

Lectin Histochemistry Reveals Changes in Carbohydrate Expression on Morphological Types of Breast Ductal Carcinoma *in situ*

Gabriela Souto Vieira-de-Mello^{1#}, Antônio Felix da Silva Filho^{1#}, Petra Barros dos Santos¹, Moacyr Jesus Barreto de Melo Rêgo¹ and Eduardo Isidoro Carneiro Beltrão^{1,2*}

¹Laboratório de Imunopatologia Keizo Asami (LIKA), Universidade Federal de Pernambuco, Cidade Universitária, Recife, Pernambuco, Brazil.

²Departamento de Bioquímica, Centro de Ciências Biológicas, Universidade Federal de Pernambuco, Cidade Universitária, Recife, Pernambuco, Brazil.

Both authors contributed equally

Abstract

Breast Ductal Carcinoma *in situ* (DCIS) show a high degree of architectural heterogeneity and what kind of DCIS lesions will progress to invasive disease remains unknown. Thus, its characterization remains a complex process. In addition, in the same tumor we can find different types of lesions that presented different biological behaviour and morphology. Carbohydrate expression changes play crucial roles in carcinogenesis of the breast, becoming the study these changes a useful tool for an accurate predictor. In this context, this work aimed analyzed the differential expression of specific carbohydrates among the DCIS lesion through lectin histochemistry and its correlation with its behaviors. Glycobiology expression of 218 DCIS samples of human breast cancer was investigated by MAL-II, PNA, WGA, PHA-L and SNA lectins. MAL-II was more expressed ($p=0.0191$) in cribriform (positive in 100%) than in comedo (positive in 41.2%) and solid lesions ($p=0.0440$). However, PNA profile showed a higher reactivity in comedo ($p=0.0052$) and micropapillary ($p=0.0010$) lesions when compared to solid one. The other lectins showed different expression degrees in all lesions analyzed but none was statistically relevant. Our results reveals different carbohydrate patterns concerning different DCIS morphological subtypes indicating that these lesions could act as independent biological entities.

Keywords: Ductal carcinoma *in situ*; Breast cancer; Glycobiology; Lectin histochemistry; Tumoral morphology

Introduction

Among women, breast cancer is the most common type and is the leading cause of cancer deaths in world [1]. In past few decades the mammography screening programs have design a new breast tumors profile frequency, when Ductal Carcinoma *in situ* (DCIS) stands out among them showing an increase in their occurrence due to the increase of early diagnosis. However this type of lesion is still poorly studied. The few studies available show contradictory results when compared to invasive ductal carcinoma, especially regarding to whether or not subtypes of DCIS lesions may progress to the invasive disease [2-4]. DCIS study is challenged by the presence of a high degree of architectural and morphological heterogeneity in their lesions. In this scenario their lesions are segregated into different histopathological groups classified as comedo (which often presents central necrosis and calcification), solid (presenting ducts filled by neoplastic cells where there is no necrosis), cribriform (with small holes or open spaces in ducts) and micropapillary (featuring finger-like projections) [5]. Commonly, a mix of these lesions is found in the same tumor; however each of these histological lesion subtypes has a different prognosis [6].

In homeostasis, cells express surface proteins that undergo post-translational modifications which glycosylation stands as one of the most important event mediated by the action of glycosyltransferases. This carbohydrate profile is implicated in the regulation of several important physiological processes such as adhesion, signal transduction and biological recognition besides mediate cell-cell interactions and cell-matrix [7].

The human glycode is of particular interest in cancer research given that altered glycans, produced by an interrupted glycosylation, are a common feature of a broad variety of tumor types [7,8]. Lectin histochemistry has been used in the medical and biological areas as a tool to investigate these altered surface carbohydrates [9], in meningotheial

tumour [10], in fetal minor salivary glands [11] and parotid gland mucoepidermoid carcinoma [12]. Thus, a better understanding of the cell surface glycode of each DCIS lesion is an initial step in identifying the reasons that lead some lesions to progress to invasive behavior. For this, the present study evaluated the expression pattern of different types of carbohydrates in DCIS subtype lesions using lectin histochemistry.

Materials and Methods

Specimens

We performed a per-lesion basis analysis of breast ductal carcinoma *in situ*. The 218 analyzed lesions were classified according to the architectural pattern as comedo, solid, cribriform and micropapillary according to Bellamy [13] and Page [14]. Samples and clinic-histopathological data were obtained from the tissue archives of Pathology Service at Hospital das Clínicas (HC) from the Federal University of Pernambuco (UFPE) after approval by Health Sciences Centre Ethics Board. Cases of DCIS associated with IDC were excluded.

Lectin histochemistry

Four micrometer thick specimens sections were deparaffinized in xylene and hydrated in graded series of alcohol (100-70%). Slices were

***Corresponding author:** Dr. Eduardo Beltrão, Universidade Federal de Pernambuco, Laboratório de Imunopatologia Keizo Asami-LIKA/UFPE, Av. Prof. Moraes Rego, s/n, Campus Universitário, Recife, Pernambuco, 50670-490, Brazil, Fax: + 55-81-2126-8000; E-mail: ebeltrao@hotmail.com

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treated with 0.1% (w/v) trypsin solution for 15 min at 37°C and with a 0.3% (v/v) methanol-H₂O₂ solution for 30 min at 25°C according to Beltrão [10]. After that, samples were incubated, separately, with the biotin conjugated lectins (*Maackia amurensis* agglutinin (MAL-II), *Arachis hypogaea* agglutinin (PNA), Wheat germ agglutinin (WGA), *Phaseolus vulgaris* leucoagglutinin (PHA-L) and *Sambucus nigra* Agglutinin (SNA)-Vector Laboratories Inc., Burlingame, CA, USA) at 70 µg/mL for 2 h at 4°C. For PHA-L lectin after trypsin treatment slides were incubated with a 0.1 U/mL neuraminidase solution from *Clostridium perfringens* (Sigma Aldrich, Missouri, USA), for 1 h at 37°C. After washes with 100mM phosphate buffer solution (PBS), pH 7.2, containing 150mM NaCl, slides were incubated with streptavidin-peroxidase polymer (Sigma Aldrich, Missouri, USA) for 45 min at 25°C. The reaction was revealed with diaminobenzidine (DAB) and counterstained with hematoxylin (PBS was used to prepare all solutions and as washing solution between each step). Negative controls were performed replacing the lectin for PBS. Histochemistry staining was independently analyzed by two pathologists and cases with discrepant scores were reevaluated jointly. The staining intensity was determined by previously study established by Lima [15] as the pattern observed in at least 20% of cells with cytoplasm or membrane staining in four categories: 0=no staining, 1=weak staining, 2=moderate staining and 3=intense staining. For statistical analysis, we dichotomized as negative (0 and 1+) and positive (2+ and 3+) following previously studies [16,17]. Tissue sections were examined using Olympus microscope optic Eclipse 50i (Tokyo, Japan) and an Image Analyses System (software NIS-Elements F version 2.30-Nikon, USA) was used for image acquisition.

Statistical analysis

Data analysis was performed with GraphPad Prism 6.0 (San Diego, CA, USA) using a p-value<0.05 as significant. The relationships between lectin staining and the DCIS morphological classification were analyzed by two-tailed Fisher Exact Test.

Results

The distribution of the lesions per lectin is showed in (Table 1). MAL-II and PNA presented a significant correlation between lectin staining and lesions subtypes. MAL-II results (Figures 1 and 2) revealed that α-2,3-sialic acid expression was increased (p=0.0191) in cribriform (positive in 100% of analyzed lesions) in comparison to comedo (positive in 41.2%) subtype, in addition, the expression of this carbohydrate also increased in cribriform compared to solid (44.4% positive samples) lesions (p=0.0440). The relationship between the staining pattern of the four subtypes of DCIS lesions and PHA-L, SNA and WGA showed no significant correlation.

Lectins	Status	Comedo	Micropapillary	Cribriform	Solid
		n (%)	n (%)	n (%)	n (%)
PHA-L	Positive	16 (94.1)	4 (100)	6 (100)	6 (100)
	Negative	1 (5.9)	0 (0)	0 (0)	0 (0)
MAL-II	Positive	7 (41.2)	4 (100)	6 (100)	4 (44.4)
	Negative	10 (58.8)	0 (0)	0 (0)	5 (55.6)
PNA	Positive	17 (73.9)	11 (91.7)	6 (66.7)	3 (23.1)
	Negative	6 (26.1)	1 (8.3)	3 (33.3)	10 (76.9)
SNA	Positive	14 (93.3)	4 (100)	5 (83.3)	7 (87.5)
	Negative	1 (6.7)	0 (0)	1 (16.7)	1 (12.5)
WGA	Positive	17 (70.9)	6 (60)	10 (71.4)	7 (63.6)
	Negative	7 (29.1)	4 (40)	4 (28.6)	4 (36.4)

Semi-quantitative analysis of the lectin histochemistry staining to morphological subtypes of DCIS.

Table 1: Lectin histochemistry per CDIS lesions status.

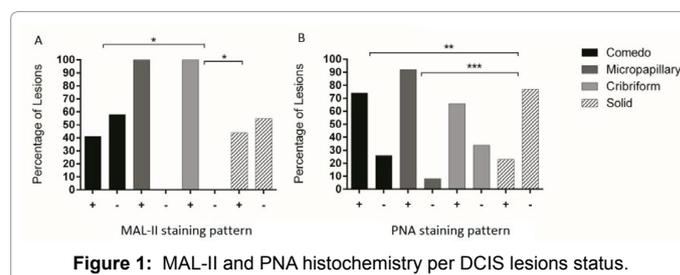


Figure 1: MAL-II and PNA histochemistry per DCIS lesions status.

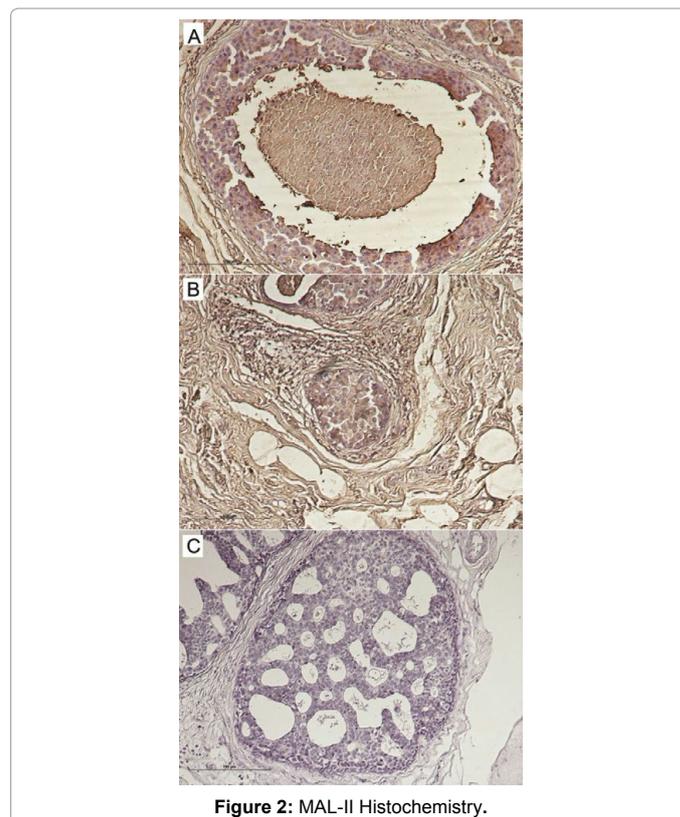


Figure 2: MAL-II Histochemistry.

The expression of β-1-3-N-acetylgalactosamine, recognized by PNA (Figure 3), was increased in both lesions comparisons, comedo (positive in 73.9%) versus solid (positive in 23.1%) with a p=0.0052 and micropapillary (positive in 91.7%) versus solid (positive in 23.1%) with a p=0.0010.

Discussion

Our results showed that different types of DCIS lesions exhibit not only a broad range of differentiation in terms of conventional histologic grades, but also a specific pattern of carbohydrate expression which may help to differentiate the subtypes in a histochemistry way. The presence of necrosis in DCIS lesions, characteristic of comedo/high-grade type, pursue a different course of evolution [18], presenting a more aggressive behavior compared with non-comedo lesions. Comedo type DCIS showed a much higher rate of short-term local recurrence and it is more histologically similar to invasive disease than is the non-comedo-type [2,19].

The comedo-type cell behavior may be related to the carbohydrate expression pattern changes found among the lesions as shown by our results. Besides individual genome alterations in cycle cell controlling

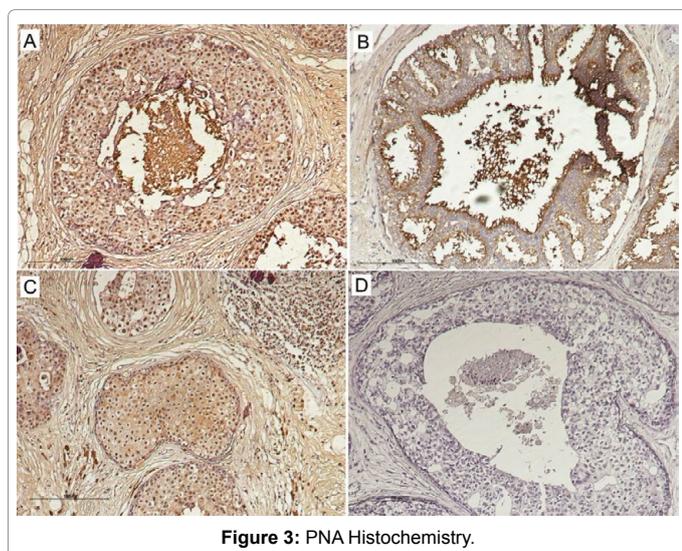


Figure 3: PNA Histochemistry.

genes glycosyltransferases and glycosylhydrolases genes, responsible for the addition and removal of sugars on proteins in the ER and Golgi apparatus, can change activity during oncogenesis, causing different oligosaccharide structures to emerge on cell surface glycoproteins [20].

Our results suggest that α -2,3-sialic acid (recognized by MAL-II) and β -1,3-N-acetylgalactosamine (recognized by PNA) expression patterns changed in response to the necrosis presence. The first carbohydrate exhibited a decrease in its expression on comedo lesions compared to cribriform lesions (non-comedo), contrasting with an increase in expression of β -1,3-N-acetylgalactosamine in comedo compared to solid lesions.

Altered sialylation is one of the most striking features of several tumors and the specific α -2,6 sialylation has most often been associated with poor prognosis, once that is directly connected with migration and invasion [21,22]. In our results α -2,3 sialylation, recognized by MAL-II, was predominant in cribriform lesions, while SNA results showed a predominance of α -2,6 sialylation in comedo and micropapillary lesions, despite the fact that presented no statistical relevance.

The results suggest that DCIS may have a prognosis when an early high degree of α -2,6 sialylation is observed. Moreover, since it is not possible to predict individually whether or not a lesion will progress to an invasive form lectin histochemistry presents that the different carbohydrate pattern found in DCIS can help to understand a possible behavior and further can help to classify the tumor as a unique entity despite the broad diagnosis of DCIS.

The altered expression of both carbohydrates recognized by PHA-L and WGA has already been shown to be involved in numerous cellular pathways as adhesion, motility, angiogenesis, and apoptosis [23]. A higher expression of these carbohydrates was observed in all subtypes of lesions.

According to Allred [18] in the majority of DCIS cases there is a gradual change from well-differentiated to poorly differentiated lesions, suggesting diversity within individual lesions at some point. Our results provide new insights into DCIS subtypes demonstrating that the different carbohydrate profile between them may help to understand the progression and relationship between benign and malignant lesions.

The present work showed that the DCIS subtypes present not only a

morphological difference but also that using lectin histochemistry these tumors present a differential carbohydrate expression. Such feature may, to some extent, suggest an individual identity, especially in the presence of necrosis. In addition, the results reinforce the importance of lectins as important scientific tools for detection of changes in phenotype of breast tumors helping to clarify the existence, or not, of a linear progression between low-grade and high-grade DCIS lesions.

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