Loss of Core 1-derived O-Glycans Decreases Breast Cancer Development in Mice*

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Background: Abnormal mucin-type O-glycosylation is in human breast cancer tissues with unclear in vivo functions. Results: Core 1 β1,3-galactosyltransferase (C1galt1) is critical to O-glycosylation. Genetic deletion of C1galt1 in the mammary epithelium reduces tumor development in breast tumor mouse models. Conclusion: Lacking core 1-derived O-glycans retards breast cancer development in mice. Significance: Core 1-derived O-glycans are important during mammary tumorigenesis.

Mammary gland epithelial cells are polarized glandular cells with specialized apical and basolateral membrane domains. This ductal-lobular system is surrounded by a layer of myoepithelial cells, which provide support and propulsive force for the contents of the ducts. The majority of breast cancers arise from mammary gland epithelial cells. Both normal and malignant mammary epithelia express large amounts of secreted and membrane-bound glycoproteins that are primarily modified by mucin-type O-glycosylation (1).

Mucin-type core 1-derived O-glycans, one of the major types of O-glycans, are highly expressed in mammary gland epithelium. Abnormal O-glycans such as Tn antigen are found in over 90% of breast cancers; however, the in vivo role of these aberrant O-glycans in the etiology of breast cancer is unclear. We generated mice with mammary epithelial specific deletion of core 1-derived O-glycans. By crossing with two spontaneous mouse breast cancer models, we determined that loss of core 1-derived O-glycans delays the onset and progression of breast cancer development. Deficiency of core 1 O-glycosylation impaired the localization of Muc1, a major O-glycoprotein, on the apical surfaces of mammary epithelium. Signaling mediated by Muc1, which is critical for breast cancer development, was also defective in the absence of core 1 O-glycans. This study reveals an unexpected role of core 1-derived O-glycans in breast cancer development in mice.

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Mucin-type O-glycosylation (hereafter referred to as O-glycosylation) starts with addition of N-acetylglalactosamine (GalNAc)4 to either serine or threonine. The resulting O-linked structure is called Tn antigen (Fig. 1A), which is a biosynthetic intermediate to all O-glycans. Core 1 O-glycan is the most common structure expressed in mammary gland epithelium (1).

In normal mammary epithelial cells, core 1 O-glycan (also named T antigen) is often capped with sialic acids and/or branched to form a diverse array of O-glycans, which include sialylated core 2 O-glycans (2). However, in breast cancer, truncated O-glycans are prevalently expressed (3, 4). Aberrant expression of several early biosynthetic intermediates has been reported (1, 2, 4, 5). For example, Tn is detected in almost 90% of breast cancers while it is rarely detected in normal tissue. The occurrence of its sialylated form sTn is associated with poor prognosis of breast cancer (4, 5). Recently, genetic mutations or epigenetic aberration in glycosylation pathways of tumor cells have been identified to cause abnormal expression of Tn and sTn (6, 7), but it is unclear whether this alteration plays a causative role in breast tumor development.

Biosynthesis of core 1 O-glycan is exclusively controlled by the enzyme core 1 β1, 3-galactosyltransferase (C1galt1, T-synthase, Fig. 1A). Lack of C1galt1 activity results in exposure of Tn in vivo. In human breast cancer, higher C1galt1 gene expression has been found in ductal breast carcinomas when compared with non-cancerous tissues (8, 9). Higher C1galt1 expression in tumor tissue is associated with advanced tumor stages and poor survival in breast cancer patients (9, 10). Yet, the in vivo role of core 1 O-glycan and its derivatives (core 1-derived O-glycans)

The abbreviations used are: GalNAc, N-acetylgalactosamine; C1galt1, Core 1 β1,3-galactosyltransferase.
in breast tumor development remains to be defined. To address this question, we generated mice lacking C1galt1 specifically in mammary gland epithelial cells (ME C1galt1−/−). After crossing with two commonly used murine breast tumor models, we unexpectedly found that ME C1galt1−/− mice exhibited delayed onset and retarded progression of breast tumors.

**Experimental Procedures**

**Mice**—To generate the ME C1galt1−/− mice, we developed mice in which C1galt1 was flanked by loxP sites (C1galt1fl/fl mice) (11). C1galt1fl/fl mice were bred with a transgenic line expressing Cre recombinase specifically in mammary gland epithelial cells, under control of the mouse mammary tumor virus (MMTV) promoter (12). To determine the contribution of core 1 O-glycan to breast cancer development, we bred ME C1galt1−/−, respectively with two well-established transgenic mouse models of breast cancer: MMTV-PyMT transgenic mice expressing the activated ErbB2 oncogene controlled by the MMTV promoter (12); MMTV-PyMT transgenic mice expressing polyoma virus middle T antigen from the MMTV promoter (13). All mouse experiments were carried out by comparison between littermates on mixed background. Experimental protocols were approved by the Institutional Animal Care and Use Committee of the Oklahoma Medical Research Foundation.

**Orthotopic Mammary Gland Tumor Implantation**—Mammary gland tumor implantation experiments were carried out based on published methods (14). Briefly, 8-week-old immune-deficient females (NOD.Cg-Rag1tm1Morm Il2rgtm1Wjl/SjI, NRG) were used as recipients. Mouse mammary tumors from 14-week-old MMTV-PyMT females with or without mammary gland core 1 O-glycan deficiency were dissected into small and triangle-shaped pieces (2 mm × 1 mm in size). Each of these tumor masses was implanted into the fourth mammary glands of NRG mice (n = 20 mice). Since the MMTV-PyMT mice develop multifocal and heterogeneous adenocarcinomas, we collected the same number of implants from the first, second, third and fourth mammary glands of littermates. The tumor volume was measured by a caliper and calculated with the formula: \( V = \frac{a \times b^2}{2} \) (mm³), where ‘a’ and ‘b’ are the largest and smallest perpendicular tumor diameters, respectively.

**Immunofluorescent Staining**—For immunofluorescent staining, 5-μm thick deparaffinized sections were blocked, after heat-induced antigen retrieval (Dako), with 0.3% Triton X-100, 3% donkey serum, 3% goat serum, and 3% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) and incubated overnight at 4 °C with specific combinations of the following primary antibodies: rabbit polyclonal anti-Muc1 cytoplasmic tail (Abcam), mouse anti-β-catenin (Cell Signaling), rabbit anti-Ki67 (clone SP6; Millipore), rat anti-α-SMA (Sigma), and rat anti-CD45 (Abcam). After washing in PBS, sections were incubated with the appropriate secondary antibodies conjugated to AlexaFluor (AF)-488, DyLight (DL)-549, or DL-649 (Jackson ImmunoResearch). The sections were mounted in mounting medium with or without DAPI (4,6 diamidino-2-phenylindole; Invitrogen) and analyzed by confocal scanning microscopy using a DSU spinning-disk head mounted on an Olympus IX81 inverted microscope with a Hamamatsu ORCA-R2 camera.

**Immunohistochemical Analysis**—Paraffin-embedded tissue sections (5 μm in thickness) were deparaffinized and rehydrated, endogenous peroxidase activity was quenched with 3% H₂O₂ for 10 min, antigen retrieval was performed using antigen unmasking solution (Vector Laboratories), and nonspecific binding was blocked using a nonspecific protein block (Dako) for 1 h. Slides were incubated with primary antibodies against Ki67 (Millipore), sTn (clone 3F1 (SBH Sciences) or biotinylated mAb against sTn antigen (mouse IgG, clone TKH2)), T antigen (clone 3C9; SBH Sciences; after neuraminidase treatment (0.5 units/ml, Roche)) or biotinylated mAb against Tn antigen (mouse IgM, clone Ca3638) at 4 °C overnight. After washing, sections were incubated with an appropriate secondary antibodies conjugated to horseradish peroxidase. Immunoreactivity was visualized using a peroxidase-diaminobenzidine kit (Vector Laboratories).

**Results**

**C1galt1 Deficiency in Mammary Epithelial Cells Delays Breast Cancer Progression in the ErbB2 Model**—ME C1galt1−/− mice were fertile and have no significant gross morphological abnormalities when compared with wild-type littermate controls (WT or C1galt1+/+ mice). Histological examinations of multiple organs, including brain, liver, lung, salivary gland, intestine, kidney, and spleen, revealed no significant differences between ME C1galt1−/− mice and C1galt1+/+ mice (data not shown). Lack of C1galt1 results in the abnormal exposure of Tn antigen, which is known as a common tumor-associated carbohydrate antigen. However, ME C1galt1−/− mice did not exhibit spontaneous development of mammary tumors (data not shown), suggesting that expression of Tn is not sufficient to induce breast cancer formation.

To further address this issue, we crossed ME C1galt1−/− mice with MMTV-ErbB2, a transgenic mouse model that develops spontaneous breast cancer resembling the sporadic human breast cancer, to generate ME C1galt1−/−;MMTV-ErbB2 mice (ME C1galt1−/−/Erb) (Fig. 1B) (12). Unexpectedly, ME C1galt1−/−/Erb mice exhibited a reduction in breast tumor development as indicated by pooled primary tumor volume in comparison with MMTV-ErbB2 littermates (Fig. 1, C and D). Accordingly, Kaplan-Meier analysis indicated that tumor-free survival in parous females of ME C1galt1−/−/Erb mice was much longer than that of C1galt1+/+/Erb (Fig. 1E). In addition, lung metastasis was dramatically reduced in ME C1galt1−/−/Erb mice compared with C1galt1+/+/Erb mice (Fig. 1F).
Mammary Epithelial Cell-specific Deficiency of C1galt1 Delays the Onset of Tumors in PyMT Model—Breast cancer development in the MMTV-Erb2 model has a long latent period (~12 months) (13). In contrast, breast tumor develops as early as 2.5 months of age in MMTV-PyMT transgenic mice, another well-established breast cancer mouse model (13). To determine if lack of core 1-derived O-glycans alters the onset of mammary tumors, ME C1galt1f/f mice were crossed with MMTV-PyMT to generate ME C1galt1f/f/PyMT mice (ME C1galt1f/f/PyMT mice) (Fig. 2, A and B). ME C1galt1f/f/Py MT mice exhibited a significant delay in breast tumor development compared with C1galt1f/f/Py littermates (Fig. 2C). Gross analysis revealed that the C1galt1-deficiency decreased the number of mammary nodules compared with control littermates at the early stage of tumor formation, suggesting a decrease in progression of mammary intraepithelial neoplasia in ME C1galt1f/f/Py mice (Fig. 2D). Furthermore, C1galt1f/f/Py mice developed grossly detectable and histologically confirmed lung metastases by 22 weeks of age; whereas, ME C1galt1f/f/Py littermates exhibited no detectable lesions within the lung (data not shown). C1galt1f/f/Py ducts also had high levels of cytoplasmic/nuclear β-catenin and few α-SMA positive myoepithelial cells consistent with neoplastic transformation (Fig. 2E) (15); however, ME C1galt1f/f/Py ducts exhibited cell membrane-associated staining pattern of β-catenin and α-SMA positive myoepithelial layers, both consistent with normal mammary epithelial architecture (Fig. 2F).

Decreased Tumor Development in Core 1 O-Glycan-deficient Mice Is Not Attributed to the Adaptive Immune Responses—Exposed Tn antigen in the absence of core 1 O-glycan may elicit immune responses, which may contribute to the delayed tumorigenesis. To address this question, we stained sections of different stages of tumors for the presence of infiltrating immune cells. Our analysis did not detect obvious differences of CD45+ cells in premalignant and malignant tumor tissues between ME C1galt1f/f/Py mice and WT littermate controls (data not shown). This result suggests that immune responses...
to Tn antigen may not contribute to the delayed tumor development in ME C1galt1−/−/Py mice.

To definitively address this question, we transplanted same-sized breast tumor grafts from ME C1galt1−/−/Py mice or C1galt1f/f/Py littermates orthotopically into the 4th mammary gland of immune-deficient NRG mice, which lack adaptive immunity (14). The tumor implant growth was monitored for 7 weeks after implantation. Consistent with the retarded growing nature in the donor ME C1galt1−/−/Py mice, ME C1galt1−/−/Py tumor implants grew significantly slower than that of C1galt1f/f/Py tumor implants as demonstrated by gross and histologic analyses (Fig. 3). These results indicate that potential immune responses to Tn antigen, if there are any, do not significantly contribute to breast cancer development in our mammary gland core 1-derived O-glycan-deficient mice.

Lack of Core 1 O-Glycosylation Decreases Proliferation of Mammary Epithelial Cells—Decreased ductal neoplasia formation in C1galt1-deficient tumor bearing mice suggested a defect in proliferation; therefore, we stained mammary epithelial ducts with Ki67, a marker for cellular proliferation (16). We found decreased Ki67-positive epithelial cells within the ducts of ME C1galt1−/− mice when compared with WT C1galt1f/f/Py littermates (Fig. 4A). We next analyzed whether core 1 O-glycan deficiency alters mammary epithelial cell proliferation in the PyMT transgenic mouse tumor model by monitoring Ki67 expression at different stages of breast tumor development. We found that when compared with C1galt1f/f/Py mice, ME C1galt1−/−/Py mice exhibited significantly reduced Ki67-positive cells at all time points corresponding to different tumor stages (Fig. 4, B and C), i.e. hyperplasia, adenoma, and invasive carcinoma, which correlates with human tumor development (13); notably, cells lining ductal structures of ME C1galt1−/−/Py mice were largely Ki67-negative.

Antibody to Tn antigen detected robust signals in the ME C1galt1−/−/Py breast epithelium and tumors, confirming the deletion efficiency. Antibody to sTn antigen showed no signals. Antibody to T antigen detected positive staining in C1galt1f/f/Py mammary epithelial cells after desialylation, suggesting that most T antigens were sialylated. Anti-T exhibited negative staining of ME C1galt1−/−/Py ductal-like structures but not in other cell types, indicating the specificity and efficiency of the deletion (Fig. 4D).

Importantly, lack of core 1 O-glycosylation did not affect growth of the primary mammary ductal network, based on the extent of the ductal growth in relation to the nipple, lymph node, and the end of the fat pad although there was a modest reduction of secondary branching in the absence of core 1 O-glycans. This result suggests that primary mammary developmental defects are unlikely the cause of the delayed development of breast cancer in our model (Fig. 5).
Altered Apical Expression and Associated Downstream Signaling of Muc1 in Core 1 O-Glycan-deficient Mice—We next sought to better understand the molecular mechanism underlying delayed mammary gland tumorigenesis in the absence of core 1-derived O-glycans. Mucin Muc1 is a predominant O-glycoprotein in the mammary gland. Altered Muc1 expression is found in over 90% of breast cancers and has been associated with increased tumor growth and metastasis (17–19). However, whether loss of core 1-derived O-glycans alters function and/or expression pattern of Muc1 in vivo is unclear (20). The expression of Muc1 is confined to the apical surface at relatively low levels under normal condition, and at very high levels following tumor transformation (21). We analyzed mammary glands of 4- to 5-week-old ME C1galt1f/f/Py mice, a time point prior to palpable tumor formation (13). Immunofluorescent staining results showed that Muc1 was primarily detected on the apical surface of epithelium and in the duct lumens in 4–5-week-old ME C1galt1f/f/Py mammary tissues. In contrast, Muc1 lost this apical localization and was found diffusely expressed in ME C1galt1+/−/Py mammary glands (Fig. 6A). Similarly, apical localized or diffused expression patterns of Muc1 were found in mammary glands of C1galt1f/f or ME C1galt1+/−/Py mice, respectively (Fig. 6B). These data support that core 1 O-glycosylation is critical for proper expression and localization of Muc1.

To determine how core 1 O-glycan regulates Muc1 expression, we immunoblotted WT breast tissue with an antibody against the cytoplasmic tail of Muc1. Under non-denaturing and non-reducing conditions, a single band of Muc1 corresponding to the cytoplasmic tail was detected in both WT and ME C1galt1+/−/Py tissues (Fig. 6C). These results suggest that core 1 O-glycosylation is necessary for the proper expression and localization of Muc1.

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O-Glycosylation and Breast Cancer Development

Expression of aberrant forms of O-glycans such as Tn and sTn is frequently observed in breast cancer and considered to play a causal role in tumor development (7). In this study, we unexpectedly found that loss of core 1-derived O-glycans and subsequent expression of Tn in mammary epithelial cells delayed the onset and progression of breast tumor based on results from two well-established spontaneous breast tumor mouse models. Deficiency of core 1 O-glycans altered expression patterns of the major O-glycoprotein, Muc1, in the mammary epithelial cells and impaired cell proliferation associated with Muc1-mediated signaling.

Loss of core 1 O-glycosylation may affect the expression and/or functions of many O-glycoproteins that regulate mammary gland proliferation. However, as the predominant O-glycoprotein, Muc1 is known to play a critical role in mammary gland development in both human and mouse. Muc1 is comprised of two subunits, Muc1-N and Muc1-C (22). The larger subunit (Muc1-N) of the ectodomain primarily consists of tandem repeats, which are highly O-glycosylated. Muc1-N is connected to the transmembrane and cytoplasmic tail, Muc1-C, to be located on the apical surface of normal polarized breast epithelia. In breast cancer cells, the Muc1-N/Muc1-C complex is found over the entire cell membranes. Altered expression of Muc1 in breast cancer is associated with increased interactions between Muc1-C and receptor tyrosine kinases, such as epidermal growth factor receptor (EGFR) of the ErbB protein family (17, 19). This interaction contributes to activation of the PI3K-AKT and mitogen-activated protein kinase (MEK/ERK) pathway (18, 19). Our observation that activation of these signaling pathways was reduced in core 1 O-glycan-deficient mammary glands. Our data support that altered expression of Muc1 in the absence of core 1 O-glycans impairs pro-proliferative signals, which supports that Muc1 requires core 1-derived O-glycans for its proper surface expression, stability, and/or to interact with receptor tyrosine kinases.
The immune system plays a critical role in cancer development and/or spreading (24, 25). Tn antigen, which is known as a tumor-associated carbohydrate antigen, may elicit immune responses that potentially retard tumor growth and metastasis in our models. However, our data show no differences in the number of immune cell infiltrates between Tn-positive breast tumors (lack of core 1 O-glycan) and Tn-negative breast tumors (WT). Furthermore, orthotopic breast tumor implantation experiments using immune-deficient NRG mice as recipients demonstrate immune responses do not significantly contribute to the retarded tumor development in our mouse models lacking mammary gland core 1-derived O-glycans.

Human breast cancer cells show increased expression of sialyltransferase ST6GalNAc1, which can modify Tn to form sialyl Tn (sTn) (26, 27). Therefore, deficiency of C1Galt1 may cause compensatory increase of sTn. However, our immunostaining of sTn in C1Galt1-deficient mammary tissue yielded negative results, suggesting that most Tn antigens are not converted to sTn in our models. Moreover, T antigen and sT (core 1 structure and sialylated core 1 structure) were commonly expressed in breast cancers (2, 4, 28, 29). Consistent with this, T and sT were found in PyMT tumor tissues but not in ME C1galt1+/−/Py tumors. These data indicate that deletion of C1galt1 eliminates core 1-derived O-glycans, which include sT and core 2 O-glycans. Together, our data show that exposure of Tn antigen and elimination of core 1-derived O-glycans in the absence of C1Galt1 retard breast tumor development in our models.

Our results are consistent with recent reports that show higher C1galt1 gene expression in human breast cancer relative to non-cancerous breast tissue (8, 9). In addition, increased C1galt1 expression in tumor tissue has been associated with enhanced Muc1 function and advanced tumor stages (9). Our study provides evidence that elimination of core 1 O-glycosylation alters the expression pattern of Muc1 in both non-transformed and transformed mammary epithelial cells in vivo and results in decreased Muc1 signaling associated with cell proliferation. Furthermore, impaired apical localization of Muc1 and decreased β-catenin on mammary epithelial cells as well as increased α-SMA on surrounding myoepithelial cells in ME C1galt1−/− tissues suggests that loss of core 1 O-glycans decreases the transformation of mammary epithelial ducts into invasive carcinoma. This study provides valuable insights into the role of core 1-derived O-glycans and Tn/sTn antigen in mammary gland tumorigenesis.

Author Contributions—K. S., B. H., and L. X. designed, analyzed, and interpreted data in the study. K. S. and B. H. performed most of the animal experiments as well as data presentation. M. S. performed animal experiments and helped in statistical analyses. M. M. performed mammary gland transplantation experiments. M. S., K. B., Y. K., S. M., and X. C. performed mouse breeding, genotyping, technical support, and analysis of data. J. F., P. L., and H. C. provided important discussions and contributed to interpretation of data in the study. K. S., B. H., and L. X. wrote the manuscript.

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References

1. Brockhausen, I. (2006) Mucin-type O-glycans in human colon and breast cancer: glycodynamics and functions. EMBO Rep. 7, 599–604
2. Picco, G., Julien, S., Brockhausen, I., Beatson, R., Antonopoulos, A., Haslam, S., Mandel, U., Della, A., Pinder, S., Taylor-Papadimitriou, J., and Burchell, J. (2010) Over-expression of ST3Gal-I promotes mammary tumorigenesis. Glycobiology 20, 1241–1250
3. Müller, S., and Hanisch, F. G. (2002) Recombinant MUC1 probe authentically reflects cell-specific O-glycosylation profiles of endogenous breast cancer mucin. High density and prevalent core 2-based glycosylation. J. Biol. Chem. 277, 26103–26112
4. Cazet, A., Julien, S., Bobowski, M., Burchell, I., and Delannoy, P. (2010) Tumour-associated carbohydrate antigens in breast cancer. Breast Cancer Res. 12, 204
5. Springer, G. F. (1997) Immunoreactive T and Tn epitopes in cancer diagnosis, prognosis, and immunotherapy. J. Mol. Med. 75, 594–602
6. Ju, T., Lanneau, G. S., Gautham, T., Wang, Y., Xia, B., Stowell, S. R., Willard, M. T., Wang, W., Xia, J. Y., Zuna, R. E., Laszik, Z., Benbrook, D. M., Hanigan, M. H., and Cummings, R. D. (2008) Human tumor antigens Tn and sialyl Tn arise from mutations in Cosncer. Cancer Res. 68, 1636–1646
7. Radhakrishnan, P., Dabelsteen, S., Madsen, F. B., Francavilla, C., Kopp, K. L., Steentoft, C., Vakhruhev, S. Y., Olsen, J. V., Hansen, L., Bennett, E. P., Woetmann, A., Yin, G., Chen, L., Song, H., Bak, M., Hlday, R. A., Peters, S. L., Opavsky, R., Thode, C., Qvortrup, K., Schjoldager, K. T., Clausen, H., Hollingsworth, M. A., and Randall, H. H. (2014) Immature truncated O-glycophotype of cancer directly induces oncogenic features. Proc. Natl. Acad. Sci. U.S.A. 111, E4066–4075
8. Richardson, A. L., Wang, Z. C., De Nicolao, A., Lu, X., Brown, M., Miron, A., Liao, X., Iglehart, J. D., Livingston, D. M., and Ganesan, S. (2006) X chromosomal abnormalities in basal-like human breast cancer. Cancer Cell 9, 121–132
9. Chou, C. H., Huang, M. J., Chen, C. H., Shyu, M. K., Huang, J., Hung, J. S., Huang, C. S., and Huang, M. C. (2015) Up-regulation of C1GALT1 promotes breast cancer cell growth through MUC1-C signaling pathway. Oncotarget 6, 6123–6135
10. Milde-Langosch, K., Schütze, D., Oliveira-Ferrer I, Wikman H, Müller V, Lebok P, Pantel K, Schröder C, Witzel I, Schumacher U. (2015) Relevance of βGal-βGalNac-containing glycans and the enzymes involved in their synthesis for invasion and survival in breast cancer patients. Breast Cancer Res. Treat. 151, 515–528
11. Fu, J., Wei, B., Wen, T., Johansson, M. E., Liu, X., Bradford, E., Thomson, K. A., McGee, S., Mansour, L., Tong, M., McDaniel, J. M., Sierra, T. J., Turner, J. R., Chen, H., Hansson, G. C., Braun, J., and Xia, L. (2011) Loss of intestinal core 1-derived O-glycans causes spontaneous colitis in mice. J. Clin. Invest. 121, 1657–1666
12. Muller, W. J., Sinn, E., Pattengale, P. K., Wallace, R., and Leder, P. (1988) Single-step induction of mammary adenocarcinoma in transgenic mice bearing the activated c-neu oncogene. Cell 54, 105–115
13. Guy, C. T., Cardiff, D. R., and Muller, W. J. (1992) Induction of mammary tumors by expression of polyomavirus middle T oncogene: a transgenic mouse model for metastatic disease. Mol. Cell Biol. 12, 954–961
14. DeRose, Y. S., Wang, G., Lin, Y. C., Bernard, P. S., Buys, S. S., Ebert, M. T., Factor, R., Matsen, C., Milash, B. A., Nelson, E., Neumayer, L., Randall, R. L., Stijlemans, J. J. W., Welm, B. E., and Welm, A. L. (2011) Tumor grafts derived from women with breast cancer authentically reflect tumor pathology, growth, metastasis and disease outcomes. Nat. Med. 17, 1514–1520
15. Lopez-Knowles, E., Zardawi, S. J., McNeil, C. M., Millar, E. K. A., Crea, P., Musgrove, E. A., Sutherland, R. L., and O’Toole, S. A. (2010) Cytoplasmic localization of beta-Catenin is a Marker of Poor Outcome in Breast Cancer Patients. Breast Cancer Res. Treat. 119, 311–322
16. Scholten, T., and Gerdes, J. (2000) The Ki-67 protein: from the known and the unknown. J. Cell Physiol. 182, 311–322
17. Hollingsworth, M. A., and Swanson, B. J. (2004) Mucins in cancer: protection and control of the cell surface. Nat. Rev. Cancer 4, 45–60
18. Kufe, D. W. (2009) Mucins in cancer: function, prognosis and therapy. Nat. Rev. Cancer 9, 874–885
19. Kufe, D. W. (2013) MUC1-C oncoprotein as a target in breast cancer: activation of signaling pathways and therapeutic approaches. *Oncogene* **32**, 1073–1081
20. Gouyer, V., Leteurtre, E., Zanetta, J. P., Lesuffleur, T., Delannoy, P., and Huet, G. (2001) Inhibition of the glycosylation and alteration in the intracellular trafficking of mucins and other glycoproteins by GalNAc-O-bn in mucosal cell lines: An effect mediated through the intracellular synthesis of complex GalNAc-O-bn oligosaccharides. *Front. Biosci.* **6**, D1235-D1244
21. Lakshminarayanan, V., Thompson, P., Wolfert, M. A., Buskas, T., Bradley, J. M., Pathangey, L. B., Madsen, C. S., Cohen, P. A., Gendler, S. J., and Boons, G. J. (2012) Immune recognition of tumor-associated mucin MUC1 is achieved by a fully synthetic aberrantly glycosylated MUC1 tripartite vaccine. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 261–266
22. Cao, R., Wang, T. T., DeMaria, G., Sheehan, J. K., and Kesimer, M. (2012) Mapping the protein domain structures of the respiratory mucins: a mucin proteome coverage study. *J. Proteome Res.* **11**, 4013–4023
23. Al Masri, A., and Gendler, S. J. (2005) Mucl affects c-Src signaling in PyV MT-induced mammary tumorigenesis. *Oncogene* **24**, 5799–5808
24. Denkert, C. (2013) Diagnostic and therapeutic implications of tumor-infiltrating lymphocytes in breast cancer. *J. Clin. Oncol.* **31**, 836–837
25. Loi, S., and MacCallum, P. (2014) Host antitumor immunity plays a role in the survival of patients with newly diagnosed triple-negative breast cancer. *J. Clin. Oncol.* **32**, 2935–2937
26. Sewell, R., Bäckström, M., Dalziel, M., Gschmeissner, S., Karlsson, H., Noll, T., Gätgens, J., Clausen, H., Hansson, G. C., Burchell, J., and Taylor-Papadimitriou, J. (2006) The ST6GalNAc-I sialyltransferase localizes throughout the golgi and is responsible for the synthesis of the tumor-associated Sialyl-Tn O-glycan in human breast cancer. *J. Biol. Chem.* **281**, 3586–3594
27. Julien, S., Adriaenssens, E., Ottenberg, K., Furlan, A., Courtand, G., Verroust-Edouart, A. S., Hanisch, F. G., Delannoy, P., and Le Bourhis, X. (2006) ST6GalNAc I expression in MDA-MB-231 breast cancer cells greatly modifies their O-glycosylation pattern and enhances their tumourigenicity. *Glycobiology* **16**, 54–64
28. Springer, G. F., Desai, P. R., Murtby, M. S., and Scanlon, E. F. (1979) Human Carcinoma-Associated Precursor Antigens of the Nm Blood-Group System. *J. Surg. Oncol.* **11**, 95–106
29. Burchell, J., Poulson, R., Hanby, A., Whitehouse, C., Cooper, L., Clausen, H., Miles, D., and Taylor-Papadimitriou, J. (1999) An α2,3 sialyltransferase (ST3Gal I) is elevated in primary breast carcinomas. *Glycobiology* **9**, 1307–1311