Sialylation of the Fas Death Receptor by ST6Gal-I Provides Protection against Fas-mediated Apoptosis in Colon Carcinoma Cells*

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The glycosyltransferase, ST6Gal-I, adds sialic acid in an α2–6 linkage to the N-glycans of membrane and secreted glycoproteins. Up-regulation of ST6Gal-I occurs in many cancers, including colon carcinoma, and correlates with metastasis and poor prognosis. However, mechanisms by which ST6Gal-I facilitates tumor progression remain poorly understood due to limited knowledge of enzyme substrates. Herein we identify the death receptor, Fas (CD95), as an ST6Gal-I substrate, and show that α2–6 sialylation of Fas confers protection against Fas-mediated apoptosis. Intriguingly, differences in ST6Gal-I activity do not affect the function of DR4 or DR5 death receptors upon treatment with TRAIL, implicating a selective effect of ST6Gal-I on the Fas receptor. Using ST6Gal-I knockdown and forced overexpression colon carcinoma cell models, we find that α2–6 sialylation of Fas prevents apoptosis stimulated by FasL as well as the Fas-activating antibody, CH11, as evidenced by decreased activation of caspases 8 and 3. We also show that α2–6 sialylation of Fas does not alter the binding of CH11, but rather inhibits the capacity of Fas to induce apoptosis by blocking the association of FADD with Fas cytoplasmic tails, an event that initiates death-inducing signaling complex formation. Furthermore, α2–6 sialylation of Fas inhibits Fas internalization, which is required for apoptotic signaling. Although dysregulated Fas activity is a well known mechanism through which tumors evade apoptosis, the current study is the first to link Fas insensitivity to the actions of a specific sialyltransferase. This finding establishes a new paradigm by which death receptor function is impaired for the self-protection of tumors against apoptosis.

The ability to evade apoptosis is one of the defining characteristics of a malignant tumor cell (1). Escape from cell death can be accomplished through alterations in various cellular components, including dysregulation of oncogenes and tumor suppressors, and mutations in apoptotic and anti-apoptotic signaling machinery. The TNF family of death receptors (TNFRs),3 including TNFR1, DR4, DR5, and Fas (CD95), represents one category of signaling molecules that is commonly disrupted in human tumors and has been strongly implicated in tumor cell survival (2, 3).

The Fas death receptor, like other TNFRs, is a homotrimmeric transmembrane receptor that activates multiple intracellular signaling cascades, one of which directs apoptosis. Upon association with activating ligands, Fas undergoes higher order clustering, which facilitates the binding of cytosolic proteins to the Fas cytoplasmic tails. The first protein recruited to the Fas tails is FADD, which binds to Fas through a region known as the death domain. Several other proteins, including procaspase 8 and procaspase 10, are then recruited to the Fas/FADD complex, and together these proteins form the death-inducing signaling complex (DISC). This complex is internalized through clathrin-mediated endocytosis and allows for further DISC formation required for apoptotic signaling (4). This enhanced formation of the DISC leads to the autolytic cleavage and activation of procaspase 8, which goes on to cleave the effector caspase, caspase 3, ultimately resulting in apoptotic endpoints such as membrane blebbing and DNA fragmentation (2).

Diminished Fas expression and activity are well established as mechanisms responsible for the apoptotic resistance of tumor cells. A myriad of studies have reported alterations in the expression of pro- and anti-apoptotic components involved in the Fas pathway such as c-FLIP, BAX, and BCL-2 (5–7). Additionally, in many tumor types there is up-regulation of the endogenous ligand for the Fas receptor, FasL, which is thought to provide a mechanism of self-conservation for tumor cells through FasL-directed killing of tumor-invading immune cells (8). However, despite the extensive research focused on changes in the expression level of Fas and associated signaling molecules, very few studies have investigated molecular mechanisms that alter Fas function independent of variant protein expression, for example, post-translational modifications such as glycosylation.

The Fas receptor is modified with both O- and N-linked glycans (9), although the functional significance of these glycoconjugates in Fas signaling has received minimal attention. It has been known for decades that tumor cells express an altered profile of cell surface oligosaccharides, and in fact there is a specific subset of glycosylating enzymes exhibiting aberrant activity in human cancers. The ST6Gal-I sialyltransferase is one of the enzymes up-regulated in multiple types of cancer (10–15), and high ST6Gal-I levels are associated with increased metastatic potential (12, 15–17). ST6Gal-I is responsible for the addition of the negatively charged sugar, sialic acid, in an α2–6

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3 The abbreviations used are: TNFR, TNF family of death receptors; DISC, death-inducing signaling complex; IPG, immobilized pH gradient; MAA, Maackia amurensis agglutinin; PECAM, Platelet Endothelial Cell Adhesion Molecule; SNA, Sambucus nigra agglutinin.
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linkage to the N-linked glycans of cell surface or secreted glycoproteins. In the current study we have identified Fas as an ST6Gal-I substrate and further determined that α2–6 sialylation of Fas inhibits Fas-mediated cell death. These collective results elucidate a novel mechanism by which tumor cells evade apoptosis.

EXPERIMENTAL PROCEDURES

Cell Culture—HD3 colon epithelial cells expressing onco-

genic ras were developed as previously reported (18). These

HD3 cells were then transduced with lentivirus (purchased

from Sigma) containing either shRNA sequence against

ST6Gal1 (HD3.sh) or an empty vector (HD3.ev). A pooled pop-

ulation of clones stably expressing shRNA was generated by

puromycin selection. Down-regulation of ST6Gal-1 expression

was confirmed by Western blot (19). SW48 cells were pur-

chased from ATCC. These cells were transduced with either

lentivirus containing rat liver ST6Gal-I cDNA (SW.ST6) or len-

tivirus containing an empty vector (SW.ev). The original

ST6Gal-I plasmid was obtained from Dr. Karen Colley (Univer-

sity of Illinois, Chicago), and lentiviral vectors developed from

this plasmid were constructed by Dr. John Wakefield (Open-

Biosystems, Inc.). SW.ST6 cells represent a pooled population

of stably transduced clones, isolated by puromycin selection.

Confirmation of expression and functionality of ST6Gal-I has

been published previously (20). HD3 cells were maintained in

low glucose (1 g/liter) DMEM with 7% FBS and 1% antibiotic/

antifungal containing penicillin G, streptomycin sulfate, and

amphotericin B (Invitrogen). SW48 cells were maintained in

low glucose (1 g/liter) DMEM with 10% FBS and 1% antibiotic/

antifungal containing penicillin G, streptomycin sulfate, and

amphotericin B (Invitrogen). CH11 (Millipore) at 0.5 mg/ml or TRAIL (Biomol International) at 2

mg/ml was incubated overnight with 50 μl of immobilized SNA-1 or MAA lectin (EV Laboratories) with rotation at 4 °C. The α2–6 sialylated proteins complexed with SNA or α2–3 sialylated proteins complexed with MAA lectin were collected by brief centrifugation and washed. Sialylated proteins were released from the complexes by boiling in SDS-PAGE sample buffer. The proteins were then resolved by SDS-PAGE and immunoblotted to detect Fas.

Two-dimensional Gel Electrophoresis—HD3 whole cell

lysates were prepared by lysing in lysis buffer with 50 mM Tris-

HCl (pH 7.4) with 1% Triton X-100 and protease inhibitors

(Roche Applied Bioscience). Lysates were then diluted in iso-

electric focusing rehydration buffer containing 7 M urea, 2 M

thiourea, 4% CHAPS, 30 mM DTT, 0.5% ampholytes, and trace

bromphenol blue. Samples were used to rehydrate 11-cm, 3–10

linear gradient IPG strips for 16 h in Drystrip Reswelling tray.

For the first dimension, IPG strips were focused using Amer-

sham Biosciences IPGphor II isoelectric focusing unit at 50 μA

per strip at 20 °C. For the second dimension, strips were equili-

brated in DTT equilibration buffer containing 6 M urea, 20%

glycerol, 50 mM Tris-HCl, pH 8.8, 2% SDS, 65 mM DTT, and

trace bromphenol blue for 30 min in I2A equilibration buffer

containing 6 M urea, 20% glycerol, 50 mM Tris-HCl, pH 8.8, 2%

SDS, 2.5% iodoacetamide, and trace bromphenol blue for 15

min. Strips were then electrophoresed on 12.5% criterion gels

(Bio-Rad) at a 100-V constant in the Bio-Rad Criterion gel box.

Proteins were then transferred to PVDF membranes and

immunoblotted for Fas (Santa Cruz Biotechnology).

Flow Cytometry—Cells were harvested non-enzymatically

trom tissue culture dishes using Cell Stripper solution (Cellgro).

Cells were then resuspended into ice-cold PBS containing 0.2%

heat-denatured BSA. For the antibody binding assay: cells were

treated with CH11 (Millipore) at 20 μg/ml or IgM control for

1 h at 4 °C. Cells were then incubated with FITC-tagged sec-

ondary antibodies. For the CH11 binding curve, the same

experiment was performed using a range of CH11 concen-

trations, including 1, 10, 20, or 40 μg/ml. For Fas expression: cells were treated with FITC-conjugated anti-human CD95 (BD Pharmingen) for 1 h at 4 °C. After labeling, cells were washed in PBS/BSA and then analyzed with a FACSCalibur (BD Biosciences) at the University of Alabama at Birmingham Arthritis and Musculoskeletal Center Analytic and Preparative Core Facility.

DISC Immunoprecipitation—DISC components were im-

munoprecipitated by treating 8.0 × 10^6 cells with 1.0 μg/ml

CH11 (Millipore) in media at 4 °C (control) or 37 °C for 30 min.

Treatment was stopped by the addition of ice-cold PBS. Cells were centrifuged to remove unbound antibody and washed with ice-cold PBS. Cells were then subjected to lysis buffer containing 50 mM Tris-HCl (pH 7.4) with 1% Triton X-100 and protease inhibitors (Roche Applied Bioscience) on ice for 15
min. Samples were centrifuged, and supernatant was collected and rotated overnight with 40 μl of prewashed anti-IgM-conjugated agarose beads (Sigma). Precipitated proteins were released from the complexes by boiling in SDS-PAGE sample buffer. Proteins were resolved by SDS-PAGE and immunoblotted for the Fas-binding protein, FADD (Cell Signaling Technology). The PVDF membrane was then stripped with Restore Western Blot Stripping Buffer (Thermo Scientific) and reprobed with Fas (Santa Cruz Biotechnology) as previously described.

Internalization Assay—Cells were lifted from the tissue culture plastic with trypsin, and trypsin activity was stopped with trypsin inhibitor. Cells were resuspended in media with 1.0 μg/ml CH11 (Millipore) at 4 °C (control) or were warmed to 37 °C for 30 min to activate internalization. Cells were washed and resuspended in 3.7% formaldehyde in PBS and fixed for 15 min at room temperature. Cells were then washed and resuspended in media with 1.25 μg/ml anti-mouse IgM Alexa 488 (Invitrogen) at 4 °C for 1 h. Stained cells were then washed and resuspended in Vectashield (Vector Laboratories, Burlingame, CA) and plated on a glass microscope slide. Slides were imaged with the Zeiss LSM 710 laser confocal scanning microscope.

RESULTS

α2–6 Sialylation Confers Protection against Ligand-induced Fas-mediated Apoptosis in Two Cell Models—Although the CH11 antibody is commonly used to stimulate Fas-mediated apoptosis, it was important to examine the effects of ST6Gal-I on apoptosis induced by the biologic ligand for the Fas receptor, FasL. HD3.par, HD3.sh, and additionally, an empty vector control cell line (HD3.ev) were treated with either CH11 or FasL and immunoblotted for cleaved caspase 3. As shown in Fig. 3A, HD3.sh cells displayed higher levels of cleaved caspase 3 in response to both FasL and CH11 as compared with HD3.par and HD3.ev cells, confirming that diminished α2–6 sialylation renders cells more susceptible to two independent Fas activators.

Having shown that ST6Gal-I knockdown enhances Fas-mediated apoptosis, we next evaluated whether overexpression of the enzyme in cells with low endogenous ST6Gal-I would inhibit Fas-induced apoptosis. To this end we monitored cell death in the SW48 colon epithelial cell model, which lacks any detectable expression of ST6Gal-I (21). Previously we generated an SW48 cell line that stably expresses ST6Gal-I and verified protein expression and enzyme activity (20). SW48 parental cells (SW.par), which have no ST6Gal-I, SW48 cells with forced expression of ST6Gal-I (SW.ST6), and an SW48 cell line transduced with an empty vector lentivirus (SW.ev) were treated with CH11 or FasL and surveyed for apoptosis (Fig. 3B). These experiments revealed substantially lower levels of activated caspase 3 in SW.ST6 cells compared with SW.par and
SW.ev cells. Hence, the combined results in Fig. 3 establish that ST6Gal-I activity confers protection against apoptosis induced by both the Fas-activating antibody, CH11, and also the endogenous ligand, FasL, in two distinct colon carcinoma cell models.

**Fas Is a Target for ST6Gal-I α2–6 Sialylation**—We next sought to determine if the Fas receptor is a direct target for α2–6 sialylation by ST6Gal-I, given that this receptor is known to have two possible N-linked glycosylation sites (22, 23). To address this, cell lysates were incubated with agarose-conjugated SNA, a lectin that binds specifically to α2–6-linked sialic acids. Samples were centrifuged to selectively precipitate α2–6 sialylated proteins, and α2–6 sialylated proteins were then immunoblotted for the Fas receptor. As depicted in Fig. 4A, the band representing Fas in the SNA precipitates from HD3.par cells is denser than that noted in precipitates from HD3.sh cells, indicating more sialylated Fas in the presence of high ST6Gal-I expression. However, no differences were observed in the amount of Fas present in whole cell lysates (representing total Fas protein), indicating that variant ST6Gal-I expression alters Fas sialylation but not Fas protein expression. Also, MAA lectin precipitation (specific for α2–3 sialic acids) revealed that ST6Gal-I activity does not protect against DR4/5-directed apoptosis (orange, FLICA staining of cleaved caspases 3 and 7: blue, Hoechst staining for nuclei). D, percentage of apoptosis was quantified by counting FLICA-positive versus Hoescht-stained cells from multiple microscopic fields. *, p < 0.01; **, p < 0.001.

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**FIGURE 2.** FLICA staining of cleaved caspases 3 and 7 demonstrates that ST6Gal-I protects cells from Fas- but not DR4/DR5-mediated apoptosis. A, HD3.par and HD3.sh cells were stained with FLICA in the absence of CH11 treatment (panels 1 and 2), which revealed negligible activation of caspases 3 and 7 in both cell lines. Upon treatment with CH11, there is more activated caspase 3 and 7 staining in HD3.sh (panel 4) as compared with HD3.par (panel 3). There are also changes in colony and individual cell morphology, indicating apoptosis. Additionally, as can be seen in B, there is nuclear condensation verifying apoptosis (white arrows) in the HD3.sh after treatment with CH11, C, to induce DR4/DR5 signaling, cells were treated with TRAIL. Minimal FLICA staining was observed in the absence of treatment (panels 1 and 2), whereas extensive and comparable amounts of cleaved caspases 3 and 7 were observed in HD3.par (panel 3) and HD3.sh (panel 4) cells. As with HD3 cells, variant expression of ST6Gal-I did not alter total Fas protein levels.

To further confirm Fas as an ST6Gal-I substrate, whole cell lysates from HD3.ev and HD3.sh cells were resolved using two-dimensional electrophoresis to separate proteins by both molecular weight and isoelectric point, and then immunoblotted for Fas receptor. Several Fas isoforms were revealed by this assay; however, a higher molecular weight, more negatively charged band was missing from the HD3.sh lysates. These results are consistent with the loss of a sialylated Fas isoform in cells with ST6Gal-I knockdown. We also used flow cytometry to show that variant Fas sialylation did not change the levels of cell surface Fas expression (Fig. 4D).
there was substantial, and equivalent, binding of CH11 to Fas expressed by the HD3.par and HD3.sh cell lines. To further investigate Fas-activator binding, we examined CH11 binding at a range of concentrations from 1 to 40 μg/ml. HD3.par and HD3.sh cell lines showed comparable mean fluorescent intensity at every examined concentration (Fig. 5B). Thus, the strong inhibitory effect of α2-6 sialylation on Fas-dependent apoptosis cannot be attributed to diminished binding of the Fas-activating antibody. These data point to a role for sialylation in modulating some aspect of Fas receptor activation rather than ligand binding.

To examine the effect of ST6Gal-I sialylation on Fas signaling, HD3 cells were treated with CH11, and whole cell lysates were immunoblotted for cleaved caspase 8. Caspase 8, an initiator caspase, was evaluated because of its early recruitment to the DISC after Fas activation. As shown in Fig. 5C, the amount of cleaved caspase 8 was dramatically increased in CH11-treated HD3.sh cells as compared with the HD3.par cells, suggesting that Fas sialylation alters signaling at some step upstream of caspase 8 activation.

FADD is the initial protein recruited to the DISC that binds directly to the cytoplasmic tail of the Fas receptor after activation. Therefore we examined the amount of FADD associated with the Fas receptor tails by using co-immunoprecipitation experiments. HD3 cells were treated with CH11 at 37 °C to activate DISC formation, and then the Fas receptor and the associated DISC complex were immunoprecipitated. As a control, cells were incubated with CH11 antibody at 4 °C, a treatment that does not induce DISC formation. Immunoprecipitates were then blotted for associated FADD (Fig. 5D). These experiments showed that a basal amount of FADD was bound to the Fas cytoplasmic tails in control cells, and no apparent increase in Fas/FADD association was observed in the HD3.par cells upon activation by CH11. In marked contrast, CH11 treatment of HD3.sh cells at 37 °C induced a substantial increase in Fas/FADD association, indicative of DISC formation. These results suggest that sialylation of Fas somehow alters the accessibility of Fas cytoplasmic domains for binding to FADD and consequently regulates the first step in DISC formation.

α2-6 Sialylation Inhibits Fas Receptor Internalization—The internalization of Fas after receptor activation is necessary for
Therefore, to further characterize the effects of receptor sialylation, we examined Fas internalization after treatment with CH11. We treated cells with CH11 at either 4 °C (as a control) or 37 °C (to allow for signaling and internalization), fixed the cells, and then used anti-mouse IgM Alexa-fluor 488 (Invitrogen) to visualize the Fas receptor remaining on the cell surface of non-permeabilized cells. As shown in Fig. 6, no major differences were noted in surface Fas levels upon CH11 treatment at 4 °C; however, upon receptor activation at 37 °C, substantially more Fas was internalized in HD3.sh cells. These data suggest that α2–6 sialylation of Fas inhibits receptor internalization, thus limiting Fas-dependent apoptosis.

DISCUSSION

There are multiple sialyltransferases that add sialic acid in an α2–3 linkage to N-glycans, however ST6Gal-I is the predominant enzyme that elaborates the α2–6 linkage of sialic acid to
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N-glycosylated proteins (24, 25). Another α2–6 sialyltransferase, ST6Gal-II, has been identified, however this enzyme is localized primarily to the brain and preferentially sialylates oligosaccharides rather than glycoproteins (26, 27). There are also several ST6GalNAc enzymes that add α2–6 sialic acid to the GalNAc residue of O-linked glycans or gangliosides (reviewed in Ref. 28). The ST6Gal-I enzyme is overexpressed in at least 13 different types of cancers, including colon, breast, esophageal, oral, ovarian, cervical, leukemias, and brain tumors (10–15, 29–31), and high ST6Gal-I levels are associated with metastasis and poor patient prognosis (15–17). The functional contribution of ST6Gal-I to tumor progression has not been widely investigated, however increased α2–6 sialylation has been linked to enhanced tumor cell migration and invasion (12, 18–20, 32). We and others have reported that ST6Gal-I expression is increased by oncogenic ras (reviewed in Ref. 33), which is found in over 30% of human cancers, and Piller’s group showed this up-regulation takes place through the ralGEF pathway (34). Studies of ST6Gal-I in colon carcinoma have revealed that 90% of colon tumors screened had up-regulated ST6Gal-I expression (11) and 70% of colorectal cancers were positive for the α2–6 sialic acid modification added by ST6Gal-I (35). Animal studies also support a role for ST6Gal-I up-regulation in tumor progression. Human (16) and murine (36) cancer cells with high levels of α2–6 sialylation were more metastatic to liver following splenic injection in nude mice and enzymatic removal of sialylation from tumor cells prior to injection inhibited metastasis. Furthermore, Varki’s group reported that ST6Gal-I-null mice bred to a spontaneous breast cancer model displayed tumors that were more differentiated than tumors from wild-type mice, suggesting that ST6Gal-I activity contributes to the poorly differentiated phenotype of more advanced cancers (37).

Despite these compelling results, there is still a limited understanding of the mechanism by which ST6Gal-I-directed sialylation regulates tumor cell behavior, because of the sparse knowledge of ST6Gal-I substrates, as well as the lack of information regarding the effects of α2–6 sialylation on the function of specific proteins. The current investigation provides a significant advance toward defining the role of ST6Gal-I in tumor progression by showing that the Fas receptor is α2–6 sialylated by ST6Gal-I. Furthermore, we show that α2–6 sialylation of Fas inhibits Fas signaling through both 1) blocking the binding of FADD to the Fas cytoplasmic tail, which is the first step in DISC formation, and 2) inhibiting internalization of the stimulated Fas receptor, which is necessary for Fas apoptotic signaling (4). Sialylation can alter receptor function through several mechanisms, including conformational alteration (38), clustering (39), and differential internalization rate, depending on the specific receptor. Consistent with our work, the CD45 and PECAM receptors have been shown to be targets for α2–6 sialylation by ST6Gal-I, and this sialylation affects internalization in both cases (39–41).

The role of α2–6 sialylation in tumor progression has most often been associated with effects on tumor cell migration and invasion, however it is emerging that ST6Gal-I may be a major regulator of tumor cell survival. This sialylation-dependent survival benefit is likely mediated through multiple molecular pathways. Studies by our group and others have shown that ST6Gal-I-directed α2–6 sialylation of selected receptors serves as a key negative regulator of galectin-induced apoptosis (39, 42, 43). Additionally, the diminished internalization of PECAM due to α2–6 sialylation allows anti-apoptotic signaling from PECAM for a longer time interval (40). It has also been determined that ST6Gal-I levels are up-regulated after radiation treatment in mice (44), and these higher levels confer protection against radiation-induced apoptosis. Our novel finding that α2–6 sialylation inhibits Fas signaling adds to the growing body of literature suggesting that ST6Gal-I modifies a select group of substrates to regulate a multiplicity of apoptotic signaling cascades, thus providing a strong selective advantage for tumor cells.

Changes in expression of the Fas receptor and associated signaling molecules have long been regarded as a mechanism by which tumors evade apoptosis. Several types of cancers are known to down-regulate the Fas receptor as a protective measure, including colon (45), testicular (46), and hepatoma (47). In addition, increased FasL expression on the tumor cell surface has been reported in several cancers (8) and is thought to play a role in tumor evasion from the immune system. Expression levels of downstream effectors in the Fas-induced apoptotic cascade are also altered in tumor tissue, providing additional mechanisms for protection against Fas-mediated apoptosis. Alterations include up-regulation of the anti-apoptotic Bcl and c-FLIP and down-regulation of pro-apoptotic Bax (5–7). Interestingly, Fas is highly expressed on the surface of many tumor types that are not susceptible to Fas-mediated apoptosis (48–51). Results presented herein implicate α2–6 sialylation as a newly identified mechanism by which tumor cells disable Fas signaling. A role for sialylation in regulating the Fas receptor was previously suggested by studies in lymphocytes (52, 53). Keppler et al. reported that highly sialylated subclones of the BJAB B cell line were less susceptible to Fas-mediated apoptosis, although α2–3 and α2–6 sialic acids were not distinguished in this study (52). Consistent with these results, Peter et al. determined that enzymatic removal of all cell sialic
acids (i.e. both α2–3 and α2–6 sialylation) increased the vulnerability of B and T cells to Fas-mediated apoptosis (53). Although these prior studies clearly linked sialylation to Fas activity, they provided limited information regarding the mechanism given that there are more than 20 different sialyltransferases. In the current study we show that Fas activity is regulated through α2–6 sialylation by a distinct sialyltransferase, ST6Gal-I, and of equal importance, this type of sialic acid modification has physiologic relevance given the known up-regulation of ST6Gal-I in cancer. Indeed we previously reported that another ST6Gal-I substrate, the β1 integrin, expresses elevated α2–6 sialylation in 100% of human colon tumors (20). It is also noteworthy that the total levels of sialylation on the two cell model systems evaluated in this study are quite different; HD3 cells express α2–3 sialyltransferases (19), whereas SW48 cells express no endogenous sialyltransferases (21). Thus, ST6Gal-I-mediated sialylation of Fas blocks Fas signaling in cells with both extensive α2–3 cell surface sialylation, including α2–3 sialylation of the Fas receptor (HD3), and cells with no surface sialylation other than that directed by exogenously expressed ST6Gal-I (SW48). These results imply a unique functionality imparted by the α2–6 sialic acid modification added by ST6Gal-I. Further highlighting the specificity of this novel molecular pathway, ST6Gal-I activity appears to have no effect on apoptotic signaling by the DR4 or DR5 death receptors. Intriguingly, work by Ashkenazi’s group has shown O-glycosylation of the DR5 receptor regulates sensitivity to the DR5 ligand, TRAIL, but does not affect signaling by Fas (54). Moreover, the sites for O-glycosylation on DR5 are not conserved in the Fas receptor, and correspondingly, the DR5 receptor does not contain consensus sequences for N-glycosylation. A fundamental concept highlighted by these observations is that there is specificity in the effects of certain glycan structures on the function of distinct death receptors.

In conclusion, the current study is the first to demonstrate that a specific sialic acid modification, elaborated by an enzyme known to be up-regulated in cancer, inactivates signaling through the Fas receptor. These results have several important translational implications, including the potential for α2–6 sialylation to serve as a biomarker for Fas insensitivity. In addition, the finding that forced down-regulation of Fas α2–6 sialylation sensitizes tumor cells to apoptosis, even in cells expressing the powerful ras oncogene, suggests that ST6Gal-I may be a promising therapeutic target. Finally, ST6Gal-I-dependent regulation of Fas suggests a new paradigm in death receptor signaling, and it more broadly highlights an emerging role for ST6Gal-I as a critical mediator of multiple cell survival pathways.

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