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Glycan-related gene expression signatures in breast cancer subtypes; relation to survival

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ABSTRACT

Alterations in glycan structures are early signs of malignancy and have recently been proposed to be in part a driving force behind malignant transformation. Here, we explore whether differences in expression of genes related to the process of glycosylation exist between breast carcinoma subtypes – and look for their association to clinical parameters. Five expression datasets of 454 invasive breast carcinomas, 31 ductal carcinomas in situ (DCIS), and 79 non-malignant breast tissue samples were analysed. Results were validated in 1960 breast carcinomas. 419 genes encoding glycosylation-related proteins were selected. The DCIS samples appeared expression-wise similar to carcinomas, showing altered gene expression related to glycosaminoglycans (GAGs) and N-glycans when compared to non-malignant samples. In-situ lesions with different aggressiveness potentials demonstrated changes in glycosaminoglycan sulfation and adhesion proteins. Subtype-specific expression patterns revealed down-regulation of genes encoding glycan-binding proteins in the luminal A and B subtypes. Clustering basal-like samples using a consensus list of genes differentially expressed across discovery datasets produced two clusters with significantly differing prognosis in the validation dataset. Finally, our analyses suggest that glycolipids may play an important role in carcinogenesis of breast tumors – as demonstrated by association of *B3GNT5* and *UGCG* genes to patient survival.
In conclusion, most glycan-specific changes occur early in the carcinogenic process. We have identified glycan-related alterations specific to breast cancer subtypes including a prognostic signature for two basal-like subgroups. Future research in this area may potentially lead to markers for better prognostication and treatment stratification of breast cancer patients.
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1. Introduction

It is believed that changes in glycan structure take place early during the malignant transformation. Glycosylation is increasingly recognized as one of the most important modifications of proteins and lipids in mammalian cells, but is still a very challenging process to study. The fundamental mechanism of glycosylation involves sequential addition of monosaccharides to a target structure by a number of glycosyltransferases, ultimately resulting in a covalently bound oligosaccharide. Further chemical modifications and branching produce a great variety of structures. The various glycosylation pathways appear to be intricately regulated and the different products are involved in a substantial number of cellular processes including adhesion and regulation of growth and growth factor receptor modulation.

Due to their widespread distribution, shedding and early alteration in carcinogenesis, glycans have been recognized as both potential biomarkers and a great opportunity as targets for cancer vaccines (Gilewski et al., 2007; Miles et al., 2011; Slovin et al., 2005; Tarp and Clausen, 2008) and possibly for antitumor antibodies (Rabu et al., 2012). Research investigating these potentials in a variety of carcinomas, including breast, has yielded encouraging results, although no therapeutic strategies have yet reached the clinic. Since complexity, lack of understanding and failure to adequately select patient groups which might benefit from the administered treatment have all been cited as main obstacles for successful management of the disease (Cazet et al., 2010), further characterization of glycosylation as well as identification of carcinoma subgroups based on their glycan gene signature is of high value.

We have previously shown that gene expression of glycosylation-related genes is highly different in breast carcinomas compared to non-malignant tissue of the breast (Potapenko et al., 2010). Breast cancer is a complex and heterogeneous disease, and over the last decade research aiming at classification and description of breast tumors based on gene expression (Fan et al., 2006; Sorlie et al., 2003; Sørlie et al., 2001), chromosomal aberrations (Curtis et al., 2012; Russnes et al., 2010) and methylation (Fleischer et al., 2014; Holm et al., 2010; Rønneberg et al., 2011) has led to important findings that can add to the histological and pathological characterization in defining clinically relevant patient groups. However, little is known about the variation of glycan epitopes in different subgroups of breast carcinomas. This prompted us to investigate whether any alterations in mRNA expression of glycosylation related genes could be found between and within breast carcinomas of different expression subtypes, histopathological types and relevant for clinical outcomes.

Results demonstrate that the PAM50 intrinsic subtypes of breast cancer (as defined by Parker et al. (Parker et al., 2009) and Sørlie et al. (Sørlie et al., 2001)) seem to differ in expression of genes related to glycosylation, and in particular genes coding for glycan binding proteins. Some of the genes implicated in glycan metabolism and binding were shown to have a strong association with survival and have therefore a potential as prognostic markers.

2. Materials and methods

2.1. *Patient cohorts*

Data from five patient cohorts (MicMa, DBCG, ULL, MDG and DCIS) were chosen for discovery and the METABRIC cohort for validation purposes.

The Micrometastases (MicMa) cohort consists of 115 breast carcinoma samples from patients originally included in the Oslo Micrometastasis Project. Specimens were collected from patients treated for localized breast cancer (stage I and II) between 1995 and 1998 (Wiedswang et al., 2003). Total RNA was isolated from fresh frozen tumors using TRIzol reagent (Invitrogen), and then applied to Agilent 44k expression one-color oligo microarrays. Microarrays were scanned using an Agilent DNA microarray scanner and processed in GenePix Pro v 4.1. Patients were followed for an average of 85 months (range 0.8–127) (Enerly et al., 2011; Naume et al., 2007).

The Danish Breast Cancer Cooperative Group (DBCG) dataset is comprised of tumor tissue from 195 high-risk patients collected as part of the Danish Breast Cancer Group trials 82 b and c (Nielsen et al., 2006). High risk was defined as presence of tumor size greater than 5 cm and/or positive axillary lymph nodes and/or local invasion of skin or pectoral muscle fascia. Collection of specimen was done at total mastectomy during the period of 1982–1985, and follow-up time was on average 9 years (range 0.5–22). Total RNA was extracted with Qiagen Midi kit Extraction column procedure (Qiagen) and analyzed using Applied Biosystem Human Genome Survey Microarray v.2.0 (Myhre et al., 2010).

The third tumor cohort is designated “ULL” (Ullevål cohort) and contains data from 80 sequentially collected breast cancer samples (Langerød et al., 2007). Samples were acquired during a four-year period between 1990 and 1994 at the Oslo University Hospital Ullevål and snap frozen. Isolation of total RNA was done using TRIzol reagent (Invitrogen). Expression levels were then assessed by cDNA arrays, data from which was processed in GenePix Pro v 4.1. Breast cancer patients participating in this study were on average followed for 96 months (range 6–178).

The Mammographic Density and Genetics (MDG) dataset contained expression data from 64 breast carcinoma samples, and 79 non-malignant breast tissues (Haakensen et al., 2011). These were collected between 2002 and 2007 from patients who were referred by a general practitioner or as a result of suspicious mammographic findings during participation in the National mammography screening program in Norway. RNA was extracted using the RNeasy Mini Protocol (Qiagen) and hybridized to Agilent 44k arrays, scanned with an Agilent scanner and processed using Feature Extraction 9.1.3.1 software. Survival data was not available at the time of writing.

The DCIS dataset used in our analyses originated as a subset of a larger study carried out between 1986 and 2004 in Uppsala, Sweden. A total of 31 ductal carcinoma *in situ* (DCIS) samples were analyzed with Agilent 44k microarrays. Scanning was performed using an Agilent microarray scanner

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