O-Glycosylation Regulates LNCaP Prostate Cancer Cell Susceptibility to Apoptosis Induced by Galectin-1

Hector F. Valenzuela,1 Karen E. Pace,1 Paula V. Cabrera,1 Rachel White,1 Katja Pörvari,3 Helena Kaija,3,4 Pirkko Vihko,3,4 and Linda G. Baum1,2

1Department of Pathology and 2Jonsson Comprehensive Cancer Center, School of Medicine, University of California at Los Angeles, Los Angeles, California; 3Research Center for Molecular Endocrinology and WHO CCR, Biocenter Oulu, University of Oulu, Oulu, Finland; and 4Department of Biological and Environmental Sciences, Division of Biochemistry, University of Helsinki, Helsinki, Finland

Abstract

Resistance to apoptosis is a critical feature of neoplastic cells. Galectin-1 is an endogenous carbohydrate-binding protein that induces death of leukemia and lymphoma cells, breast cancer cells, and the LNCaP prostate cancer cell line, but not other prostate cancer cell lines. To understand the mechanism of galectin-1 sensitivity of LNCaP cells compared with other prostate cancer cells, we characterized glycan ligands that are important for conferring galectin-1 sensitivity in these cells, and analyzed expression of glycosyltransferase genes in galectin-1–sensitive, prostate-specific antigen–positive (PSA+) LNCaP cells compared with a galectin-1–resistant PSA– LNCaP subclone. We identified one glycosyltransferase, core 2 N-acetylglucosaminyltransferase, which is down-regulated in galectin-1–resistant PSA– LNCaP cells compared with galectin-1–sensitive PSA+ LNCaP cells. Intriguingly, this is the same glycosyltransferase required for galectin-1 susceptibility of T lymphoma cells, indicating that similar O-glycan ligands on different polypeptide backbones may be common death trigger receptors recognized by galectin-1 on different types of cancer cells. Blocking O-glycan elongation by expressing α2,3-sialyltransferase 1 rendered LNCaP cells resistant to galectin-1, showing that specific O-glycans are critical for galectin-1 susceptibility. Loss of galectin-1 susceptibility and synthesis of endogenous galectin-1 has been proposed to promote tumor evasion of immune attack; we found that galectin-1–expressing prostate cancer cells killed bound T cells, whereas LNCaP cells that do not express galectin-1 did not kill T cells. Resistance to galectin-1–induced apoptosis may directly contribute to the survival of prostate cancer cells as well as promote immune evasion by the tumor.

[Conflict of Interest Disclosure]

Introduction

Specific alterations in the glycosylation pattern of cell-surface glycoproteins and glycolipids occur during cellular transformation and tumor progression (1–6). These novel "glyco-epitopes" are proposed to facilitate tumor progression by several mechanisms, which include promoting cellular detachment from basement membrane and migration to sites of metastasis, masking of tumor cells to avoid an immune response, and protecting tumor cells from endogenous inducers of apoptosis (1, 5, 7, 8). Altered tumor cell glycosylation as well as changes in expression of glycan-binding lectins by tumor cells and stroma has been described in prostate cancer, the most common cancer in men and the second highest cause of cancer deaths in Western society (3, 4, 9–17). Changes in prostate cancer cell glycosylation have been proposed to affect prostate cancer cell growth, invasion and metastasis, and progression to androgen independence. For example, prostate-specific antigen (PSA) from cancer patients typically bears increased levels of α2,3-linked sialic acid compared with PSA from patients with benign prostatic hyperplasia (18). Similarly, the tumor cell-surface glycoprotein prostate mucin antigen, which bears abundant O-glycans, is expressed on prostate tumor cells but not on normal prostate cells (19). O-Glycans on prostate cancer cells often bear the Tn antigen (GalNAc-O-Ser/Thr), a glyco-epitope that has been proposed as a target for tumor immunotherapy (20), and the T antigen on O-glycans (Galβ1,3GalNAc) plays a role in prostate cancer cell adhesion to endothelium (21).

Several members of the galectin family of lectins are aberrantly expressed in prostate cancer and have been implicated in the process of prostate cancer progression (9–17, 21–26). Galectins are soluble carbohydrate-binding proteins that can remain intracellular, where they regulate cell signaling and cell survival, or can be secreted via a nonclassic pathway to mediate cell-cell and cell–matrix interactions (27). Galectin-8, also known as prostate carcinoma tumor antigen 1, is selectively expressed in prostate carcinoma, compared with normal or benign hyperplastic prostate (16, 17). As galectin-8 modulates cell adhesion to extracellular matrix proteins such as fibronectin, galectin-8 may be involved in tumor cell invasion. Galectin-3 is proposed to have both intracellular and extracellular functions in prostate cancer. Cytoplasmic galectin-3 promotes prostate cancer cell resistance to apoptosis, anchorage-independent growth, and invasion into extracellular matrix, whereas extracellular galectin-3 mediates prostate cell attachment to endothelial cells (9–12, 21–25).

Galectin-1 acts extracellularly to induce apoptosis of normal and transformed T lymphocytes, as well as breast and trophoblast tumor cells (27, 28). Whereas specific types of cell-surface glycosylation are known to create or mask the glycan ligands required for galectin-1–induced T-lymphocyte cell death (8, 29–31), nothing is known about features of cell-surface glycosylation that regulate epithelial cancer cell susceptibility to galectin-1. Expression of galectin-1 by LNCaP prostate cancer cells, either by treatment with sodium butyrate or transfection with rat galectin-1 cDNA, induced apoptosis of this cell line, although these studies did not examine secretion of galectin-1 by the LNCaP cells, nor were glycan ligands for galectin-1 characterized (26). In contrast, the prostate cancer cell lines DU145 and PC-3 synthesize and secrete abundant galectin-1, implying that these cell lines are resistant to death triggered by galectin-1 (25). Similarly, a PSA–
LNCaP subclone showed a 25-fold increase in expression of galectin-1 mRNA and increased expression of galectin-1 protein, compared with androgen-dependent PSA− LNCaP cells (32, 33). Because loss of PSA expression is correlated with progression to a more aggressive tumor, the robust expression of galectin-1 by PSA− LNCaP cells suggested a correlation between increasing galectin-1 expression and prostate cancer cell progression. Similarly, Castronovo and colleagues have found that increasing expression of galectin-1 in prostate tumor stroma correlates with tumor aggressiveness and poor prognosis in prostate cancer patients, implying that tumor cell acquisition of resistance to galectin-1−induced cell death carries a selective advantage to tumor cells that can evade galectin-1−induced cell death (14). Moreover, recent work in a murine melanoma model showed that melanoma expression of galectin-1 induced death of infiltrating T cells, whereas suppression of tumor cell expression of galectin-1 enhanced CD8 T-cell attack of the tumor (34). We have directly shown that galectin-1 in extracellular matrix can kill infiltrating T cells (35). Thus, resistance to galectin-1 apoptosis may enhance prostate cancer cell metastasis as well as evasion of an immune response.

As mentioned above, the specific glycan ligands bound by galectin-1 to trigger cell death have not been identified in prostate cancer cells. In the present study, we identify a specific glycan structure required for galectin-1 to induce death of LNCaP cells. Importantly, this glycan ligand on LNCaP cells that is required for susceptibility to galectin-1−induced cell death is the same structure required for T-cell susceptibility to galectin-1 (29, 30); as prostate cancer cells do not express the T-cell glycoprotein receptors that participate in galectin-1−induced cell death (CD45, CD43, and CD7; refs. 30, 36, 37), these data indicate that galectin-1−induced death of epithelial and mesenchymal cells involves a common saccharide ligand that can be attached to different polypeptide backbones on different cell types. Finally, we find that prostate cancer cells expressing galectin-1 are potent inducers of T-cell apoptosis, supporting the model that galectin-1 expression by tumor cells promotes evasion of an immune response (34, 35, 38).

Materials and Methods

Cell lines. The human prostate cell lines LNCaP, PC-3, and DU145 and the human T-cell line CEM were obtained from the American Type Culture Collection. CEM, LNCaP and the PSA− and PSA+ LNCaP subclones (32) were grown in 100 × 20 mm tissue culture dishes in 10-mL RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 100 mg/mL streptomycin, and 100 units/mL penicillin. DU145 was grown in DMEM with 10% FBS and PC-3 was grown in Ham’s F12K medium with 7% FBS (Life Technologies, Inc.). Cells were maintained in a humidified incubator at 37°C with 5% CO2.

Galectin-1 detection. For immunofluorescence microscopy, LNCaP, DU145, and PC-3 were grown for 48 h on glass coverslips until 50% confluent. Cells were fixed with 2% paraformaldehyde in PBS for 30 min on ice, washed with PBS, stained with polyclonal rabbit anti-human galectin-1 antibody (1:500), and bound antibody was detected with fluorescein-conjugated goat anti-rabbit immunoglobulin G (IgG; 1:300; Jackson Immunonochemical) exactly as previously described (30). Slides were analyzed using an Axioskop 2 plus microscope and Axiovision 3.1 software (Zeiss).

For immunoblotting, subconfluent monolayers of prostate cells were removed from plates with PBS-EDTA (5 mmol/L). Cells were lysed in lysis buffer (50 mmol/L Tris-Cl, 1% NP40, 5 mmol/L EDTA, 1 mmol/L phenylmethylsulfonyl fluoride, 10 μg/mL aprotinin, 10 μg/mL leupeptin, 150 mmol/L NaCl) for 30 min on ice, samples centrifuged to pellet nuclei, and protein concentrations of the supernatants determined by Bio-Rad Protein Assay (Bio-Rad). Equal amounts of total cell protein (20 μg/lane) were separated by SDS-PAGE and transferred to nitrocellulose membranes; membranes were blocked with 10% nonfat milk and incubated overnight at 4°C with rabbit polyclonal antibody to galectin-1 (1:1,000). Membranes were washed in TBS with 0.5% Tween and incubated with horseradish peroxidase−conjugated goat anti-rabbit reagent (1:1,000; Bio-Rad). Samples were visualized with Hyperfilm enhanced chemiluminescence (Amersham). To detect equal loading, blots were stripped and reprobed with anti-actin (1:5,000; Sigma-Aldrich).

ELISA quantification of cell-surface galectin-1 was done exactly as in ref. 35, with 0.02 × 10^6 to 2 × 10^9 of the indicated cells added per well, using anti-galectin-1 IgG purified from rabbit polyclonal antisera to galectin-1. Galectin-1 concentration was determined based on a standard curve using purified recombinant galectin-1.

Galectin-1 prostate cell death assays. Subconfluent monolayers of prostate cell lines grown in 35-mm six-well plates were treated with 20 μmol/L galectin-1 in a final volume of 2-mL PBS with 1.2 mmol/L DTT for 5 h at 37°C. Control samples included cells treated with PBS/1.2 mmol/L DTT only (galectin-1 buffer control) and cells treated with 100 mmol/L lactose for 5 min before galectin-1 to show saccharide-specific galectin-1−induced cell death. The supernatant was removed and adherent cells were treated with 2 μL of 0.25% trypsin-EDTA (Life Technologies) and collected with a plastic scraper, resuspended by gentle pipetting in PBS, and labeled by terminal deoxynucleotide dUTP nick end labeling (TUNEL) using the APO-DIRECT kit (PharMingen). Data were acquired on a FACScan flow cytometer (Becton Dickinson) to detect FITC-labeled DNA and analysis was done with CellQuest software. Single cells were discriminated by analysis of DNA width versus DNA area, and only single cells were analyzed for TUNEL labeling.

Flow cytometric analysis of cellular glycosylation. Subconfluent LNCaP cells were treated with 2 mmol/L benzyl-α-N-acetylgalactosamine (benzyl-α-GalNac) in ethanol, with 25 mmol/L deoxynojirymycin (DMN) dissolved in media (Calbiochem), or with appropriate buffer controls for 72 h at 37°C. Cells were resuspended with PBS-EDTA and washed with PBS. LNCaP cells (1 × 10^6) were stained with 5 μL (1 mg/mL) of biotin-conjugated peanut agglutinin (PNA) or phytohemagglutinin (PHA) lectins (EY Laboratories) for 30 min on ice. Cells were washed and bound lectin was detected with phycoerythrin-conjugated streptavidin for 20 min on ice. Cells were washed and analyzed by flow cytometry. Data were acquired using a FACScan flow cytometer and analyzed with CellQuest software.

Analysis of glycosyltransferase gene expression in PSA− and PSA+ LNCaP cells. Microarray analysis of differentially expressed genes in two LNCaP-derived prostate cell cancer lines was previously described (32). To validate differences in expression of the core 2 β1,6-N-acetylglucosamyltransferase (core 2 GT) in the two cell lines, reverse transcription-PCR (RT-PCR) analysis was done. Briefly, RNA was isolated using Qiagen RNA isolation kit (Qiagen) and RT-PCR was done according to the protocol provided in the SuperScript One-Step RT-PCR with Platinum Taq (Invitrogen) using core 2 GT forward (GCAATGAGTGCAAACTGGAAA) and core 2 GT reverse (AATTGCGCGTAATTGTCAGTT) primers. Core 2 GT RT-PCR products were quantified by spectrophotometric analysis (Ultraspec 2000, Pharmacia Biotech) on dilutions done until a linear range of product was observed. Values were normalized by comparison to expression of β-actin (forward primer GCTGGTCTGTCGACAGGCTCT and reverse primer CAAACATGATCTGGGTCATCTT).

Expression of β-galactoside α2,3-sialyltransferase 1 in LNCaP cells. β-Galactoside α2,3-sialyltransferase 1 (ST3Gal1) cDNA (ref. 39; gift of Dr. James Paulson, The Scripps Research Institute, La Jolla, CA) was subcloned into pcDNA3.1 (Invitrogen; pST3). Subconfluent monolayers of LNCaP cells were grown for 5 days before transfection in Opti-MEM 1 media (Life Technologies). Each 100-mm dish was transfected with 16.7 μg of pST3 or vector alone (control) in Lipofectamine (Life Technologies) following the manufacturer’s protocol. Transfected cells were selected by culture in media with G418 (100 μmol/L; Life Technologies) for 3 days.

Prostate cell induction of T-cell death. CEM T cells (10^6; >98% viable by trypan blue exclusion) in RPMI were added to confluent monolayers of the indicated prostate cell lines for 8 h at 37°C. Plates were vigorously
Results

Galectin-1 expression by prostate cancer cell lines. Galectin-1 is expressed by DU145 and PC-3 prostate cancer cell lines but not by PSA+ LNCaP cells (25, 26). We confirmed that DU145 and PC-3 make abundant galectin-1 whereas LNCaP cells do not produce galectin-1 detected by immunoblotting, as previously shown (refs. 25, 26; Fig. 1A). Moreover, DU145 and PC-3 cells externalize galectin-1; cell-surface galectin-1 was detected by ELISA (Fig. 1A) and by immunofluorescent labeling of DU145 and PC-3 cells (Fig. 1B). Importantly, galectin-1 on DU145 and PC-3 cells can be released by preincubation with lactose (data not shown), showing that secreted endogenous galectin-1 is retained on the cell surface via lectin-carbohydrate interactions.

Prostate cancer cell susceptibility to galectin-1–induced cell death. Because DU145 cells but not LNCaP cells produce and externalize abundant galectin-1, we reasoned that DU145 cells would be resistant to cell death triggered by exogenous galectin-1, whereas LNCaP cells would die after binding exogenous galectin-1. As shown in Fig. 1C and D, LNCaP cells are susceptible to cell death induced by exogenous galectin-1. Importantly, the effect of galectin-1 on LNCaP cells was inhibited by addition of lactose, showing that galectin-1–induced cell death required galectin-1 binding to saccharide ligands on the surface of the LNCaP cells. Cell death was detected by the TUNEL assay, showing that the cells reached a late stage of apoptosis in which DNA degradation has occurred. In contrast to LNCaP cells, DU145 cells were resistant to galectin-1–induced apoptosis (Fig. 1D), consistent with the observation that DU145 cells synthesize and secrete abundant endogenous galectin-1.

O-Glycans on cellular glycoproteins are required for LNCaP susceptibility to galectin-1–induced death. As shown in Fig. 1, galectin-1–induced death of LNCaP cells requires galectin-1 binding to saccharide ligands on the cells. Galectin-1 preferentially binds to galactose-terminated saccharide ligands, particularly to lactosamine (Galβ1,4GlcNAc) sequences that can be found on N- or O-linked glycans on cell-surface glycoproteins (27, 29–31). These lactosamine sequences can be elongated to form polylactosamine structures that are high-avidity ligands for galectin-1. O-glycans, elongation of lactosamine sequences is typically initiated by the action of the N-acetylgalactosaminyltransferase V enzyme, whereas on O-glycans, elongation of polylactosamine sequences is typically initiated by the action of the core 2 GnT enzyme (29). Because the lactose inhibition data in Fig. 1D showed that galectin-1–induced death of LNCaP cells required galectin-1 binding to saccharide ligands on the cell surface, we asked if galectin-1–induced death of LNCaP cells required N- or O-glycan structures.

We used inhibitors to modify glycosylation of N- and O-glycans. The inhibitor DMNJ modifies N-glycans by inhibiting mannosidase II, the enzyme that trims high-mannose structures on N-glycans to allow subsequent addition of N-acetylgalactosamine residues and elongation of lactosamine sequences (31). Loss of N-acetylgalactosamine residues, particularly on the glycan branch modified by N-acetylgalactosaminyltransferase V, can be detected by loss of staining with the plant lectin PHA. As shown in Fig. 2A, treatment of LNCaP cells with DMNJ markedly reduced PHA staining, indicating the effectiveness of DMNJ treatment in reducing lactosamine addition to N-glycans. To modify O-glycan elongation,
we used benzyl-α-GalNAc that can modify O-glycan elongation in two different ways (39–41). In cells with low amounts of sialylated O-glycans, benzyl-α-GalNAc blocks elongation of O-glycans beyond the initial GalNAc residue (Fig. 3), an effect that can be detected by reduced reactivity with the plant lectin PNA. In cells with high levels of sialylated O-glycans, benzyl-α-GalNAc inhibits O-glycan sialylation, resulting in exposure of nonsialylated O-glycans and increased PNA reactivity. As shown in Fig. 2B, treatment of LNCaP cells with benzyl-α-GalNAc resulted in increased PNA binding, indicating that the inhibitor treatment increased exposure of nonsialylated O-glycans on these cells.

LNCaP cells treated with either DMNJ or benzyl-α-GalNAc were assessed for sensitivity to galectin-1–induced cell death. Treatment with DMNJ had no significant effect on LNCaP cell susceptibility to galectin-1 (Fig. 2C); as DMNJ treatment did not reduce LNCaP susceptibility to galectin-1, complex N-glycans bearing lactosamine sequences are not required for galectin-1–induced death of these cells. In contrast, treatment of LNCaP cells with benzyl-α-GalNAc, causing increased exposure of nonsialylated O-glycans, resulted in a dramatic increase in sensitivity to galectin-1–induced cell death, with virtually 100% of the cells dying in response to galectin-1 (Fig. 2D). These results indicated that O-glycans on cell-surface glycoproteins regulate LNCaP cell sensitivity to galectin-1–induced cell death.

Galectin-1–resistant PSA− LNCaP cells have decreased expression of core 2 GnT. PSA− LNCaP cells express abundant galectin-1 and are resistant to galectin-1–induced death (32), whereas both PSA− and PSA+ LNCaP cells were susceptible to Fas-induced death, indicating that PSA− LNCaP cells are not generally resistant to apoptosis.5 This implied that specific differences in expression of glycan ligands between PSA− and PSA+ LNCaP cells might be responsible for differential susceptibility to galectin-1–induced death. To identify candidate glycosyltransferase genes that would promote susceptibility to galectin-1 in LNCaP cells, we compared patterns of glycosyltransferase gene expression between PSA− LNCaP and PSA− LNCaP clones (32). We examined 120 glycosyltransferase genes in the U95 Affymetrix array, focusing on enzymes that participate in O-glycan synthesis. Of all glycosyltransferases examined, the enzyme with the most profound difference in expression between the PSA− and PSA+ LNCaP cells was core 2 GnT, which initiates branched structures on polypeptides on different cell types.

As shown in Fig. 3A, core 2 GnT expression also renders T cells resistant to galectin-1–induced death. Intriguingly, our group has previously shown that core 2 GnT expression expression in galectin-1 (Fig. 3). Our data indicated that O-glycans participate in galectin-1–induced death of LNCaP cells (Fig. 2C), suggesting that the reduction in core 2 GnT expression in PSA− LNCaP cells could render the cells resistant to galectin-1–induced cell death. Intriguingly, our group has previously shown that core 2 GnT expression also renders T cells susceptible to galectin-1–induced cell death (29, 30), indicating that galectin-1 susceptibility of various cell types may be controlled at the level of cell-surface glycosylation by common glycosyltransferase enzymes that add the same glycan ligands to different polypeptides on different cell types.

Blocking O-glycan elongation protects LNCaP cells from galectin-1–induced death. To directly address the role of O-glycans in regulating LNCaP susceptibility to galectin-1, we attempted to decrease core 2 GnT expression in LNCaP cells using small interfering RNA. However, despite repeated attempts, we were unable to reduce core 2 GnT enzyme expression below 40%

5 P. Cabrera and L. Baum, unpublished data.
of control; whereas we observed a concomitant reduction in the level of core 2 GnT protein by immunoblotting, this level of reduction of core 2 GnT enzyme activity did not appreciably alter cellular glycosylation as detected by PNA binding, nor did we detect a reduction in susceptibility to galectin-1–induced death (data not shown). Thus, as an alternate approach to block O-glycan elongation, we transfected LNCaP cells with cDNA encoding β-galactoside ST3GalI (39). This sialyltransferase competes with core 2 GnT in the Golgi, adding sialic acid to nascent O-glycans and blocking O-glycan elongation (refs. 39, 42; Fig. 3). We have previously found that overexpression of ST3GalI is an effective method of blocking O-glycan elongation in T cells (39). As shown in Fig. 5A, expression of ST3GalI in LNCaP cells reduced PNA binding to the cells, showing the effectiveness of ST3GalI expression in blocking O-glycan elongation. Furthermore, ST3GalI expression resulted in a marked reduction in LNCaP cell susceptibility to galectin-1–induced cell death (Fig. 5B and C). Cells expressing the ST3GalI showed >60% reduction in TUNEL-positive cells following galectin-1 binding, compared with cells transfected with vector alone. These results show that changes in O-glycan expression and degree of O-glycan sialylation regulate LNCaP cell susceptibility to galectin-1–induced cell death.

**Galectin-1–resistant prostate cancer cells express galectin-1 and kill adherent T cells.** Galectin-1 expression increases with epithelial cancer progression in prostate, ovarian, and squamous cell carcinomas (13, 14, 43, 44). Moreover, increased galectin-1 expression in human squamous cell carcinomas and in a mouse melanoma model resulted in decreased T-cell infiltration into the tumors (34, 44), indicating that galectin-1 expression by tumor cells that are resistant to galectin-1–induced death would enable tumor cells to evade immune attack. To ask if expression of galectin-1 by PSA− LNCaP cells and PC-3 cells enabled prostate cells to kill adherent T cells, we added human CEM T cells to monolayers of PSA− LNCaP, PSA− LNCaP, or PC-3 cells. After 8 h, T cells were removed from the prostate cell monolayers and T-cell death was assessed by TUNEL. Flow cytometric analysis of forward versus side scatter of nonadherent cells was used to discriminate T cells from nonadherent prostate cells. There was minimal TUNEL labeling of CEM cells adherent to PSA− LNCaP cells that do not express galectin-1 (Fig. 6B). In contrast, we observed a distinct population of TUNEL-positive T cells after binding to PSA− LNCaP cells that express galectin-1. Similarly, we observed robust TUNEL staining of ~50% of T cells adherent to PC-3 cells (Fig. 6C). T cells were directly treated with purified recombinant galectin-1 as a positive control (Fig. 6C). Thus, prostate cancer cells that have acquired resistance to galectin-1–induced death can express endogenous galectin-1 that can kill T cells, supporting the model that tumor expression of galectin-1 promotes tumor immune privilege.

**Discussion**

Galectin-1 can kill lymphoid and epithelial tumor cells and has the potential to synergize with other cell death–inducing agents to enhance killing of a variety of tumor cell types (27, 28, 45). Whereas glycan ligands on T cells recognized by galectin-1 have been identified, little is known about galectin-1 recognition of specific glycans on epithelial neoplasms such as breast and prostate cancer. In this report, we have shown that O-glycans on LNCaP prostate cancer cells are essential for triggering galectin-1–induced cell death, and that masking of these O-glycans by sialic acid, by overexpressing the ST3GalI sialyltransferase enzyme, renders the
cells resistant to galectin-1. Whereas ST3Gal1 expression in LNCaP cells did not completely mask all O-glycans because we still detected some PNA binding to the cells (Fig. 5A), ST3Gal1 expression reduced susceptibility of LNCaP by >60%. This suggests that critical O-glycans bound by galectin-1 to trigger cell death were preferentially modified by ST3Gal1.

Importantly, our group previously found that O-glycans on T cells are critical for susceptibility to galectin-1 (29, 30). In T cells, elongation of O-glycans by core 2 GnT modifies several cell-surface glycoproteins, including CD45 and CD43, required for conferring susceptibility of CD45+ T cells to galectin-1 (30). We have also recently determined that malignant T cells from some patients with cutaneous T-cell lymphoma have aberrant cell-surface glycosyla-

Figure 5. Sialylation of O-glycans protects LNCaP cells from galectin-1–induced cell death. A, expression of ST3GaI reduced PNA binding sites on the surface of LNCaP cells. PNA binding to LNCaP cells transfected with vector alone (LNCaP-pC) or with ST3GaI (LNCaP-pST3) is reported as mean phycoerythrin (PE) fluorescence intensity of cells treated with control reagents or PNA-biotin. B, expression of ST3GaI reduces susceptibility of LNCaP cells to galectin-1–induced death, detected by flow cytometry with streptavidin-PE and PNA (control). C, quantification of PNA binding to LNCaP cells transfected with vector alone or with ST3GaI (LNCaP-pST3) is reported as mean fluorescence intensity. *P < 0.002. Open columns, buffer control; filled columns, galectin-1 treated; hatched columns, galectin-1 plus lactose. Columns, mean of triplicate samples from one of four replicate experiments; bars, SE.

Figure 6. Prostate cells expressing cell-surface galectin-1 kill human CEM T cells. A, an example of forward versus side scatter analysis used to discriminate CEM T cells (left) from LNCaP cells (center); a 1:1 mixture of both cell types confirmed that two distinct populations could be discriminated (right). In all T-cell death experiments on prostate cell monolayers, nonadherent prostate cells represented <5% of the total cells analyzed. B, CEM T cells that are susceptible to galectin-1–induced death were added for 8 h to confluent monolayers of the parental PSA+ LNCaP cells that do not express endogenous galectin-1, or to PSA−LNCaP cells that express abundant endogenous galectin-1. CEM T cells were removed and analyzed for cell death by TUNEL staining (X-axis). PSA+LNCaP (left) cells induced minimal TUNEL staining of CEM T cells (thin line) compared with PSA−LNCaP cells (right) that triggered death of a significant population of T cells. Filled histograms, CEM T cells treated identically but added to plastic dishes in the absence of prostate cell monolayers. C, quantification of TUNEL labeling of CEM T cells treated with 20 μmol/L recombinant human galectin-1 or buffer control (open columns), or bound to the indicated prostate cancer cells (filled columns). *, P < 0.05; **, P < 0.001. Columns, mean of triplicate samples from one of five replicate experiments; bars, SE.
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and T lymphoma cells. Whereas the LNCaP cell-surface glycoproteins that can be modified by core 2 GnT are not known, it is clear that LNCaP cells do not express CD45 and CD43, the proteins that can be modified by core 2 GnT are not known, it is and T lymphoma cells. Whereas the LNCaP cell-surface glyco-

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