Differential Expression of Blood Group Precursor Antigen in Human Breast Cancer Tissue

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Abstract

There is a pressing need for biomarkers for targeted immunotherapy against breast cancer (BCA), the leading cause of cancer death in women. Previously, a blood group precursor O-core epitope gpCl was found to be highly expressed in breast circulating tumor cells (BCTCs) and BCA cell lines with cancer stem cell (BCSC) features. In this pilot study, the breast tissue distribution of gpCl was examined using tissue microarrays (TMAs). Notably, gpCl positive cells were detected in the major histological types of neoplastic breast tissues. Conversely, none of the breast tissues derived from subjects without BCA were gpCl positive. Thus, gpCl expression seems to be tumor-specific but not histological type-dependent, reflecting perhaps its characteristics as a conserved epitope of oncofetal blood group precursor antigens.

Keywords
Blood group precursors; breast cancer; circulating tumor cells; cancer stem cells; glycan marker; tissue microarray

Introduction

Worldwide, breast cancer (BCA) is the most frequent malignancy and the leading cause of cancer death in women [1, 2]. It is a heterogeneous disease at the molecular level, and the treatment concepts have evolved to include biologically directed therapies [1, 3]. There is an urgent need for biomarkers that can provide information about clinical and pathological features and prognosis to guide treatment stratification [4]. It is well known that abnormal glycosylation is involved in virtually every cancer type [5, 6]. Glycan markers take advantage of surface-exposed and easily accessible cellular features and may be important potential BCA biomarkers in the era of precision therapy. Previously, we investigated the expression of a blood group precursor O-core glyco-epitope gpCl in BCA at the cellular level and demonstrated that gpCl is frequently expressed by a number of human BCA cell lines, as well as by breast circulating tumor cells (BCTCs) in stage IV metastatic BCA.
patients [5–7]. Interestingly, in a patient with advanced triple-negative BCA, 92.5% of CTCs (37/40 CTCs) were found to be gpC1 positive [5].

The high expression rate of gpC1 in BCA suggests this blood group precursor antigen may be a BCA biomarker, and the strikingly high percentage of gpC1 positive CTCs in the advanced stage of triple-negative BCA indicate gpC1 is potentially associated with aggressive tumor behaviour. Hence, in the present study, we further investigated the expression of glyco-epitope gpC1 in tissue microarrays from BCA patients who had different histological tumor types classified at various pathological stages.

**Methods and Materials**

A key immunological probe of this investigation was the anti-tumor glycan monoclonal antibody (mAb), G1, which opposes epiglycanin, the major sialomucin glycoprotein (~500 kDa) of murine mammary adenocarcinoma TA3 cells and has been shown to specifically bind human carcinoma-associated antigen in vitro [8, 9]. As has been seen with mAb AE-3, G1 is highly specific for glyco-epitope gpC1 [6]. De-identified human BCA tissue microarrays were obtained from the Cooperative Human Tissue Network (CHTN), mid-Atlantic division (Charlottesville, VA, USA). The immunohistochemistry (IHC) study was performed on formalin-fixed, paraffin-embedded tissue microarray sections using mAb G1. An independent, blinded evaluation was performed by qualified pathologists using three parallel staining results. Statistical analyses were performed using SAS Survey Procedures (SAS 9.4, SAS Institute Inc, Cary, NC, USA). Fisher’s exact test was used for two-group comparisons.

**Results and Discussion**

The IHC study was performed on 64 specimens of the tissue microarray; four samples were excluded because there was not enough tissue for interpretation, and eight samples served as controls. A total of 52 breast tissue specimens were included in the analysis (Table 1). As shown in (Table 1), there were 13 non-neoplastic specimens: six were from patients without breast carcinoma (NB-NC), and seven were from patients diagnosed with breast carcinoma (NB-C). No gpC1 expression was observed in the NB-NC specimens (as indicated by uniformly negative G1 staining). The tissues from the NB-NC and NB-C group showed a gpC1 expression frequency of 2/13 (15.4%), and only one specimen demonstrated focal strong positive staining. The 39 remaining neoplastic specimens exhibited a 26/39 (66.7%) gpC1 positive rate, of which 12/39 (30.8%) showed diffuse positive or focal strong positive patterns. Compared to the non-neoplastic specimens, BCA neoplastic specimens had a higher rate of gpC1 expression (P = 0.0028).

We examined the pattern of gpC1 expression in neoplastic specimens with different pathological grades and histological types. As shown in (Figure 1), gpC1 was overexpressed in a subset of BCA cells within each type of positive specimens, while other BCA cells did not stain. Most gpC1 positive cells were cytoplasmic or membranous stained. Notably, gpC1-positive cancer cells and negative cancer cells co-existed in most of the BCA tissue specimens with the latter as the predominant cell populations while the former appeared like
“seeds” in the “lawn” of negatively stained cancer tissues. Clarke et al. have demonstrated a cancer stem cell (CSC) model in solid tumors [10]. BCSCs are a small subset of BCA cells and thought to have a central role in the initiation and progression of BCA and in the clinical response to therapy. The BCSC related mutation pathways have been reported to correlate with metastases and the markers CD44/CD24 and ALDH1 have been widely used; however, their expression is not always consistent [11, 12]. The gpC1, which is a blood group precursor-based oncofetal antigen, may be explored as a BCSC biomarker candidate, and it showed no cross-reactivity with normal breast tissue in our study [5–7]. In fact, the selective gpC1 expression in NB-C specimens may indicate gpC1 could serve as a cancer-specific target for use in early detection of BCA even before neoplastic changes.

In conclusion, these findings suggest the gpC1 blood group precursor-based oncofetal antigen may be useful in identifying a subset of BCA cells. Further investigation of gpC1 expression level, its relationship with BCA progression, and comparisons with traditional BCSC markers are needed. Further exploration of glycan markers for BCTCs/BCSCs is likely to provide new insight into precision medicine and targeted immunotherapy of BCA.

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Figure 1:
Immunohistochemical study of gpC1 expression in primary and metastatic BCA tissue with different pathological grades and histological types by antibody G1. White arrows show positive staining, and yellow arrows show negative staining. A) Diffuse positive staining in high-grade DCIS (DCIS-H). B) Positive vs. negative staining in an area of DCIS-H. C) Diffuse positive staining in low-grade IDC (IDC-L) (grade 1 and 2). D) Local strong positive vs. negative staining in an area of IDC-L. E) Diffuse positive staining in ILC. F) Positive vs. negative staining in an area of ILC. G) Diffuse positive staining in LNM. H) Positive vs. negative staining in an area of LNM.
Table 1:

Results of breast tissue microarray immunohistochemical analysis of gpC1 expression.

|                      | gpC1 Positive (%) | gpC1 Negative (%) | gpC1 Diffuse or focal strong positive (%) |
|----------------------|-------------------|-------------------|------------------------------------------|
| Non-neoplastic tissue|                   |                   |                                          |
| NB-NC (n = 6)        | 0(0%)             | 6(100%)           | 0(0%)                                    |
| NB-C (n = 7)         | 2(28.6%)          | 5(71.4%)          | 1(14.3%)                                 |
| Non-neoplastic total | 2(15.4%)          | 11(84.6%)         | 1(7.7%)                                  |
| Neoplastic tissue    |                   |                   |                                          |
| DCIS-L (n = 7)       | 5(71.4%)          | 2(28.6%)          | 2(28.6%)                                 |
| DCIS-H (n = 7)       | 4(57.1%)          | 3(42.9%)          | 2(28.6%)                                 |
| DCIS total           | 9(64.3%)          | 5(35.7%)          | 4(28.6%)                                 |
| IDC-L (n = 6)        | 5(83.3%)          | 1(16.7%)          | 3(50%)                                   |
| IDC-H (n = 7)        | 6(85.7%)          | 1(14.3%)          | 2(28.6%)                                 |
| IDC total            | 11(84.6%)         | 2(15.4%)          | 5(38.5%)                                 |
| ILC (n = 5)          | 3(60%)            | 2(40%)            | 2(40%)                                   |
| LNM (n = 7)          | 3(42.9%)          | 4(57.1%)          | 1(14.3%)                                 |
| Neoplastic total     | 26(66.7%)         | 13(33.3%)         | 12(30.8%)                                |

NB-NC: Non-neoplastic breast tissue from patients without breast carcinoma; NB-C: Non-neoplastic breast tissue from breast carcinoma patients; DCIS-L: Ductal carcinoma in situ, low grade; DCIS-H: Ductal carcinoma in situ, high grade; IDC-L: Invasive ductal carcinoma, grade 1 or 2; IDC-H: Invasive ductal carcinoma, grade 3; ILC: Invasive lobular carcinoma; LNM: Breast carcinoma metastatic to regional lymph nodes.