α2-6–Linked Sialic Acids on N-Glycans Modulate Carcinoma Differentiation *In vivo*

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Abstract

Sialic acids on vertebrate cell surfaces mediate many biological roles. Altered expression of certain sialic acid types or their linkages can have prognostic significance in human cancer. A classic but unexplained example is enhanced α2-6-sialylation on N-glycans resulting from overexpression of the Golgi enzyme β-galactoside:α2-6-sialyltransferase (ST6Gal-I). Previous data supporting a role for the resulting Siaα2-3Galβ1-4GlcNAc (Sia6LacNAc) structure in tumor biology were based on *in vitro* studies in transfected carcinoma cells, in which increased Sia6LacNAc on β1-integrins enhanced their binding to ligands, and stimulated cell motility. Here, we examine for the first time the *in vivo* role of the ST6Gal-I enzyme in the growth and differentiation of spontaneous mammary cancers in mice transgenic for a mouse mammary tumor virus promoter–driven polyomavirus middle T antigen, a tumor in which β1-integrin function is important for tumorigenesis and in maintaining the proliferative state of tumor cells. Tumors induced in *St6gal1*-null animals were more differentiated compared with those in the wild-type background, both by histologic analysis and by protein expression profiles. Furthermore, we show the *St6gal1*-null tumors have selectively altered expression of genes associated with focal adhesion signaling and have decreased phosphorylation of focal adhesion kinase, a downstream target of β1-integrins. This first *in vivo* evidence for a role of ST6Gal-I in tumor progression was confirmed using a novel approach, which conditionally restored *St6gal1* in cell lines derived from the null tumors. These findings indicate a role for ST6Gal-I as a mediator of tumor progression, with its expression causing a less differentiated phenotype, via enhanced β1-integrin function. [Cancer Res 2008;68(2):388–94]

Introduction

Sialic acids are acidic sugars typically terminating the outer ends of cell surface glycan chains (1). Enhanced expression of α2-6-linked sialic acids on N-glycans in the sequence Siaα2-6Galβ1-4GlcNAcβ1-R (Sia6LacNAc units) often correlates with human cancer progression, metastatic spread, and poor prognosis. Increased Sia6LacNAc is explained by up-regulation of the *ST6GALI* gene encoding the enzyme β-galactoside:α2-6-sialyltransferase (ST6Gal-I). Increased expression is reported in carcinomas of the colon, breast and cervix, choriocarcinomas, acute myeloid leukemia, and in some brain tumors (2–11). Interpretation of expression levels is complicated by the genomic complexity of the *ST6GAL1* gene (formerly called *SIAT1*), with multiple promoters generating tissue-specific and developmentally regulated transcripts (12–14). Regardless, all transcripts encode the identical enzyme polypeptide, which is highly conserved from mice to humans, and functions in the Golgi apparatus to add terminal α2-6-sialic acids onto N-glycans of newly synthesized proteins.

Although integrin-mediated adhesion is primarily based on protein interactions, binding can be significantly modulated by the glycosylation status of the integrin (15, 16). Increased Sia6LacNAc on β1-integrins has been reported in several transformed cell types and postulated to alter integrin function by enhancing its activation state and binding to collagen (17, 18). In these studies, increases in Sia6LacNAc levels have been correlated with enhanced cell motility and invasiveness *in vitro*.

Some apparently conflicting literature about Sia6LacNAc in cancer is likely explained by the traditional approach of isolating subclones of tumor lines with varying amounts of a molecule of interest. Malignant lines are genetically unstable, and a cloned subline may not retain the overall genotype or phenotype of the original population. These and other problems make it necessary to reexamine the role of Sia6LacNAc in an *in vivo* model, in which the only change is in the *St6gal1* gene. Here, we study the role of the Sia6LacNAc structure in mammary tumors arising in mice genetically deficient in ST6Gal-I. We hypothesized that if hypersialylation could promote conformational changes involved in β1-integrin activation and signaling, then the absence of Sia6LacNAc could favor differentiation.

Materials and Methods

Mice

C57Bl6 mice expressing the PyV-mT antigen (19) were from Dr. Carol Macleod (originally from Prof. William J. Muller). *St6gal1*-null mice were from Dr. J. Marth (20). All mice were bred in a congenic C57Bl/6 background and maintained according to university guidelines.

Histology and Immunostaining

Tumors were processed for frozen or paraffin sections. Sections were stained with H&E for histology review, and adjacent sections were evaluated by immunostaining. Before staining, mouse tumor sections were air-dried for 30 min, fixed in 4% paraformaldehyde, and nonspecific binding sites were blocked with 5% bovine serum albumin (BSA) in PBS for 30 min. Endogenous peroxidase activity was blocked by incubating the sections with 0.01% H_2O_2 in PBS for 30 min and washed with PBS. Subsequently, sections were overlaid with antibodies diluted in 1% BSA/PBS for 30 min at room temperature. Rabbit polyclonal anti-mouse focal adhesion kinase (FAK), anti-FAK [pY^{397}], and anti-FAK [pY^{576}] were from BioSource International. Rabbit anti-laminin and anti-cytokeratin were from DAKO. Rabbit anti-mouse paxillin (H-114) was from Santa Cruz Technologies. FITC-conjugated goat anti-rabbit antibody was from Bio-Rad. Biotinylated lectins were from Vector Laboratories. Binding of horseradish peroxidase (HRP) conjugates was visualized with either Nova Red (Vector Labs) or AEC.

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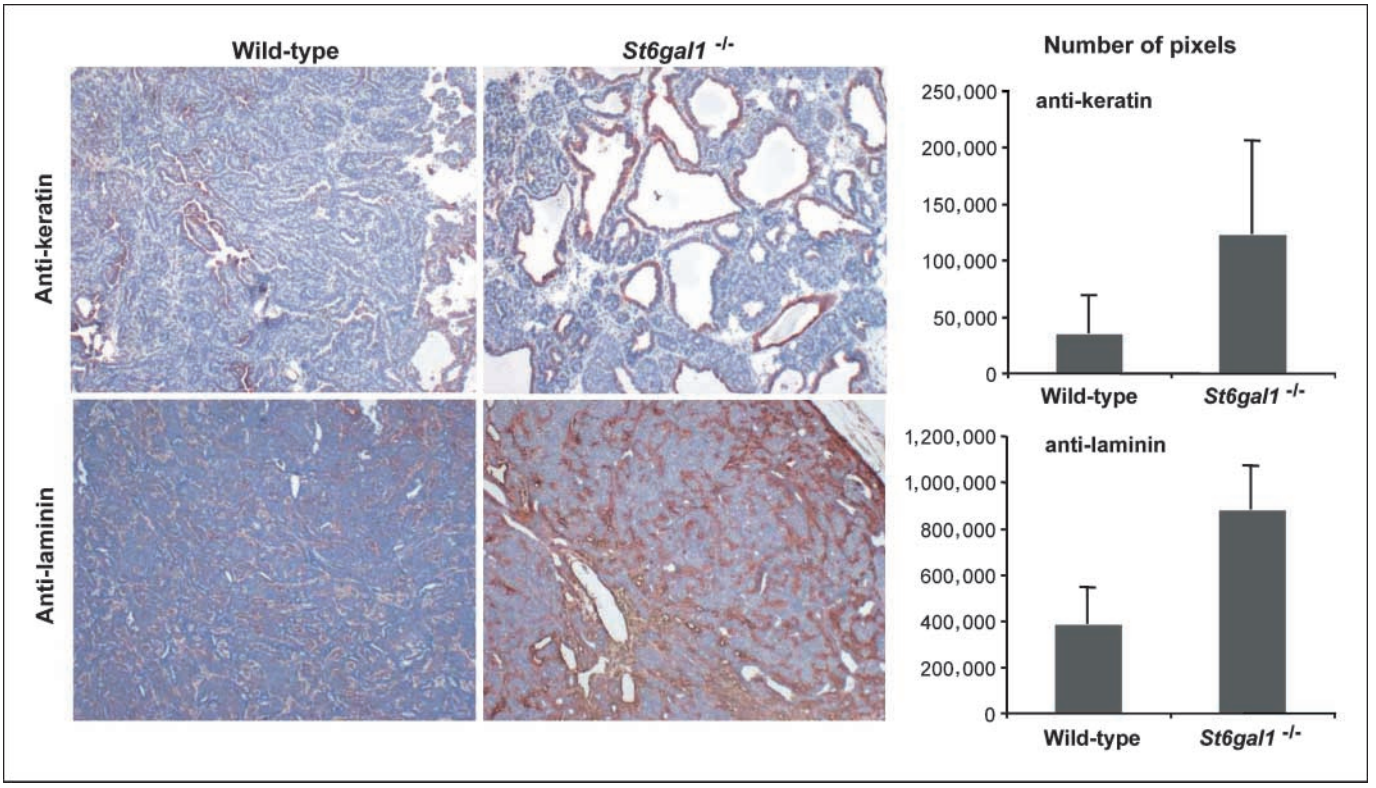

Figure 1. Enhanced epithelial differentiation of *St6gal1*-null tumors. Mice expressing the PyV-mT antigen under control of the mouse mammary tumor virus long terminal repeat promoter were used to study mammary tumors *in vivo*. Immunohistochemistry of *St6gal1*$^{-/-}$ and wild-type tumors using an anti-cytokeratin or anti-laminin antibody and a secondary HRP-conjugated antibody. Nuclei were stained using aqueous Mayer's hematoxylin. Quantitation of positive staining was performed using Adobe Photoshop. *St6gal1*$^{-/-}$ tumors (n = 7) show enhanced expression of both cytokeratin and laminin compared with wild-type controls (n = 8; P < 0.05 and P < 0.01, respectively). Magnification, 100×. *Brownish-red,* positive staining.

Quantitation of positive staining was performed using Adobe Photoshop (21). Minor adjustments to brightness or contrast done to best recapitulate the appearance to the human eye under the microscope were always done identically for each set of results.

RNA Isolation and Oligonucleotide Array

The tumor tissue (100 mg) was homogenized in Ultraspec (Biotecx Labs), and total RNA was extracted according to the manufacturer's protocol. Quantity and quality of final total RNA were examined with RNA 6000 Nano LabChip using the Agilent 2100 Bioanalyzer (Agilent Technologies) as well as spectrophotometrically. Purified total RNA (10 μg) was used for cDNA synthesis, followed by *in vitro* transcription to incorporate biotin labels, and subsequent hybridization to Murine Genome 430 2.0 Array GeneChip (Affymetrix) was performed by the GeneChip Microarray Core (University of California, San Diego) as described in the Affymetrix GeneChip protocol (Affymetrix). Each microarray was scanned, visualized, and analyzed for the level of each individual transcript using version 1.2 GeneChip Software (Affymetrix). The signal values were subjected to t test to identify statistically significant differences. Probes, with detection signal considered absent in all samples, were removed from the data set. Differences of least 1.2-fold expression (P < 0.05) found in both sets of microarray were further analyzed using the Webgestalt resource.[1] The data from the complete microarray have been deposited in National Center for Biotechnology Information Gene Expression Omnibus[2] and are accessible through Gene Expression Omnibus Series accession number GSE9447.

[1] http://bioinfo.vanderbilt.edu/webgestalt/
[2] http://www.ncbi.nlm.nih.gov/geo/

Plasmid Construction

Plasmids for conditional expression were produced as described (22). Briefly, the CTS region (the localization domain) of rat ST6Gal-I (accession number M18769) was amplified from the plasmid ST6Gal-1 pcDNA3.1 by PCR using primers CTS ′5 (5-CGATCTCTAGACGGTTTTTGATCATCCTGA-3) and CTS ′3 (5-AGGCCCTTACACTGCAAGCCACTAGTCCGGT-3) and digested with *Xba*I to yield a fragment encoding the first 300 amino acids of ST6Gal-I. This fragment was ligated into the plasmid pcDNA3.1-Zeo, which had been digested with *Nhe*I and *Xba*I and treated with CIP. The product pcDNA3.1-CTS was verified by sequencing. The resulting plasmid pcDNA3.1-CTS was linearized with *Xba*I and ligated with the *Xba*I-FKBP-myc-*Spe*I (*Xba*I and *Spe*I are compatible ends) to generate the final plasmid.

Catalytic domain plasmids. The plasmid pcDNA3.1-Vh was generated as described (22). The catalytic domain of ST6Gal-I (amino acids 372–1645) was PCR amplified from pcDNA3.1(+)ST6Gal-I using the primers CAT 5′ (5′-CGATCTCTAGAAGCAAGCAAGACCCTAAGGA-3′) and CAT 3′ (5′-TTCTGCATGGGTAGGGAAAGACTAGTCCGGT-3′), whereas introducing *Xba*I (5′ end) and *Spe*I (3′ end) restriction sites. This insert was cloned into the *Xba*I site of pcDNA3.1-Vh. The resulting plasmid was digested with *Xba*I and subsequently ligated with the FRB insert. This process was repeated twice, the last time using the HA-FRB insert, to generate the final plasmid HA-FRB-FRB-FRB-CAT.

Creation of mouse mammary tumor virus–polyomavirus middle T antigen cell lines. Mammary tumors from wild-type mouse mammary tumor virus (MMTV)–polyomavirus middle T antigen (PyMT$^{+/-}$) or *St6gal1*$^{-/-}$ MMTV-PyMT$^{+/-}$ mice were excised under aseptic conditions. Tumors were minced in ice-cold PBS and separated using a 20-mL syringe. The larger pieces were discarded, and the cells in the supernatant were harvested by centrifuged at 800 rpm for 4 min. The pellet was resuspended and plated onto a 10-cm tissue culture dish. The resulting cell lines were

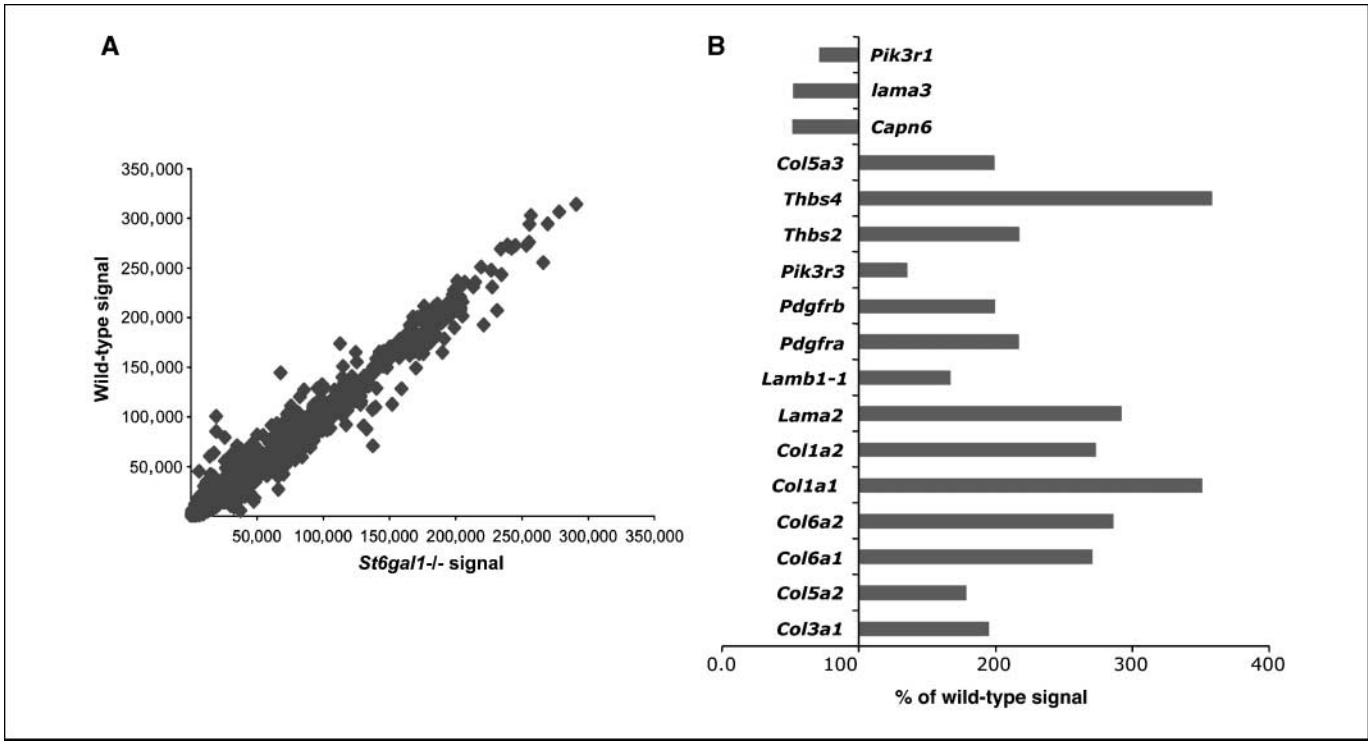

Figure 2. Altered gene expression in *St6gal1*-null tumors. Affymetrix analysis was performed by Affymetrix GeneChip Operating Software 1.2 and further analyzed using the Webgestalt online resource (http://bioinfo.vanderbilt.edu/webgestalt/). *A,* gene expression is selectively altered in tumors deficient in *St6gal1*. The majority of genes affected were found to belong to a class associated with focal adhesion signaling. *B,* altered gene expression associated with the phenotypic changes observed. Data shown are from an analysis of pooled samples from triplicate tumors presented as percentage change of signal compared with wild-type control. See text for details.

cultured in DMEM (high glucose; Invitrogen) containing 10% FCS (HyClone). Genotypes of the isolated cell lines were confirmed by isolating DNA from cell pellets and performing PCR using the following primers: ST6f1, ACT GAA TGG TGG ACT GTG G; ST6EX2r, TGT TGA AGG GAG AAT CTG TG; and ST6r1, CAT TTT GTG AGC CCC ATT AG. The PCR reaction was 94°C for 2 min; 40 cycles of 94°C for 30 s, 55°C for 45 s, and 72°C for 2 min; and 72°C for 10 min.

Cell Culture

Mouse mammary tumor cells were grown in DMEM supplemented with 10% (v/v) fetal bovine serum. Transfection was performed using Lipofectamine 2000 (Invitrogen) with plasmid DNA diluted in Optimem (Invitrogen). All experiments were also controlled by vector-alone transfections.

Flow cytometry analysis of cells. Cells were grown to 90% confluency and detached using PBS containing 2 mmol/L EDTA, at 37°C, and washed thrice with cold HBSS. After blocking with HBSS containing 1% BSA (HBSS/BSA), cells were incubated with biotinylated *Sambucus nigra* lectin (SNA) at 2 μg/mL for 30 min on ice. Tumor cells were subsequently incubated with FITC-conjugated streptavidin and incubated for 30 min on ice. Cells were washed with HBSS/BSA and analyzed by flow cytometry.

Results

Elimination of ST6Gal-I causes glandular differentiation of breast carcinomas. Mammary tumors experimentally generated by transgenic expression of a PyMT (19) were induced in *St6gal1*-null (20) or syngeneic C57Bl/6 wild-type female mice. Tumors were visible in *St6gal1*-null mice at 17.3 weeks and in syngeneic wild-type littermates at 17.1 weeks of age. When the experiment was terminated at ~21 weeks of age, the tumor burden in null mice was 2.6 ± 0.85 g compared with 2.5 ± 1.0 g in the wild-type littermates. Finding no obvious difference in growth rate, tumor size, or metastasis in these fast growing and typically necrotic tumors at 4 to 5 weeks after tumor onset, we focused on earlier events. Histologic analysis of tumors 2 weeks after onset with H&E staining showed that *St6gal1*-null tumors seemed more differentiated (Fig. 1), with focal areas of abortive gland formation and some even containing milk-like secretions staining positive with an anti-whey acidic protein antibody (not shown). Cytokeratin, a differentiation marker used to identify epithelial cells and known to be expressed in ductal epithelium, was enhanced in the majority of tumors from null mice (Fig. 1). Thus, absence of *St6gal1* enhanced differentiation of tumors.

Gene expression analysis. mRNA from tumors arising in wild-type or *St6gal1*-null littermates was used for the gene expression analysis on the Mouse Genome 430 2.0 Array (Affymetrix). Six PyV-mT *St6gal1*-null and wild-type tumor pairs were used in four independent pools of three tumors each. Data was analyzed using the Webgestalt resource.[3] Remarkably, absence of this single linkage-specific sialyltransferase had a marked effect on gene expression patterns (Fig. 2*A*). The majority of the affected genes were associated with focal adhesion signaling, which occur at sites where the cytoskeleton-associated proteins of the cell membrane interact and is one of the initial steps in integrin signaling. Many changes in gene expression agreed with the phenotypic changes observed. For instance 2-fold to 3-fold increases were observed in genes like collagens, which are involved in integrin binding (Fig. 2*B*). Thrombospondin-2, previously shown to promote tumor cell differentiation and reduce invasion (23), also showed increased

[3] http://bioinfo.vanderbilt.edu/webgestalt/

expression. Other integrin ligands, such as the laminin genes, showed increased expression, and laminin itself was present in a more organized fashion in the null tumors compared with the wild-type tumors (Fig. 1). This is consistent with previous data showing a role for laminin B in forming a laminin-containing extracellular matrix (ECM) network. Genes down-regulated in null tumors (Fig. 2) included calpain-6, a cysteine proteinase normally up-regulated in carcinomas, and laminin-5, which mediates anchorage-independent survival in breast tumors by activating extracellular signal-regulated kinase signaling (24).

Loss of FAK phosphorylation in *St6gal1*-null tumors. Muller and colleagues showed that β1-integrin expression is critical for initiation of mammary tumorigenesis in this system and for maintaining the proliferative state (25). We found that overall levels of β1-integrins were unaltered in the *St6gal1*-null tumors (not shown). Thus, we hypothesized that α2-6-sialylation of β1-integrins enhances conformational changes promoting activation during tumor progression, and lack of this linkage in null mice reduces β1-integrin function and favors differentiation.

Cellular adhesion to the ECM triggers integrin clustering, which induces phosphorylation of FAK at tyrosine residue 576 (Y^{576}) in the kinase domain. Subsequently, the c-Src binding site (Y^{397}) of the FAK molecule becomes phosphorylated. Given their important roles in integrin signaling, we investigated the phosphorylation status of these sites in tumors from *St6gal1*-null and wild-type littermates. Whereas staining of nonphosphorylated FAK revealed a similar pattern in both types of tumors, phosphorylated Y^{576} (Fig. 3) and Y^{397} (not shown) were decreased in the *St6gal1*-null tumors.

Restoration of *St6gal1* expression using a small molecule approach for inducible expression. We next wished to conditionally modulate ST6Gal-I *in vitro* and study the consequences for cell morphology and integrin-mediated signaling. A novel approach for inducible expression of the α1-3-fucosyltransferase FucT-VII (22) took advantage of the fact that effective glycosyltransferase action requires Golgi localization. Here, we successfully applied this approach to ST6Gal-I (Fig. 4*A*). The *St6gal1* cDNA was separated into two domains, one that determines localization (stem, transmembrane, and cytosolic tail) and the second coding for the intralumenal domain responsible for catalysis. Conditions causing these two proteins to associate would result in retention of the catalytic domain with the localization domain in the Golgi, thus reconstituting the enzyme's normal activity. Association of the two domains was controlled by rapamycin-mediated heterodimerization of the FKBP and FRB components of the two constructs.

Cell lines were established from the *St6gal1*-null and wild-type tumors and deliberately maintained as polyclonal mixtures to avoid selecting any rare variants, and *St6gal1* expression was restored in the null cell lines using the conditional constructs. As shown in Fig. 4*B*, addition of rapamycin caused the expression of Sia6LacNAc units (detected by the lectin SNA) in *St6gal1*-null cells transfected with constructs containing the two components of ST6Gal-I. In contrast, *St6gal1*-null cells transfected with vector alone did not show increased SNA reactivity. This provides a system to study the effects of restoration of ST6Gal-I expression.

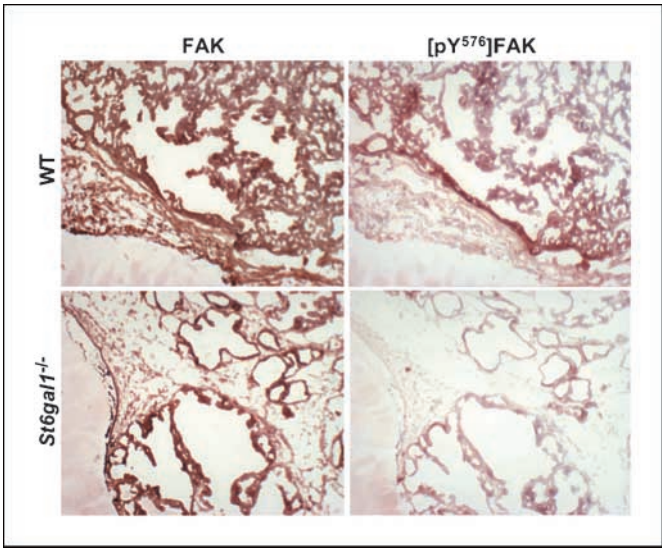

Figure 3. Reduced phosphorylation of FAK in *St6gal1*-null tumors *in vivo*. Immunohistochemistry analysis of frozen sections of mammary tumors from wild-type MMTV-PyMT$^{+/-}$ and *St6gal1*$^{-/-}$ MMTV-PyMT$^{+/-}$ mice using a rabbit anti-[pY^{576}]FAK and a secondary HRP-conjugated anti-rabbit antibody. *St6gal1*$^{-/-}$ tumors exhibit reduced phosphorylation of FAK compared with wild-type tumors. Magnification, 100×. *Brownish-red,* positive staining.

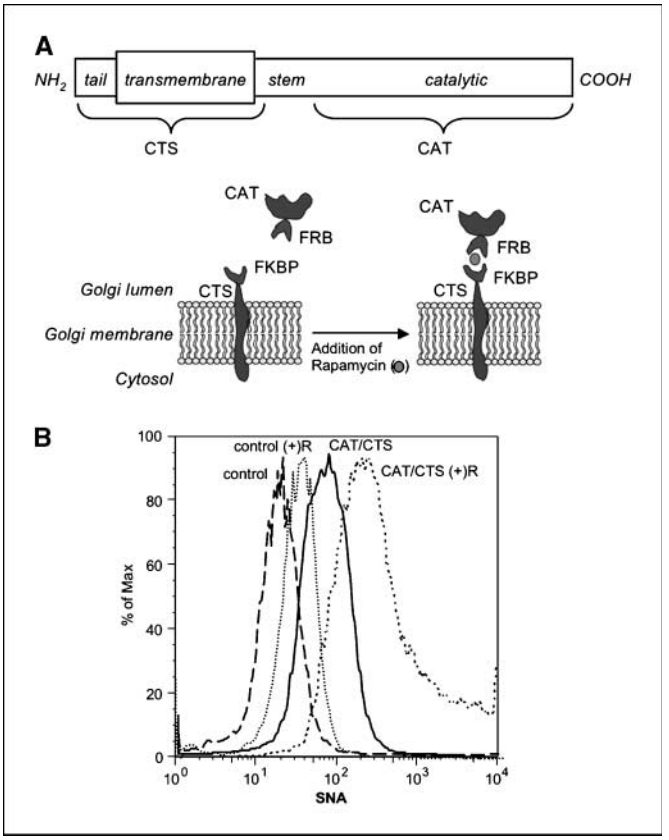

Figure 4. A small-molecule approach for conditional expression of *St6gal1*. *A,* constructs for conditional expression of *St6gal1*. Domain structure of ST6Gal-I containing an N-terminal cytosolic tail, a transmembrane domain, luminal stem region, and a C-terminal catalytic domain. The catalytic domain (*CAT*) and the localization domains (*CTS*) of the enzymes were separated and fused to FRB and FKBP, respectively. Rapamycin induces association of FKBP and FRB, thereby localizing the catalytic domain in the Golgi. *B,* enhanced SNA binding in *St6gal1*$^{-/-}$ cells transfected with the catalytic domain/localization domain (*CAT/CTS*) constructs. Flow cytometry analysis showing increased Sia6LacNAc expression (detected by SNA lectin) in the presence of rapamycin. Vector-transfected control cells did not show enhancement. The background SNA reactivity is likely due to a different sialylated structure, sialyl-Tn.

Restoration of *St6gal1* expression promotes the β1-integrin signaling pathway. To examine the effect of restoration of *St6gal1* on integrin focal adhesion signaling, such mammary tumor cells with or without induced *St6gal1* gene expression were plated on collagen I, a known ligand for the β1-integrins. Staining for phosphorylated Y^{397} (not shown) and Y^{576} was dramatically different between the two cell lines. With induction of enzyme expression, both antibodies stained focal adhesions in *St6gal1*-reconstituted cells that were essentially absent in the *St6gal1*-null cells (Fig. 5*A*). Localized focal adhesion staining with antibodies to phosphorylated FAK was not found in the absence of induction. Similar to results with the whole tumors, total nonphosphorylated FAK was present at similar levels in wild-type, *St6gal1*-null, and ST6Gal-I–reconstituted cells, although somewhat differentially distributed. In the *St6gal1*-null cells, unphosphorylated FAK accumulated in the perinuclear region, probably as a consequence of decreased phosphorylation and a decrease in peripheral localization to focal adhesions. Furthermore, the focal adhesion-associated adaptor protein paxillin was found to be localized to focal adhesions in wild-type and $St6gal1^{-/-}$ restored cells treated with rapamycin (Fig. 6), lending further support for a role of *ST6Gal-I* in activation of β1-integrin signaling in mouse mammary carcinoma cells.

Discussion

Integrins are cell-surface transmembrane glycoproteins mediating cell-ECM interactions and linking matrix proteins to the cytoskeleton (26). They play an important role in intracellular signal transduction (27), regulating various processes, such as proliferation, differentiation, apoptosis, and cell migration. Most integrins are major carriers of N-glycans, and changes in these structures can alter cell-cell and cell-ECM interactions, thereby affecting cell adhesion, migration, and tumor malignancy (for review, see refs. 16, 28–30). Binding could also be modulated by introducing a large artificial glycan wedge into one of the integrin glycosylation sites, creating a molecule with constitutively high affinity for physiologic ligands (31).

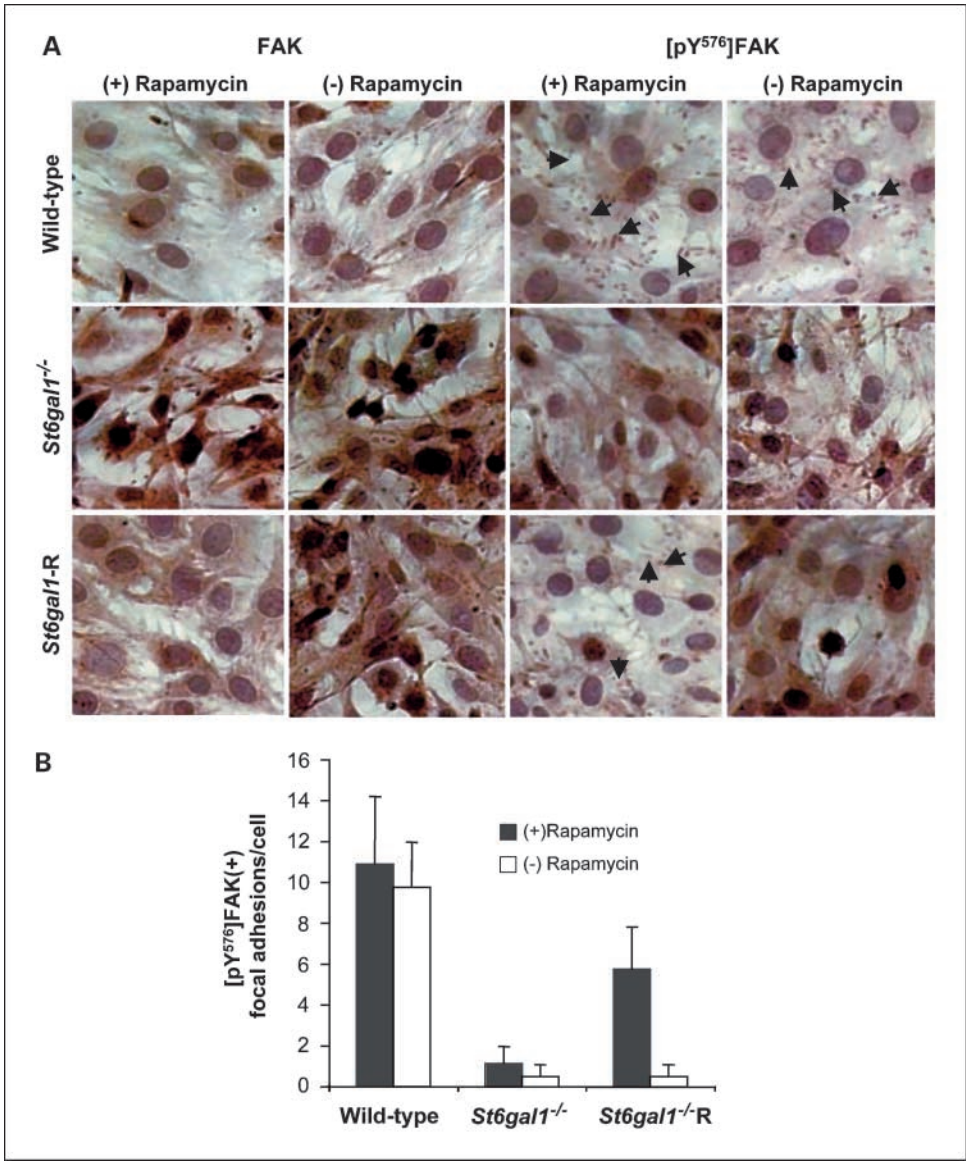

Figure 5. Reconstitution of ST6Gal-I expression in mammary tumor cell lines promotes localization of phosphorylated FAK to focal adhesions. *A,* cell lines established from wild-type or *St6gal1*-null MMTV-PyMT$^{+/-}$ mice were grown on collagen I–coated chamber slides and stained with antibodies against FAK or [pY^{576}]FAK (*top and middle*). *St6gal1*-null cells were also reconstituted with the inducible construct for reexpression of Sia6LacNac units (*ST6Gal-I-R*) Magnification, 400×. *Brownish-red,* positive staining. Arrows point to focal adhesion sites. Counterstaining of nuclei was performed using aqueous Mayer's hematoxylin. *B,* the number of focal adhesions staining positive for [pY^{576}]FAK was quantitated using Adobe Photoshop, analyzing six random high-power fields of each chamber. Addition of rapamycin significantly increased staining in *St6gal1*-reconstituted cells compared with the null cells ($P < 0.05$).

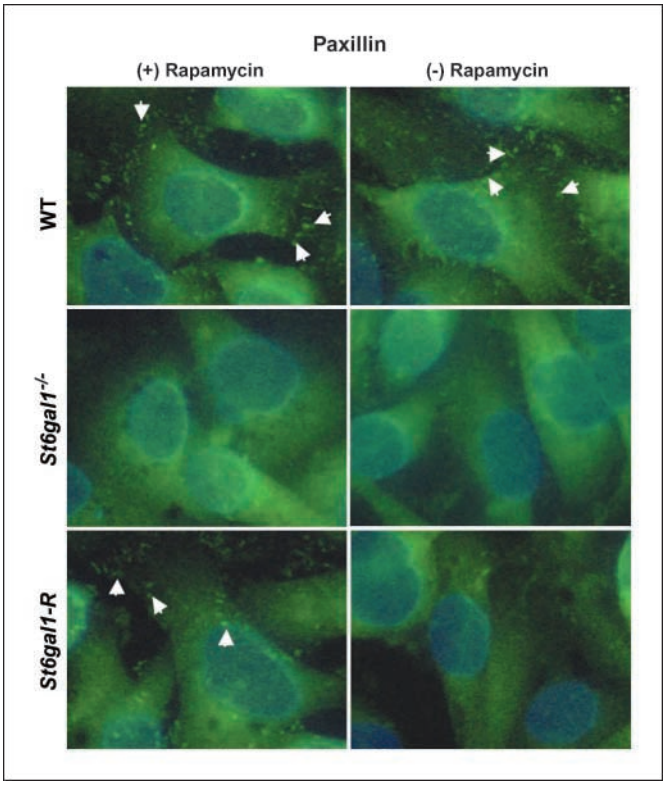

Figure 6. Increased association of the focal adhesion-associated adaptor protein paxillin with focal adhesions of *St6gal1*-restored cells. Mouse mammary tumor cells from MMTV-PyMT$^{+/-}$ mice were grown on collagen I–coated chamber slides and stained with rabbit anti-paxillin antibody and secondary FITC-conjugated goat anti-rabbit antibody. Counterstaining of nuclei was performed using mounting medium with 4′,6-diamidino-2-phenylindole. Arrows point to focal adhesion sites. The addition of rapamycin significantly increased staining paxillin in focal adhesions in *St6gal1*-reconstituted cells. Magnification, 1,000×.

Previous *in vivo* studies focused on major changes in glycan structure on integrins and other glycoproteins. For instance, removing the enzyme responsible for synthesis of the underlying LacNAc structure initiated by β1-6 *N*-acetylglucosaminyltransferase-V (encoded by *Mgat5*) resulted in tumor growth and metastasis being suppressed in *Mgat5*-deficient mice (32). Furthermore, *Mgat5*$^{-/-}$ tumor cells were deficient in membrane ruffling, actin was organized as stress fibers, and turnover was slower. The underlying LacNAc structure can be recognized by galectins, and previous studies have shown that galectin recognition can be involved in the activation of FAK and promotes tumor cell spreading (33). However, these studies did not address changes in terminal Sia6LacNAc units. Sialic acid capping, especially by α2-6-sialic acids, can reduce galectin binding (34); thus, it is possible that some effects seen here can be ascribed to enhanced galectin binding. However, one would have expected an increase in activation of FAK in the Sia6LacNAc-deficient cells or tumors. Thus, the phenotype in the *St6gal1*$^{-/-}$ background is more likely due to a direct effect on integrin function, perhaps via the enhanced binding to collagen, as reported by Bellis and colleagues (18).

Here, we have been able to study the biological significance of Sia6LacNAc structure in tumor biology and progression *in vivo*. Increased differentiation in Sia6LacNAc-deficient tumors was associated with altered expression of genes in the focal adhesion signaling pathway, many of which are downstream of β1-integrins. In this regard, β1-integrin plays a critical role in both the initiation and maintenance of mammary tumor growth *in vivo* (25). Ablation of this integrin prevented the formation of focal adhesion (35).

Given prior literature, it may not be a surprise that altered glycosylation of an integrin can modify function. However, it is remarkable that elimination of just one terminal sialic acid linkage affects the signaling through the integrin. Whereas hypersialylation may stabilize the integrin in its active conformation, removal of this sialic acid linkage from the integrin may allow normal regulation of integrin activation and downstream signaling, promoting differentiation. It is also interesting to note that, at least in this system, increased differentiation was not associated with any obvious reduction in tumor growth rate.

We realize of course that, although the genetic change made is quite subtle (elimination of a single linkage-specific sialyltransferase), integrins do not function alone and ST6Gal-I modifies several other surface glycoproteins in this system. Thus, although we feel that the β1-integrin effects remain the best explanation for the phenotype, there could be other potential effects on other cell surface receptors. Regardless, our studies provide an explanation for the frequent up-regulation of the *ST6GALI* gene in various human carcinomas and the association with progression and poor prognosis. Given the microevolutionary nature of cancer, subclones with up-regulated ST6Gal-I would be selected, being less differentiated and proliferating better. Inhibition of the enzyme might thus be useful in cancer therapy.

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