Role of N-acetylgalactosaminyltransferase 6 in early tumorigenesis and formation of metastasis

FRIEDERIKE LIESCHE¹, ALEXANDRA C. KÖLBL¹, MATTHIAS ILMER², STEFAN HUTTER¹, UDO JESCHKE¹ and ULRICH ANDERGASSEN¹

¹Department of Obstetrics and Gynaecology, Ludwig Maximilians University Munich, D-80337 Munich; ²Department of Surgery, Klinikum Grosshadern, Ludwig Maximilians University Munich, D-81377 Munich, Germany

Received October 22, 2015; Accepted February 18, 2016

DOI: 10.3892/mmr.2016.5044

Abstract. Glycosylation is one of the most important posttranslational modifications of proteins and lipids that contributes to the structural diversity of cellular molecules. Enzymes of the glycosyltransferase class are responsible for altering glycosylation patterns by adding carbohydrate chains to the respective acceptor molecules. It is well known that glycosylation is commonly altered in cancerous tissue. Therefore, the present study aimed to determine the incidence of N-acetylgalactosaminyltransferase 6 (GALNT6), a prominent member of the glycosyltransferase class, in breast cancer tissue of different developmental stages by immunohistochemistry. Although no correlation was identified between tumour characteristics and GALNT6 staining intensity, to the best of our knowledge, this is the first study to demonstrate that tissue from carcinoma in situ-tumours and metastases were more heavily stained than late-stage breast cancers. This may indicate an important role of glycosylation aberration in escaping the immune system at early phases of tumour development. The present study also hypothesised that nascent or early metastasizing tumours are normally recognized by the immune system of the patient, but glycosylation pattern changes may facilitate tumor escape from immune recognition. In follow-up studies, our group will aim to confirm and consolidate these results in a larger patient cohort that may give greater insight into breast cancer characterization as well as tumour treatment.

Introduction

Glycosylation is the most frequent post-translational modification of proteins and lipids, which adds to the large

Correspondence to: Professor Udo Jeschke, Department of Obstetrics and Gynecology, Ludwig Maximilians University Munich, 11 Maistrasse, D-80337 Munich, Germany E-mail: udo.jeschke@med.uni-muenchen.de

Key words: glycosyltransferase, breast cancer, immunohistochemistry, carcinoma *in situ*, metastasis

structural diversity of cellular molecules (1). Functionally, this process contributes to altering cell adhesion properties (2,3) and influencing intra- and intercellular communication (4). Furthermore, protein glycosylation can be essential in antigen recognition by the immune system (5).

Malignant transformation of cells is frequently accompanied by alterations in the post-translational modification of proteins (6-8). This was substantiated first in 1985 by the use of antibodies against altered carbohydrate chains (9) and was more recently confirmed in cell culture models (10). The most frequent alterations, particularly in adenocarcinomas (11), are mucin-type-O-glycosylations, giving rise to glycosylation patterns that can only be found in cancer, not in normal cells (8,12). Cancer-associated glycans form novel glycopeptide epitopes that can be targeted by the immune system (13,14). Hence, these altered glycopeptides can exhibit diverse and even opposing roles in immunogenicity (15). Conversely, they can either have immunogenic character themselves or new conformational changes (16,17), or can provoke anti-tumour responses (18). These activities can then lead to elevated antibody levels against the pancarcinoma antigen, which is regarded as a marker for increased survival (19). As a consequence, altered glycosylation is critical in early phases of tumourigenesis as well as in tumour progression. However, it also offers novel angles for diagnostic, prognostic and therapeutic strategies in cancer (20-22).

In breast cancer, altered glycosylation is frequently associated with a worse prognosis and shorter overall survival (23). Shortened O-glycans and increased sialylation are prevalently found in this tumour type. Furthermore, morphological and phenotypic transformation of epithelial cells caused by alterations in glycosylation patterns was shown, which ultimately leads to a higher incidence of remote metastasis (4).

Changes in glycosylation are predominantly caused by changes in the expression of glycosyltransferases, a class of enzymes that are responsible for the transfer of carbohydrate chains to acceptor sites on proteins and lipids (24,25). Expression profiles of glycosyltransferases have been extensively investigated (17,26-29) and it was reported that they may serve as an important marker for tumour characterization (30-32).

The present study aimed to determine the specific role of glycosyltransferase N-acetylgalactosaminyltransfera

se 6 (GALNT6) in breast tumourigenesis. Accordingly, paraffin-embedded tissue samples of primary tumours including adjacent healthy tissue as well as paired metastatic sites were immunohistochemically stained. It was demonstrated that GALNT6 is correlated with the occurrence of lymph node metastasis, local recurrence and remote metastasis (33). Moreover, GALNT6 is known to be involved in the first steps of O-glycosylation (33). The mRNA for this enzyme was shown to be expressed in breast cancer and associated with smaller tumours (T1) (34). This association was confirmed in the present study. It was hypothesised that certain glycosylation motifs may enable immune escape mechanisms of small tumours, as well as remote metastasis formation to different sites since the latter requires alterations in cell adhesion and detachment that partially depend on glycosylation changes (35,36).

Materials and methods

Tissue samples. Tissue samples for the study were obtained from the pathology archive of the Department of Gynaecology and Obstetrics, Ludwig Maximilians University of Munich, (Munich, Germany).

In total, 44 patients diagnosed and treated with surgery for primary breast cancer between 1998 and 2002 were followed up for 8 years and included in the study. Research conducted on patients was in compliance with the Helsinki declaration. The present study was approved by the ethical committee of Ludwig-Maximilians University of Munich (approval nos. 148-12 and 048-08). Patients were asked to sign written informed consent prior to the use of tissue samples for investigation by the current study.

For the current study, 10 breast cancer samples from patients with lymph node metastasis, 10 samples from patients with local recurrence and 8 samples from patients with metastasized breast cancer were included. For all these samples, tissue sections of the primary tumour as well as diseased lymph node, local recurrent tumour or remote metastases were available. The histological classification, and analyses of grading and hormone- and Her2-receptor-status were performed pathologically, subsequent to surgery.

As controls, 8 samples of breast cancer diagnosed as ductal carcinoma *in situ* and 8 samples of primary tumours without local recurrence or remote metastasis were used.

Patients had an average age of 57 years (range from 33-87 years) at time of surgery. TNM-staging of the tumours and their histology were determined postoperatively by a pathologist and location of recurrence or metastases were also noted. The intensity and amount of staining of the tumour tissues for ER, PR and Her2 were asserted and categorized (- no staining, + slight staining, ++ moderate staining, +++ strong staining). The determination and classification of staining intensity for GALNT6 for the primary tumour (PT) and tissue of affected lymph nodes (LN), recurrence (Rec.) or metastasis (Met.) were determined as described in Table I.

Immunohistochemistry. Paraffin-embedded (Merck Milipore, Darmstadt, Germany) tumour blocks were cut into 2-3 μ m sections with a sliding microtome (Leica Microsystems GmbH, Wetzlar, Germany), placed on covered microscope slides (Menzel GmbH, Braunschweig, Germany) and air-dried overnight. Samples were then incubated in Xylol (Merck Milipore). After Xylol removal, endogenous tissue peroxidase activity was inhibited by incubation of the samples in 3% H_2O_2 (VWR International, Wayne, PA, USA). Then, samples were heated for 5 min in a pressure cooker in a Na-citrate buffer (Merck Milipore) at pH 6 in order to dissolve protein crosslinks that arise from the fixation procedure. The tissue samples were washed in water and phosphate-buffered saline (Biochrom GmbH, Berlin, Germany).

The prepared slides were first incubated in normal goat serum (Vector Laboratories, Inc., Burlingame, CA, USA) to prevent unspecific binding of the primary antibody. After removal of the blocking solution (Vector Laboratories, Inc.), primary antibodies were added at concentrations determined in positive control sample staining.

Incubation with the polyclonal IgG rabbit anti-GALNT6 (1:1,000; cat. no. GTX104602; GeneTex, Inc., Irvine, CA, USA) primary antibody was conducted for 18 h at 4°C. Slides were then washed three times with phosphate-buffered saline (PBS) for 5 min and subsequently incubated with biotinylated secondary antibody solution (from the HRP100 staining kit; Zytomed Systems GmbH, Berlin, Germany) for 30 min at room temperature. When the secondary antibody (Vector Laboratories, Inc.) was removed by a subsequent wash with PBS, ABC-reagent (Vector Laboratories, Inc.) was applied to the slides for 30 min. Next, a solution of DAB-reagent (Dako; Agilent Technologies, Santa Clara, CA, USA) was added to the slides for 1 min. DAB is the substrate for biotin-coupled peroxidase, the reaction results in a brown precipitate, which can then be evaluated by Leitz Diaplan light microscope (Ernst Leitz GmbH, Wetzlar, Germany). The slides were washed under running tap water to arrest the enzyme reaction. Nuclei were counterstained with Mayer's hemalum (AppliChem, Darmstadt, Germany) for 5 min and samples were then dehydrated and embedded in Eukitt (Medite, Burgdorf, Germany). The stained samples were then evaluated or stored at room temperature.

Prior to the staining procedure on tumour tissue samples, positive and isotype control samples were stained (Fig. 1). For the positive control, a sample from a tissue (placenta, collected after birth) known to overexpress the antigen of interest was stained to test antibody function and to determine an ideal dilution of the antibody. The isotype control should reveal background staining due to primary antibody. Therefore the tissue was stained, which was also used for positive control, with an isotype control serum instead of a primary antibody solution.

Microscopy and evaluation of staining. Samples were visualized with a Leitz Diaplan light microscope. Four objectives with different magnifications (6,3X, 10X, 25X and 40X) were used.

Staining was evaluated according to the immune-reactive-score (IRS) described by Remmele and Stegner (37). The IRS is obtained by multiplication of the staining intensity with the number of stained cells. Briefly, staining intensity is classified into groups from 0 to 3 (0 means 'no staining reaction' and 3 means 'strong color reaction') and the number of stained cells is similarly classified (0 means '0% stained cells' to 4 '81-100% stained cells'). Thus, the IRS is in a range of 0 to 12.

Table I. Patient/tumour data in correlation to IRS for GALNT6.

Patient no.	Age (years)	Histology	TNM	Size (cm)	Loc. Met/Rec.	ER	PR	Her2	IRS PT	IRS LK. /Rec/Met.
1	56	Duct. Lob.	pT1a, pN0, MX	1,2 mult.	None	-	+	ND	8	None
2	67	Duct.	pTis, pNX, MX	0,8	None	ND	ND	ND	ND	None
3	49	Duct.	pTis, NX, MX	0,9	None	ND	ND	ND	8	None
4	47	Duct.	pTis, NX, MX	1,1	None	+	+	ND	8	None
5	55	Duct.	pTis, NX, MX	0,3	None	ND	ND	ND	4	None
6	45	Duct.	pTis, NX, MX	0,7	None	ND	ND	ND	12	None
7	57	Duct.	pTis, NX, MX	4,0 mult.	None	ND	ND	ND	8	None
8	72	Duct.	pTis, NX, MX	0,9	None	ND	ND	ND	8	None
9	51	Duct. + Cis	pT1c, pN0, MX, G3	1,5 mult.	None	+	+	ND	4	None
10	55	Duct.	pT1c, pN0, MX, G3	1,2	None	+	+	ND	ND	None
11	57	Duct.	pT1c, pN0, MX, G2	1,4 mult.	None	+	+	ND	4	None
12	59	Lob.	pT2, pN0, MX	2,8 mult.	None	-	+	ND	4	None
13	75	Duct.	pT1c, pN0, MX, G3	1,3	None	+	+	ND	4	None
14	49	Duct.	pT2, pN0, MX, G3	2,7	None	-	-	ND	4	None
15	77	Duct. + Cis	pT2, pN0, MX, G2	2	None	+	+	ND	4	None
16	55	Duct.	pT2, pN0, MX, G2	3,2	None	+	+	ND	4	None
17	58	Lob.	pT1c, pN2, MX	1,4 mult.	LN	+	+	ND	4	ND
18	33	Duct.	pT1c, pN1bi, MX, G2	1,5	LN	+	+	ND	4	4
19	63	Duct.	pT2, pN1biii, MX, G3	2,5	LN	+	+	ND	12	8
20	60	Lob.	pT1c, pN2, MX, G3	3,5	LN	+	-	ND	8	8
21	45	Duct.	pT1c, pN2, MX, G3	1,2	LN	-	+	ND	4	4
22	54	Duct.	pT2, pNbiii, MX, G2	3,3	LN	+	+	ND	12	8
23	77	Duct.	pT1c, pN1biii, MX, G2	1,2	LN	+	+	ND	8	8
24	48	Duct. + Cis	pT1b, pN1bi, MX, G3	1	LN	-	-	ND	0	4
25	72	Duct.	pT2, pN1biv, MX, G3	2,1	LN	-	-	ND	8	8
26	65	Duct.	pT2, pN1biii, MX, G3	2	LN	+	+	ND	8	8
27	45	Duct. + Cis	pT1c, pN1bi, MX, G2	1,5 mult.	Thor. Wall	-	-	+++	2	4
28	64	Duct.	pT1c, pN0, MX, G2	1,9 mult.	Intra-mam.	++	-	+	12	12
29	57	Duct. + Cis	Post CTx	1	Intra-mam.	-	+	+	3	n.d.
30	87	Lob.	pT4, pNX, MX	1,5	Thor. Wall	+++	-	++	2	1
31	74	Duct.	pT1c, pN0, MX, G2	1,8	Thor. Wall	+	+	-	8	3
32	53	Duct. + Cis	pT1a, pNX, MX	0,1 mult.	Ulc.	-	-	+++	8	4
33	39	Duct. + Cis	pT2, pNbiii, MX, G2	3	Intra-mam.	+++	++	+++	4	4
34	38	Duct.	pT1c, pNX, MX, G2	1,5 mult.	Thor. Wall	+++	+	+++	4	8
35	58	Med.	pT1c, pN0, MX	1,8	Intra-mam.	-	-	+++	1	4
36	52	Duct. + Cis	pT1, pN1bi, MX	1	Intra-mam.	-	-	+++	4	4
37	51	Lob. + LCIS	pT1c, pN1a, M1	1,8	Skin	-	+	++/+++	3	4
38	78	Lob.	pT1c, pN1biii, M1, GND	1,5 mult.	Contr. Ax. LN	+	-	+++	4	12
39	45	Duct. + Cis	pT1c, pN1bi, M1, G2	1,5 mult.	Contr. Ax. LN	-	-	+++	2	4
40	71	Duct. + Cis	pT2, pN1biv, M1, G2	2	Contr. Ax. LN	-	-	+++	8	8
41	33	Small cell Ca.	pT1c, pN1bii, M1	10	Ovary	-	+	+	12	12
42	38	Duct.	pT2, nP1a, M1, G2	2,5	Contr. Ax. LN	-	-	++	ND	12
43	58	Med.	pT1c, pN0, M1	1,8	Contr. Ax. LN	-	-	+++	3	1
44	41	Duct.	pT4d, pN1bii, M1	1,3 mult.	Contr. Ax. LN	+	+	+++	8	8

No. 1-8, DCIS; no. 9-16, primary tumour; no. 17-26, tumour with lymph node affection; no. 27-36, tumour with recurrence; no. 37-44, tumour with metastasis; Duct, ductal; Lob, lobular; Med, medullar; Small cell Ca, small cell carcinoma; pT, tumour size; pN, nodal staging; pM, metastatic staging; G, grading; CTx, chemotherapy, ND, not detected; mult, multifocal; LN, lymph node affection; IRS PT, immune-reactive-score primary tumour; IRS LN/Rec/Met, immune-reactive-score of affected lymph nodes, recurrence or metastasis tissue; TNM, tumor size, nodal involvement, metastasis; Thor. Wall, Thoracical wall; Intra-mam, within the breast; Ulc, ulcerated; Contr. Ax.LN, axillar lymph nodes of the contrary side.

Statistical analysis. Statistical analysis was conducted using SPSS version 20.0 (IBM, Armonk, NY, USA). Since patient samples are not normally distributed, non-parametric Mann-Whitney-U-Test comparing two variables was applied; in cases of more variables, Kruskal-Wallis Test was used. A P \leq 0.05 was considered to indicate a statistically significant difference.

Results

Staining intensity correlation with patient data. Breast cancer tissues at different phases of disease progression (Cis, primary tumour tissue, tumour with lymph node infiltration, recurrent tumours and metastasized tumours) were immunhistochemically stained for GALNT6 (Fig. 1). IRS 4312

Table II. GALNT6 staining intensity of tissue samples.

Type of sample	Number of samples	Average IRS	Minimum IRS	Maximum IRS	Standard deviation
DCIS	7	8.00	4.00	12.00	2.309
Primary tumour	7	4.00	4.00	4.00	0.000
Tumour with lymph node affection	10	6.80	0.00	12.00	3.795
Affected lymph nodes	10	6.00	0.00	8.00	2.828
Tumour with recurrence	10	4.80	1.00	12.00	3.458
Recurrence tissue	9	4.89	1.00	12.00	3.219
Tumour with remote metastasis	7	5.71	2.00	12.00	3.684
Metastasis tissue	8	7.63	1.00	12.00	4.274

GALNT6, N-acetylgalactosaminyltransferase 6; DCIS, ductal carcinoma in situ; IRS, immune-reactive-score.



Figure 1. Light microscopy images of control and tumour tissue samples taken with 10X objective.

was determined by light microscopy by two independent investigators. Subsequently, IRS was correlated with patient and tumour characteristics in order to detect associations between tumour features and the GALNT6 staining intensity (Table I). The following parameters were included in the study: Age of the patient at primary diagnosis, tumour histology, TNM-staging of the tumour, tumour size and hormone (estrogen and progesterone)- and Her2-receptor status (if available). Extensive statistical analysis revealed no significant correlation between these investigated patient-related factors and the staining intensity of GALNT6.

Staining intensity of different tumour stages. It was then evaluated whether mean IRS-values for GALNT6 of the samples was correlated with different developmental stages of the tumour samples (Table II). Notably, the highest staining intensity was demonstrated in precursor lesions (Cis-tumours) with an average IRS of 8.00 and in metastases tissue samples

Table III. Statistical comparison of GALNT6-IRS.

Group	P-value ^a		
Tumours with affected lymph nodes	0.546		
Tumours with recurrence	0.172		
Tumours with remote metastasis	0.443		
Tumours with affected lymph nodes/recurrence/metastasis	0.541		

^aCompared with control (ductal cell in situ or primary tumor). GALNT6, N-acetylgalactosaminyltransferase 6; IRS, immune-reactive-score.

with an average IRS of 7.63. Thus, the pre-invasive form of breast cancer intraductal carcinoma (also Cis), newly arising small tumours and metastatic lesions display the highest incidence of GALNT6.

Statistical analysis of GALNT6-staining. Lastly, the IRS values of the control group (DCIS- and primary tumours) were statistically compared with the more advanced tumour stages (Table III). No significant differences were identified between the control group and tumours with infiltrated lymph nodes (P=0.546). In addition, no statistical significance was identified when comparing the control group with the recurrence-group (P=0.172) or the metastasis group (P=0.443). Finally, comparisons of the control group with all other tumour stages together did not reveal any statistically significant results (P=0.541).

Discussion

The present data did not identify a direct correlation between GALNT6-staining and the age of primary diagnosis, TNM-staging, tumour size and histology, and hormone (estrogen and progesterone)-receptor and Her2-status. However, the results presented here may be regarded as preliminary, as they were collected from a small patient collective.

Notably a coherence between GALNT6-staining and different developmental stages of the tumours was identified, as Cis-tumours and metastatic tumour mass are highly stained. This is consistent with a previous study (38) that demonstrated a correlation between the occurrence of glycosyltransferases and tumour size and grade. It could be shown, that glycosyltransferases, particularly GALNT6, are identified in recently formed tumours. Knockdown of GALNT6 by small interfering RNA slowed breast cancer cell growth by increasing cell adhesion. Furthermore GALNT6 was shown to be important for the stabilization of the MUC1-oncoprotein (33). GALNT6 is critical in the fibronectin pathway, regulating cellular morphology and morphological changes, which are required for the transformation of a normal epithelial cell into a tumour cell, and is thereby an important component for breast cancer development and progression (39). Consistently, a recent study in lung cancer research demonstrated that cigarette smoke induces the glycosylation of MUC1 via GALNT6, and that inhibition of GLANT6 leads to the maintenance of cellular polarity and cell adhesion (33), which are critical steps in the early stages of tumourigenesis (40).

A possible reason for the correlation identified may be that at early stages of development, tumour cells activate an immune response. In order to spread and grow, the cells use glycosylation alterations to escape these mechanisms. By contrast, glycosylation may also be key in ensuring that an established tumour is no longer attacked by the immune system. Therefore, the presence of GALNT6 may also be an important marker in tumour diagnosis and tumour staging. To further address this hypothesis, our future studies will investigate a larger panel of tumour samples of different stages with matched metastasis in order to have a sample that is large enough for meaningful statistical analysis which in turn would consolidate these working hypotheses. Furthermore, we plan on staining a different and broader panel of glycosylation enzymes in the tumour samples. With the aim of identifying different steps in glycosylation, which are important for tumourigenesis and immune escape of the tumour. Thereby, mechanisms of tumourigenesis may be further clarified and could lead to the development of novel treatment strategies for patients with breast cancer. Furthermore, it is desirable to identify the precise chronology of glycosylation enzyme activation and expression during the course of breast cancer tumourigenesis, as this knowledge may aid in staging tumours.

Acknowledgements

The authors would like to thank Dr Sabine Heublein for help with the statistical analysis.

References

- Skelton TP, Zeng C, Nocks A and Stamenkovic I: Glycosylation provides both stimulatory and inhibitory effects on cell surface and soluble CD44 binding to hyaluronan. J Cell Biol 140: 431-446, 1998.
- Diamond MS, Staunton DE, Marlin SD and Springer TA: Binding of the integrin Mac-1 (CD11b/CD18) to the third immunoglobulin-like domain of ICAM-1 (CD54) and its regulation by glycosylation. Cell 65: 961-971, 1991.
- McEvoy LM, Sun H, Frelinger JG and Butcher EC: Anti-CD43 inhibition of T cell homing. J Exp Med 185: 1493-1498, 1997.
- Dennis JW, Granovsky M and Warren CE: Glycoprotein glycosylation and cancer progression. Biochim Biophys Acta 1473: 21-34, 1999.
- 5. Rudd PM, Elliott T, Cresswell P, Wilson IA and Dwek RA: Glycosylation and the immune system. Science 291: 2370-2376, 2001.
- Hakomori S: Aberrant glycosylation in cancer cell membranes as focused on glycolipids: Overview and perspectives. Cancer Res 45: 2405-2414, 1985.
- 7. Hakomori S: Tumor malignancy defined by aberrant glycosylation and sphingo (glyco)lipid metabolism. Cancer Res 56: 5309-5318, 1996.
- 8. Springer GF: T and Tn, general carcinoma autoantigens. Science 224: 1198-1206, 1984.
- 9. Feizi T: Demonstration by monoclonal antibodies that carbohydrate structures of glycoproteins and glycolipids are onco-developmental antigens. Nature 314: 53-57, 1985.
- Julien S, Adriaenssens E, Ottenberg K, Furlan A, Courtand G, Vercoutter-Edouart AS, Hanisch FG, Delannoy P and Le Bourhis X: ST6GalNAc I expression in MDA-MB-231 breast cancer cells greatly modifies their O-glycosylation pattern and enhances their tumourigenicity. Glycobiology 16: 54-64, 2006.
- Taylor-Papadimitriou J, Burchell J, Miles DW and Dalziel M: MUC1 and cancer. Biochim Biophys Acta 1455: 301-313, 1999.
- Hakomori S: Carbohydrate-to-carbohydrate interaction in basic cell biology: A brief overview. Arch Biochem Biophys 426: 173-181, 2004.

- 4314
- 13. Sørensen AL, Reis CA, Tarp MA, Mandel U, Ramachandran K, Sankaranarayanan V, Schwientek T, Graham R, Taylor-Papadimitriou J, Hollingsworth MA, et al: Chemoenzymatically synthesized multimeric Tn/STn MUC1 glycopeptides elicit cancer-specific anti-MUC1 antibody responses and override tolerance. Glycobiology 16: 96-107, 2006.
- 14. Tarp MA, Sørensen AL, Mandel U, Paulsen H, Burchell J, Taylor-Papadimitriou J and Clausen H: Identification of a novel cancer-specific immunodominant glycopeptide epitope in the MUC1 tandem repeat. Glycobiology 17: 197-209, 2007.
- 15. Madsen CB, Petersen Č, Lavrsen K, Harndahl M, Buus S, Clausen H, Pedersen AE and Wandall HH: Cancer associated aberrant protein O-glycosylation can modify antigen processing and immune response. PLoS One 7: e50139, 2012.
- Apostolopoulos V, Pietersz GA, Xing PX, Lees CJ, Michael M, Bishop J and McKenzie IF: The immunogenicity of MUC1 peptides and fusion protein. Cancer Lett 90: 21-26, 1995.
- 17. Wandall HH, Blixt O, Tarp MA, Pedersen JW, Bennett EP, Mandel U, Ragupathi G, Livingston PO, Hollingsworth MA, Taylor-Papadimitriou J, *et al*: Cancer biomarkers defined by autoantibody signatures to aberrant O-glycopeptide epitopes. Cancer Res 70: 1306-1313, 2010.
- Xu Y, Gendler SJ and Franco A: Designer glycopeptides for cytotoxic T cell-based elimination of carcinomas. J Exp Med 199: 707-716, 2004.
- 19. Blixt O, Bueti D, Burford B, Allen D, Julien S, Hollingsworth M, Gammerman A, Fentiman I, Taylor-Papadimitriou J and Burchell JM: Autoantibodies to aberrantly glycosylated MUC1 in early stage breast cancer are associated with a better prognosis. Breast Cancer Res 13: R25, 2011.
- Dabelsteen E: Cell surface carbohydrates as prognostic markers in human carcinomas. J Pathol 179: 358-369, 1996.
- Hollingsworth MA and Swanson BJ: Mucins in cancer: Protection and control of the cell surface. Nat Rev Cancer 4: 45-60, 2004.
- Kim YJ and Varki A: Perspectives on the significance of altered glycosylation of glycoproteins in cancer. Glycoconj J 14: 569-576, 1997.
- 23. Miles DW, Happerfield LC, Smith P, Gillibrand R, Bobrow LG, Gregory WM and Rubens RD: Expression of sialyl-Tn predicts the effect of adjuvant chemotherapy in node-positive breast cancer. Br J Cancer 70: 1272-1275, 1994.
- 24. Joziasse DH: Mammalian glycosyltransferases: Genomic organization and protein structure. Glycobiology 2: 271-277, 1992.
- 25. Le Marer N, Laudet V, Svensson EC, Cazlaris H, Van Hille B, Lagrou C, Stehelin D, Montreuil J, Verbert A and Delannoy P: The c-Ha-ras oncogene induces increased expression of beta-galactoside alpha-2, 6-sialyltransferase in rat fibroblast (FR3T3) cells. Glycobiology 2: 49-56, 1992.
- (FR3T3) cells. Glycobiology 2: 49-56, 1992.
 26. Bennett EP, Hassan H, Mandel U, Hollingsworth MA, Akisawa N, Ikematsu Y, Merkx G, van Kessel AG, Olofsson S and Clausen H: Cloning and characterization of a close homologue of human UDP-N-acetyl-alpha-D-galactosamine: Polypeptide N-acetylgalactosaminyltransferase-T3, designated GalNAc-T6. Evidence for genetic but not functional redundancy. J Biol Chem 274: 25362-25370, 1999.
- 27. Brooks SA, Carter TM, Bennett EP, Clausen H and Mandel U: Immunolocalisation of members of the polypeptide N-acetylgalactosaminyl transferase (ppGalNAc-T) family is consistent with biologically relevant altered cell surface glycosylation in breast cancer. Acta Histochem 109: 273-284, 2007.

- 28. Freire T, Berois N, Sóñora C, Varangot M, Barrios E and Osinaga E: UDP-N-acetyl-D-galactosamine: Polypeptide N-acetylgalactosaminyltransferase 6 (ppGalNAc-T6) mRNA as a potential new marker for detection of bone marrow-disseminated breast cancer cells. Int J Cancer 119: 1383-1388, 2006.
- 29. Nomoto M, Izumi H, Ise T, Kato K, Takano H, Nagatani G, Shibao K, Ohta R, Imamura T, Kuwano M, *et al*: Structural basis for the regulation of UDP-N-acetyl-alpha-D-galactosamine: Polypeptide N-acetylgalactosaminyl transferase-3 gene expression in adenocarcinoma cells. Cancer Res 59: 6214-6222, 1999.
- Paulson JC and Colley KJ: Glycosyltransferases. Structure, localization, and control of cell type-specific glycosylation. J Biol Chem 264: 17615-17618, 1989.
- 31. Hebbar M, Krzewinski-Recchi MA, Hornez L, Verdière A, Harduin-Lepers A, Bonneterre J, Delannoy P and Peyrat JP: Prognostic value of tumoral sialyltransferase expression and circulating E-selectin concentrations in node-negative breast cancer patients. Int J Biol Markers 18: 116-122, 2003.
- 32. Recchi MA, Hebbar M, Hornez L, Harduin-Lepers A, Peyrat JP and Delannoy P: Multiplex reverse transcription polymerase chain reaction assessment of sialyltransferase expression in human breast cancer. Cancer Res 58: 4066-4070, 1998.
- 33. Park JH, Nishidate T, Kijima K, Ohashi T, Takegawa K, Fujikane T, Hirata K, Nakamura Y and Katagiri T: Critical roles of mucin 1 glycosylation by transactivated polypeptide N-acetylgalactosaminyltransferase 6 in mammary carcinogenesis. Cancer Res 70: 2759-2769, 2010.
- 34. BeroisN,MazalD,UbillosL,TrajtenbergF,NicolasA,Sastre-GarauX, Magdelenat H and Osinaga E: UDP-N-acetyl-D-galactosamine: Polypeptide N-acetylgalactosaminyltransferase-6 as a new immunohistochemical breast cancer marker. J Histochem Cytochem 54: 317-328, 2006.
- 35. Casey RC, Oegema TR Jr, Skubitz KM, Pambuccian SE, Grindle SM and Skubitz AP: Cell membrane glycosylation mediates the adhesion, migration, and invasion of ovarian carcinoma cells. Clin Exp Metastasis 20: 143-152, 2003.
- Zhang L and Ten Hagen KG: The cellular microenvironment and cell adhesion: A role for O-glycosylation. Biochem Soc Trans 39: 378-382, 2011.
- 37. Remmele W and Stegner HE: Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue. Pathologe 8: 138-140, 1987 (In German).
- Andergassen U, Liesche F., Kölbl AC, Ilmer M, Hutter S, Friese K and Jeschke U: Glycosyltransferases as markers for early tumourigenesis. Biomed Res Int 2015L 792672, 2015.
- 39. Park JH, Katagiri T, Chung S, Kijima K and Nakamura Y: Polypeptide N-acetylgalactosaminyltransferase 6 disrupts mammary acinar morphogenesis through O-glycosylation of fibronectin. Neoplasia 13: 320-326, 2011.
- 40. Zhang L, Gallup M, Zlock L, Chen YT, Finkbeiner WE and McNamara NA: Pivotal role of MUC1 glycosylation by cigarette smoke in modulating disruption of airway adherens junctions in vitro. J Pathol 234: 60-73, 2014.