27.3)	0.564
Positive	20 (71.4)	9 (69.2)		16 (72.7)	13 (68.4)		29 (69)	8 (72.7)	
HER2									
Negative	11 (39.3)	12 (92.3)	**0.001**	9 (40.9)	14 (73.7)	**0.036**	19 (50)	7 (70)	0.221
Positive	17 (60.7)	1 (7.7)		13 (59.1)	5 (26.3)		19 (50)	3 (30)	

Bold indicates *p* value <0.05

Breast DCIS is considered a set of lesions with different malignant potential [38]; Significant expression of GnT-III, that occur only in pure DCIS samples containing comedonic lesions, can not ensure formation of carbohydrate product. The lack of studies that investigate the direct relationship between the GnT-III expression and the morphological subtypes of DCIS or tumor nuclear grade prevents advance inferences about the issue but raised several hypotheses that need further studies.

Another important group of glycosyltransferases are the UPD-N-acetylgalactosamine: polypeptide N-acetylgalactosaminyltranferases (ppGalNAc-Ts), which are responsible for initial stages of *O*-glycosylation-carrying monomers [17]. The modification of the ppGalNAc-T activity can lead to truncated or high glycosylated structures formation, which in turn may favor breast cancer progression [39].

Members of this enzyme family differ in their tissue expression, enzymatic substrate specificity. Distinct isoforms of ppGalNAc-T1, −T2 and -T10, which are the most widely expressed, have a more restricted expression profile [40]. In agreement with that both ppGalNAc-Ts (−T6 and –T8) analyzed here showed different results; only the ppGalNAc-T8 presented a significant correlation with tumors from premenopausal patients.

The association of anothers ppGalNAc-Ts and prognostic factors has been already described in breast cancer. PpGalNAc-T14 was, for example, associated with histological grade [41]. However little information was presented regarding ppGalNAc-T8. Here we first described this correlation, placing the ppGalNAc-T8 expression directly associated with premenopausal status, allowing us to suggest that this enzyme as a useful biomarker for DCIS breast cancer.

A complementary way for study the carbohydrate moiety modulated by glycosyltransferases is the lectin histochemistry [19]. In our results residues of β-1-3-N-acetylgalactosamine (PNA target) presented no correlation with all analyzed clinic-histopathological parameters in both population, pure DCIS and DCIS with invasive component. On the other hand WGA and PHA-L staining was inversely correlated to HER-2 expression in DCIS with IDC component. Overexpression of this receptor leads to signal transduction prolongation through biochemical pathways which favor tumor development through the proliferation, cell cycle activation [42, 43] and escape from apoptosis [12, 44].

Also using lectin histochemistry Handerson et al. (2005) observed that a greater expression of β1,6-N-acetylglucosamine were directly associated with breast carcinoma nodal metastasis [45]. In addition this carbohydrate was associated with GnT-V activity, increasing the tumor malignancy [46]. In agreement with those facts, in our results the β1,6-GlcNAc expression was mainly present in tumors from younger women, in which tumor generally exhibit high malignance [47, 48].

PHA-L staining revealed a dualistic aspect of tumor glycobiology depending the context [49]. As indicated by Abbott et al (2008), *N*-glycosylation promoted by GnT-V, and recognized by PHA-L, is necessary for the final configuration of various proteins in mammary carcinomas, and can be directly involved in cancer progression [50].

Conclusion

Our results present ppGalNac-T8 as a biomarker in pure CDIS, β1,6-N-acetylglucosamine and N-acetylglucosamine residues as biomarkers in CDIS-CDI, and GnT-III stood out in both. Through our results was possible to observe that the GnT-III association with comedo morphological subtype in pure DCIS, and its relation with high nuclear grade tumors, function as an aggressive (poor) prognostic factor. Therefore, this work has contributed to widen the knowledge about the glycobiology of breast cancer.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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