Expression of Glycodelin in Human Breast Cancer: Immunohistochemical Analysis in Mammary Carcinoma In Situ, Invasive Carcinomas and their Lymph Node Metastases

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Abstract. Objective: Glycodelin is a 28 kDa glycoprotein, previously known as placental protein 14 (PP 14). Glycodelin displays immunosuppressive and contraceptive properties. It also suppresses the cytolytic capacity of human natural killer (NK) cells in vitro and inhibits binding of sperm cells to the zona pellucida (the outer membrane of the oocyte). Glycodelin is expressed in normal glandular epithelium of the endometrium as well as in endometrial, ovarian and cervical carcinoma cells. Glycodelin is also expressed in normal and malignant glandular cells outside the reproductive tract, like hidroadenoma, parabronchial glands, sweat glands and pancreatic cystadenoma. Recently, glycodelin was demonstrated to be expressed in normal and cancerous human breast tissue. Materials and Methods: Paraffin-embedded slides of carcinoma in situ (8 DCIS, 2 CLIS), invasive carcinomas without lymph node metastases (9 invasive duct carcinomas, 1 invasive lobular carcinoma) and invasive carcinomas (7 invasive duct carcinomas, 2 invasive lobular carcinomas, 1 mucinous carcinoma) with corresponding lymph node metastases were used. Immunohistochemical staining reaction was used to detect glycodelin expression in the different types of carcinoma in situ, in invasive carcinoma of the human breast and in metastatic carcinoma in axillary lymph nodes. Results: Glycodelin expression was found in all cases of carcinoma in situ (10/10). In the group of invasive carcinoma of the breast (ductal and lobular carcinoma) without lymph node metastases, expression of glycodelin was demonstrated in 9 cases (9/10), of whom 3 cases showed a strong and 6 cases a mediate to weak expression of glycodelin. Only 1 case was negative. In the group of invasive breast cancer with axillary lymph node metastases, in 5 cases (4 ductal and 1 lobular carcinoma) there was no expression of glycodelin, either in the primary tumour tissue, or in the metastatic infiltration of the axillary lymph nodes (5/10). In only one case (invasive ductal carcinoma) was there a strong expression of glycodelin in the primary tumour and a weak expression in the metastasis in axillary lymph node. In 4 other cases (2 ductal, 1 lobular and 1 mucinous), there was a weak staining in primary breast cancer cells and no expression in the metastatic lymph node. Conclusion: These results demonstrate that invasive breast carcinomas without axillary lymph node metastases (better prognosis) are more likely to express glycodelin. In contrast, the cases of breast cancer with metastatic infiltration of the axillary lymph nodes showed no (or weak) expression of glycodelin (worse prognosis). On the basis of these results, we speculate that glycodelin expression could be used as a prognostic marker for breast cancer. This has to be confirmed in larger studies.

Carcinoma of the breast is the most frequent malignant tumour in women, with high incidence and mortality in most developed countries. Early detection and adequate therapy are very important (1). Among many prognostic factors, the most important are the tumour size, histological grade and lymph node involvement. Lymph node involvement is a powerful prognostic factor. Numerous studies have shown that patients with axillary lymph node metastases have an independently increased risk for relapse and for cancer-associated death, compared to patients without nodal metastasis (2).

Glycodelin, previously known as placental protein 14 (PP14) (3), which is immunologically indistinguishable from chorionic α2-microglobulin (CAG-2) (4) and placental
specific α2-microglobulin (PAMG-2) (5), is expressed in glandular epithelium of the endometrium (6) and decidua (7). Glycodelin suppresses the cytolytic capacity of NK-cells (natural killer cells) in vitro (8). Our own investigations (9) suggested a relationship between serum levels of glycodelin and threatened abortion.

Table 1. Antibodies used in the study.

<table>
<thead>
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<th>Epitope</th>
<th>Antibody</th>
<th>Isotype</th>
<th>Concentration</th>
<th>Source</th>
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<tr>
<td>Glycodelin A</td>
<td>A87-D/F4</td>
<td>Mouse IgG</td>
<td>2 μg/ml</td>
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<tr>
<td>Glycodelin peptide</td>
<td>-</td>
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Figure 1. A. Expression of glycodelin in ductal carcinoma in situ (DCIS) with comedo necrosis, 10x. B. Expression of glycodelin in invasive ductal carcinoma without metastasis, 25x. C. Expression of glycodelin in invasive ductal carcinoma with lymph node metastasis, 10x. D. Expression of glycodelin in lymph node metastasis, 10x.

Figure 2. Staining intensity of glycodelin in breast cancer subtypes [carcinoma in situ (Ca in situ), invasive carcinoma without lymph node metastasis (Ca w/o LNM), invasive carcinoma with lymph node metastasis (Ca w LNM) and lymph node metastases (LNM)] determined by computerised analysis of the immunohistochemical reaction on the different tissue slides.
Glycodelin was shown to have a molecular weight of 28,000, with two identical subunits held together by noncovalent bonds and a carbohydrate content of 17.5% with a unique carbohydrate configuration, consistent with sialylated LacdiNAc structures that are very unusual for mammals (10). Julkunen and coworkers confirmed the findings of homology between glycodelin and β-lactoglobulins by deducing its complete amino acid sequence (11). Two isoforms of glycodelin have been described; glycodelin A and a similar glycoprotein, glycodelin S, which is found in seminal plasma, but is differently glycosylated from glycodelin A (12).

There are several other prognostic and predictive factors for breast cancer, which are still under investigation. The present study was designed to analyse glycodelin expression in different types of in situ and invasive breast carcinoma, and to address its role in metastatic tissue of breast cancer.

Materials and Methods

Forty formalin-fixed paraffin-embedded tissue blocks from patients, who underwent surgery for breast tumour were obtained. The tissue samples were classified according to the histological classification of breast cancer: intraepithelial neoplasia (8 patients with DCIS, 2 patients with CLIS), invasive carcinomas without metastasis (9 invasive ductal carcinomas, 1 invasive lobular carcinoma) and invasive carcinomas (7 invasive ductal carcinomas, 2 invasive lobular carcinomas, 1 invasive mucinous carcinoma) with corresponding lymph node metastases

Immunohistochemistry. Immunohistochemistry was performed on paraffin sections (7 μm) of the different breast cancer tissue specimens, as described previously (13). Briefly, sections were incubated in methanol/H2O2 (30 min) to inhibit endogenous peroxidase activity, washed in phosphate-buffered saline (PBS) (5 min) and treated with goat serum (20 min, 22°C) to reduce non-specific background staining. Incubation with the primary antibody (Table I) was done overnight at 4°C. Sections were then thoroughly incubated with the biotinylated secondary anti-mouse antibody (1 h, 22°C) and avidin-biotinylated peroxidase (45 min, room temperature). Between each step, the sections were washed with PBS (pH 7.4) three times. Peroxidase staining reaction was done with diaminobenzidine/H2O2 (1 mg/ml; 5 min) and stopped in tap water (10 min). Sections were counter stained in haemalaun (1 min) and then cover-slipped. In controls, the primary antibody was replaced with pre-immune mouse serum. Positive and negative controls were always included. All specimens were evaluated by a pathologist with experience in immunohistochemistry. The classification of the stained cells as either micrometastases or tumour cells also required histomorphological characterisation. Unspecific reactions or staining of endothelial cells in the marginal sinus of lymph nodes were not considered to be related to tumour cells.

Computerized analysis of antigen expression. The level of antigen expression was determined in a blinded fashion in one run with identical staff, equipment and chemicals. From each section, 5 digital pictures were taken at random of different areas of the tumour tissue (200-fold magnification; 3CCD colour camera; Hitachi HV-C20M; Hitachi Denshi Ltd, Japan, and Axiolab, Carl Zeiss, Germany). For standardization of the measurement, in each picture the optical density of white background colour was attuned to 250. For all sections, we assessed the mean optical density and the quantity of pixels which had a positive reaction for glycodelin using the KSRun software (imaging system KS400, release 3.0; Zeiss, Vision GmbH, Germany).

Statistical methods. The SPSS/PC software package, version 6.01 (SPSS GmbH, München, Germany), was used for collection, processing and statistical analysis of all data. All p values resulted from two-sided statistical tests and p≤0.05 was considered to be significant.

Results

Expression of glycodelin in breast cancer tissue. We found a strong expression of glycodelin in tissue slides of carcinoma in situ, with 100% of the cases (10/10) being positive for this antigen (Figure 1A). Invasive carcinoma tissue without axillary lymph node metastases (LNM) showed similar, but partially reduced, expression of glycodelin compared to carcinoma in situ (Figure 1B). We identified expression of this antigen in 90% of the invasive carcinomas without axillary LNM. A reduced expression of glycodelin was identified in tumour cells with LNM (Figure 1C). We found an expression of this antigen in 50% of the cases with node-negative breast cancer. The expression of glycodelin in LNM (Figure 1D) was comparable to that in the primary tumour.

Computerized quantification of glycodelin expression in breast cancer tissue. The computerized analysis of staining intensity (optical density) is summarized in Figure 2. We identified significant differences of glycodelin staining between carcinoma in situ and LNM (p=0.028).

Discussion

In this study, we analysed the expression of the glycoprotein glycodelin in forty formalin-fixed paraffin-embedded tissue blocks from patients who underwent surgical removal of a breast tumour. Glycodelin is mainly synthesized in secretory endometrial glands, by gestational decidua, in seminal vesicles (6, 7, 13, 14), in the ovary (15), as well as in megakaryocytic/erythroid precursors of the bone marrow (16). Furthermore, glycodelin is expressed in a variety of tumours. Investigations of glycodelin expression in ovarian cancer (15) showed that polyclonal and monoclonal antibodies against PP14 stained negative in serous cystadenomas and mucinous ovarian tumours. The same antibodies were used to investigate the glycodelin expression in breast cancer cells (17). Glycodelin expression was found in ductal carcinomas, tubular carcinomas, mucinous carcinomas, mixed ductal/tubular carcinomas and lobular carcinomas. The results were confirmed by Northern blot analysis and RT-PCR. Investigations with a polyclonal
antibody against a synthetic glycodelin peptide sequence (18) on endometrial, ovarian and cervical cancer showed that glycodelin expression is elevated in ovarian and endometrial cancer tissue. These results were confirmed at the mRNA-level by RT-PCR. In addition, transfection of glycodelin cDNA into breast cancer cells induced an alteration of the cell growth behaviour, leading to formation of acinar configurations and apoptosis. The glycodelin-expressing cells displayed strongly up-regulated expression of markers of organized epithelia. The rate of proliferation was also suppressed. These results indicate that the expression of glycodelin is accompanied by a phenotype of organized glandular epithelium in breast cancer cells (19). 

Our results seem to confirm these findings. In summary, invasive breast carcinomas without metastasis (better prognosis) are more likely to express glycodelin. In addition, no (or weak) expression of glycodelin (worse prognosis) was observed in positive breast cancer cases. On the basis of these results, we suggest that glycodelin expression could be used in the future as a prognostic marker for breast cancer. This has to be confirmed in larger studies.

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References


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