MUC1 Is a Counter-Receptor for Myelin-Associated Glycoprotein (Siglec-4a) and Their Interaction Contributes to Adhesion in Pancreatic Cancer Perineural Invasion

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Abstract

Perineural invasion in pancreatic adenocarcinoma, a common pathologic phenomenon whereby cancer cells invade and intimately contact the endoneurium of pancreatic nerves, is thought to contribute to both pain and local disease recurrence. MUC1, a type I transmembrane mucin that can affect the adhesive properties of cells, contains a large extracellular tandem repeat domain, which is heavily glycosylated in normal epithelia, but is overexpressed and differentially glycosylated in pancreatic cancer. This altered glycosylation includes the shortened core I O-glycans for monosialyl and disialyl T antigens. Myelin-associated glycoprotein (MAG), a membrane-bound protein expressed on oligodendrocytes and Schwann cells, binds myelin to neurons. MAG’s preferred ligands are derivatives of the monosialyl and disialyl T antigen. We investigated whether MUC1 is a counter-receptor for MAG and if their interaction contributed to pancreatic perineural invasion. Results showed that MAG binds pancreatic cells expressing MUC1, that this binding is sialidase-sensitive, and that MAG physically associates with MUC1. Heterotypic adhesion assays between pancreatic cancer cells and Schwann cells revealed that increased expression of MUC1 or MAG enhanced adhesion. Conversely, specific inhibition of MAG or sialyl-T MUC1 partially blocked adhesion. Immunohistochemical analysis of pancreatic perineural invasion showed the expression of both MUC1 and MAG. These results support the hypothesis that the adhesive interactions between MUC1 and MAG are of biological significance in pancreatic cancer perineural invasion. [Cancer Res 2007;67(21):10222–9]

Introduction

A common pathologic consequence of pancreatic ductal adenocarcinoma (PDAC) is perineural invasion (PNI), thought to occur in 100% of pathologic specimens (1, 2). Tumor cells invade both the epineurium and perineurium to become intimately associated in the endoneurium of both intrapancreatic and extrapancreatic nerves (3), in close association with Schwann cells and nerve axons. Perineural invasion, possibly through the actions of the nerve growth factor/TrkA (4) axis, is believed to cause significant lower back pain and discomfort associated with pancreatic cancer. Furthermore, perineural invasion may be a source of local recurrence after the resection of primary PDAC masses (5). To date, very little is known about receptor/ligand interactions that mediate pancreatic tumor adhesion during invasion and metastasis (6).

MUC1, a membrane-bound type I transmembrane mucin, affects the adhesive, differentiation, and cellular growth properties of cells (7). MUC1 is translated as a single polypeptide chain, which undergoes autoproteolytic cleavage within its SEA domain to form two subunits bound by noncovalent forces (8–10). The larger NH2-terminal subunit is extracellular and contains a signal sequence and tandem repeat domain (11). The COOH-terminal subunit contains a short extracellular domain, a transmembrane region, and a cytoplasmic tail. The tandem repeat domain of MUC1 is extensively O-glycosylated in normal tissues (12); however, MUC1 is overexpressed and aberrantly glycosylated in adenocarcinomas (13), including pancreatic cancer (14, 15). This aberrant glycosylation reveals novel and shortened oligosaccharide structures including the sialyl-T structure (15–17).

MUC1 can have both adhesive and antiadhesive effects. Antiadhesive effects occur when MUC1 binds the receptors found on the same cell surface, thereby preventing their interaction with ligands or receptors on opposing cells (18–21). Adhesive effects are mediated by MUC1 binding to receptors on opposing cell surfaces or extracellular matrices. For example, the peptide backbone core of MUC1’s tandem repeat binds intercellular adhesion molecule-1 to mediate heterotypic adhesion to endothelial cells (22). The highly varied sialylated structures of MUC1 can also bind to selectins (23, 24) and sialoadhesin (25, 26).

Myelin-associated glycoprotein (MAG, Siglec-4a), is a member of the sialic binding, immunoglobulin-like lectin (siglec) family (27). A membrane-bound protein, MAG contains five extracellular immunoglobulin-like domains, and the NH2-terminal V-type immunoglobulin domain also contains the lectin domain (28). In the peripheral nervous system, MAG is expressed by myelinating Schwann cells in the periaxonal membrane, the paranodal loops, the Schmidt-Lanterman incisures, and the mesaxon membranes (29), where it functions as an adhesive molecule to compact myelin and bind it to axons (30). Known ligands for MAG include both proteins and glycolipids. MAG on Schwann cells bind to gangliosides on axons to mediate adhesion and inhibit nerve regeneration (31, 32). Both monosialylated and disialylated derivatives of the T antigen bind MAG with high affinity (33).

Here, we report that MAG binds pancreatic tumor cells in a sialic acid–dependent manner. MUC1 and MAG can be
commonly precipitated, demonstrating a direct interaction. Furthermore, MUC1 on pancreatic tumor cells contributes to the adhesion of MAG on Schwann cells. Finally, we show that MUC1 and MAG are coexpressed in pancreatic cancer perineural samples.

**Materials and Methods**

**Materials.** The cell line S2-013 is a cloned subline of a human pancreatic tumor cell line (SUIT-2, ref. 34). Panc-1 (human pancreatic tumor cell line) and TR68C1 (mouse Schwannoma cell line) were obtained from the American Type Culture Collection and the European Tissue and Cell Collection, respectively. The rat Schwann cell lines Schwann-LMAG (28) and parental Schwann were kind gifts from Dr. Marie Filbin, Hunter College, New York City, NY. MAG-Fc was produced and purified as described previously (35). Monoclonal antibodies (mAb) were obtained from the following sources: anti-MAG clone 513 and anti-sLea clone KM231 were purchased from Chemicon, anti-MAG clone B11F7 (36) was a gift from Dr. Jacques Bara (Centre National de la Recherche Scientifique, Paris, France), anti-sLex clone CSLEX and anti-MUC1 clone B27.29 were gifts from Dr. Jacques Bara (Centre National de la Recherche Scientifique, Paris, France), anti-sLex clone KM231 and anti-MUC1 clone B27.29 were gifts from the Biomira Corporation (Edmonton, Alberta, Canada), anti-MUC1 HMFG-2 was provided by Dr. Sandra Gendler (Mayo Clinic, Scottsdale, AZ), and anti-FLAG clone M2 was purchased from Sigma. HEPES was purchased from Fischer Biotech. Calcein AM was purchased from Invitrogen. Unless otherwise specified, all other reagents were from Sigma.

Tissue samples (*n* = 9) were obtained at the time of rapid autopsy from patients with pancreatic cancer and immediately placed in formalin under the approval of the University of Nebraska Medical Center’s Institutional Review Board. The median age for patients was 69.6 years (range, 59–81).

**Cell culture.** The transfected pancreatic tumor cell lines S2-013.MUC1F, S2-013.NEO (14), S2-013.MUC1F-ΔTR (37), Panc-1-MUC1F, and Panc-1.Neo as well as Schwann-LMAG were maintained in DMEM supplemented with 10% heat-inactivated fetal bovine serum (Valley Biomedical), penicillin/streptomycin (Bio Whittaker), and 200 μg/mL of geneticin (G418; Invitrogen). The parental Schwann cell line and parental S2-013 cells were maintained in DMEM with 10% fetal bovine serum and penicillin/streptomycin. TR68C1 cells were maintained in DMEM with 10% fetal bovine serum, 10% tryptophane phosphophate broth, and penicillin/streptomycin. All cell lines were grown at 37°C in a 5% CO₂ humidified chamber.

**Flow cytometry analyses.** Tumor cell lines were grown to ~80% confluence and then released from tissue culture plates by incubation for 30 min in PBS containing 0.5 mM EDTA, and 0.1% bovine serum albumin. Cells (5 × 10⁶) were washed and stained at 4°C with 50 μg/mL of MAG-Fc in staining media (DMEM containing 0.1% bovine serum albumin and 0.1% sodium azide), washed twice with staining media, stained with a secondary antibody, FITC-conjugated goat anti-mouse IgG, washed twice with staining media then fixed for 10 min in 2% formaldehyde. Ten thousand cells, resuspended in PBS containing 0.1% bovine serum albumin and 0.1% sodium azide, were analyzed on a Becton Dickinson fluorescence-activated cell sorting machine. For sialidase treatment, cells were incubated with sialidase (Boehringer Mannheim) for 30 min at 37°C. Where indicated, cells were preincubated with antibodies for 60 min at 25°C. Antibodies were used at the following concentrations: anti-MAG 513 50 μg/mL, anti-FLAG M2 50 μg/mL, anti-sLea KM231 20 μg/mL, anti-MUC1 B27.29 20 μg/mL, anti-sLex CSLEX 20 μg/mL, and anti-sLex T 19-3LE diluted 1:5 (ascites). S2-013.MUC1F cells were treated with V. cholerae neuraminidase in DMEM plus HEPES (pH 6.8) for 3 h at 37°C prior to labeling with calcine AM and the adhesion assay was done at either 37°C or 4°C.

**Immunohistochemistry.** For immunohistochemical evaluation of MAG in pancreatic adenocarcinoma perineural invasion, 5-μm-thick, paraffin-embedded tissue sections were assayed using the Dako Envision + Cytomation polymer–based protocol (DAKO Cytomation). Briefly, tissue sections were deparaffinized through xylene and an alcohol gradient. Antigen retrieval was then done with Antigen Unmasking Solution (Vector Labs) according to the manufacturer’s protocol. Sections were blocked in normal goat serum for 20 min, then incubated for 40 min with primary antibodies at room temperature: anti-MAG mAb B11F7 (1 μg/mL), anti-MUC1 mAb HMFG-2 (1:5 dilution), and anti-FLAG mAb M2 (1 μg/mL). The slides were rinsed with PBS-T (TB with 0.2% (v/v) Tween 20) and incubated with polymer-labeled 2” Abb for 40 min. The slides were rinsed with PBS-T and incubated for 5 to 10 min with 3,3’-diaminobenzidine substrate, observing closely for color to develop. The slides were counterstained for of phenylmethylsulfonyl fluoride. Insoluble material was pelleted at 10,000 × g for 10 min. The lysate was precleared for 30 min with 50 μL of protein A-agarose and lysate fractions corresponding to 10⁶ cells were incubated overnight with 50 μg of MAG-Fc, M2 mAb, or murine IgG₁, isotype control. Fifty microliters of protein A-agarose beads were added and after 1 h of incubation, the resin was washed thrice with wash buffer [50 mMol/L Tris-HCl (pH 8.0) containing 0.1% NP40, 150 mMol/L NaCl, 5 mMol/L EDTA, 0.1 mMol/L phenylmethylsulfonyl fluoride] and bound material was eluted in reducing SDS-PAGE loading buffer by boiling for 5 min. Samples were resolved on 6% SDS-PAGE and analyzed by autoradiography.

**Western blot analysis.** Immunoprecipitated proteins were resolved on 6% polyacrylamide gels with 3% polyacrylamide stacking gel. Proteins were transferred to polyvinylidine difluoride membranes electrothermally and blocked in PBS containing 5% dry milk overnight. Primary and secondary antibodies were diluted in PBS containing 5% dry milk (HMFG-2, 1:500) / alkaline phosphatase–conjugated goat anti-murine IgG₁ + IgM (1:10,000; Pierce). Membranes were incubated with primary antibody for 2 h at 4°C with gentle shaking followed by three 10-min washes and incubation with secondary antibody for 2 h at 4°C followed by three 10-min washes. ECF substrate (Amersham Pharmacia Biotech) was used for the detection of immunoreactive proteins. Membranes were analyzed on a Luminex Image Analyzer (LAS-1000plus; FUJIFILM).

**Tumor cell–Schwann cell adhesion assay.** Schwann cell (Schwann-LMAG, and Schwann) and TR68C1 Schwannoma monolayers were plated at 2.5 × 10⁶ cells per well in 96-well cell culture plates and grown to confluency (~48 h). Prior to the adhesion assay, these confluent monolayers were washed twice with serum-free DMEM with 25 mMol/L of HEPES (pH 7.4) and incubated for 30 min in adhesion media prior to the assay. To these plates, calcine AM (Molecular Probes, Invitrogen) labeled S2-013 cells (MUC1F, Neo) and Panc1 cells (MUC1F, Neo) were added at 2.5 × 10⁴ cells/50 μL/well. The plates were immediately spun at 35 × g for 5 min at room temperature and read on a Gemini EM fluorescent microplate reader (Molecular Devices; pre-FL), followed by incubation at 37°C for 30 min to allow for adhesion. Nonadherent tumor cells were removed by vigorous agitation twice on a shaker at 178 rpm for 2 min (22). Remaining adherent cells were read a second time on fluorescent microplate reader (post-FL). Adhesion was calculated as the following:

\[
\text{Percent adhesion} = \left( \frac{\text{post-FL} - \text{media-FL}}{\text{pre-FL} - \text{media-FL}} \right) \times 100
\]

Where indicated, cells were preincubated with antibodies for 60 min at 25°C. Antibodies were used at the following concentrations: anti-MAG 513 50 μg/mL, anti-FLAG M2 50 μg/mL, anti-sLea KM231 20 μg/mL, anti-MUC1 B27.29 20 μg/mL, anti-sLex CSLEX 20 μg/mL, and anti-sLex T 19-3LE diluted 1:5 (ascites). S2-013.MUC1F cells were treated with V. cholerae neuraminidase in DMEM plus HEPES (pH 6.8) for 3 h at 37°C prior to labeling with calcine AM and the adhesion assay was done at either 37°C or 4°C.
30 s with Meyer’s hematoxylin (Vector Labs) and for 5 min in 0.038 mol/L of ammonium hydroxide, dehydrated, and mounted with VectaMount (Vector Labs) and coverslips and analyzed under a microscope (Nikon Eclipse 90i; Nikon). Images were captured using a digital camera (Nikon Digital Sight DS-5M; Nikon) and imaging software (Nikon ACT-2U).

Perineural invasion was defined as invasion through the perineurium and tumor cell contact with the endoneurium (39). Tumor nerve involvement was defined as tumor cells in contact with the perineurium, but not invading through it.

Statistics. Adhesion assay results were compared with the paired Student’s \( t \) test. Binding of different clones of the same experimental group of pancreatic cancer cell lines (e.g., S2-013.MUC1F.16 and S2-013.MUC1F.26 versus S2-013.Neo) to Schwann cells were compared using one-way ANOVA followed by the Tukey HSD test. \( P < 0.05 \) was considered statistically significant.

Results

S2-013 cells bind to MAG. MAG-Fc soluble fusion chimeras were used to determine if MAG (MAG-Fc) could bind to a pancreatic adenocarcinoma cancer cell line, S2-013. Flow cytometry analysis showed that MAG-Fc binds to S2-013 and S2-013.MUC1F cells (Fig. 1A and B, black line). Cells were then stained with FITC-conjugated goat anti-human IgG only (solid gray) or MAG-Fc (black line). Cells were then stained with FITC-conjugated goat anti-human IgG for detection of MAG-Fc chimera. Inhibition of MAG-Fc binding by pretreatment of S2-013 cells with sialidase (light gray line).

Figure 1. Pancreatic cancer cells bind to MAG via sialic acids. Flow cytometry analysis of S2-013 parental cells (A) and S2-013 cells transfected with MUC1F (S2-013.MUC1F, B) were stained with FITC-conjugated goat anti-human IgG only (solid gray) or MAG-Fc (black line). Cells were then stained with FITC-conjugated goat anti-human IgG for detection of MAG-Fc chimera. Inhibition of MAG-Fc binding by pretreatment of S2-013 cells with sialidase (light gray line).

S2-013–associated MUC1 interacts with MAG. To determine if MAG-Fc interacts with MUC1, S2-013.MUC1F cells were assayed by immunoprecipitation and Western blotting. Lysates from S2-013.MUC1F cells were immunoprecipitated with MAG-Fc, M2 (anti-FLAG) mAb (which binds to FLAG-tagged MUC1 expressed in S2-013.MUC1F cells), or murine IgG1 isotype control. The immunoprecipitated proteins were analyzed by Western blot with an antibody (HMFG-2) that recognizes the tandem repeat domain of MUC1. HMFG-2 reactivity was detected in MAG immunoprecipitates from S2-013.MUC1F cells (Fig. 2A) and from lysates immunoprecipitated with M2 (anti-FLAG). This shows that MAG binds to MUC1.

To determine if MAG-Fc binds to glycomolecules other than MUC1 in S2-013 pancreatic cancer cells, metabolic labeling and removal of \( \alpha 2,3-, \alpha 2,6-, \) and \( \alpha 2,8- \) sialic acid. S2-013 and S2-013.MUC1F cells treated with neuraminidase did not bind to MAG-Fc (Fig. 1A and B, light gray line).

Figure 2. MAG binds MUC1. A, S2-013.MUC1F cells were immunoprecipitated with MAG-Fc, anti-FLAG mAb (M2), or murine IgG1 isotype control and immunoprecipitates were separated by 6% SDS-PAGE, transferred to polyvinylidene difluoride membrane and analyzed by Western blot with a MUC1 tandem repeat reactive mAb (HMFG-2). B, S2-013 parental cells (expressing moderate levels of endogenous MUC1) and S2-013 cells transfected with MUC1F (S2-013.MUC1F) were metabolically labeled with \( ^{3}H \)glucosamine. These cell lysates were immunoprecipitated with MAG-Fc, anti-FLAG mAb (M2), or murine IgG1 isotype control, separated by 6% SDS-PAGE and analyzed by autoradiography. C, CHO parental cells (expressing no MUC1) and CHO cells transfected with MUC1 (CHO.MUC1) were metabolically labeled with \( ^{3}H \)glucosamine. These cell lysates were immunoprecipitated with MAG-Fc, anti-FLAG mAb (M2), or murine IgG1 isotype control, separated by 6% SDS-PAGE and analyzed by autoradiography.
immunoprecipitation experiments were done. Parental S2-013 cells (expressing moderate amounts of endogenous MUC1) and S2-013.MUC1F cells (overexpressing MUC1) were metabolically radiolabeled with 6-[3H]glucosamine. [3H]Glycoproteins were immunoprecipitated from cell lysates with MAG-Fc, M2 (anti-FLAG) mAb, or murine IgG1 isotype control. Immunoprecipitates were separated by SDS-PAGE and analyzed by autoradiography (Fig. 2B). MAG immunoprecipitated a >250,000 molecular weight glycoprotein in both parental S2-013 and S2-013.MUC1F cells (Fig. 2B). M2 (anti-FLAG) mAb immunoprecipitated MUC1F in S2-013.MUC1F cells. The M2 (anti-FLAG) immunoprecipitate has the same electrophoretic properties as the MAG-Fc immunoprecipitates from the same cell line. Therefore, it seems that MAG-Fc primarily binds to molecules with a molecular weight of >250,000 in S2-013 parental and transfected cell lines, and that this molecule is likely MUC1.

To confirm that MUC1 binds to MAG, Chinese hamster ovary (CHO) cells (which contain no endogenous MUC1), and CHO cells transfected with MUC1 were tested by metabolic labeling and immunoprecipitation. [3H]Glycoproteins were immunoprecipitated with MAG-Fc, M2 (anti-FLAG) mAb, or murine IgG, isotype control from metabolically labeled cell lysates of CHO and CHO.MUC1.
cells. MAG-Fc immunoprecipitated a glycoprotein (>250,000 molecular weight) from the lysates of CHO cells transfected with MUC1 (Fig. 2C). No immunoprecipitated MUC1 was detected by MAG-Fc from parental CHO cells. Also, as expected, no immunoprecipitated MUC1 was detected by M2 (anti-FLAG) mAb. These results support the conclusion that MAG binds specifically to MUC1.

Adhesion of pancreatic tumor cells to Schwann cells correlates with MAG and MUC1 expression. The contribution of MUC1 and MAG to adhesion between pancreatic tumor cells and Schwann cells was assessed using a heterotypic cell adhesion assay. Three Schwann cell lines with different degrees of MAG expression were employed (Fig. 3A). The parental Schwann cells expressed little MAG at the cell surface, whereas TR6Bc1 cells expressed moderate levels of MAG, and Schwann-LMAG cells expressed high levels of MAG at the cell surface. Pancreatic tumor cell adhesion to parental Schwann cells (Fig. 3B), TR6Bc1 cells (Fig. 3C), and Schwann-LMAG cells (Fig. 3D) was measured. As the level of MAG surface expression increased in Schwann cell lines (Fig. 3B–D), the percentage of pancreatic tumor adhesion also increased. The correlation between MAG expression (x) and S2-013.MUC1F-R5Schwann-LMAG cell adhesion (y) was plotted linearly (y = 0.028x + 27.556) and showed a correlation coefficient of 0.7018.

Overexpression of MUC1 in two pancreatic cancer cell lines, Panc1 and S2-013, showed a statistically significant (P < 0.05) increase in adhesion to TR6Bc1 (Fig. 3C) and Schwann-LMAG monolayers (Fig. 3D). Panc1.Neo and S2-013.Neo cells express low to moderate levels of endogenous MUC1. The degree of increase in adhesion that occurred with the overexpression of MUC1 was similar in both cell lines, resulting in an increase of ~10% to 15%.

Inhibition of adhesion between pancreatic tumor cells and Schwann cells. Selected blocking antibodies and sialidase treatment were used to determine if sialyl-T glycans and MAG mediate the heterotypic adhesion between pancreatic tumor cells and Schwann cells. Pretreatment of Schwann-LMAG cells with anti-MAG (clone 513) antibody significantly inhibited the adhesion of S2-013.MUC1F cells (Fig. 4A), whereas control antibody had no effect.

We hypothesized that the sialyl T-glycans on MUC1 bind to MAG, and tested this possibility by using selected antibodies to inhibit binding between these structures and MAG. Antibody 19-5LE, an IgM antibody against sialyl-T, had a small inhibitory effect on adhesion when preincubated with S2-013.MUC1F (Fig. 4B). Because structures exposed on the protein backbone tandem repeat of MUC1 are known to mediate cell adhesion (22), it was important to investigate the contribution of this domain. Importantly, antibody B27-29 against the tandem repeat protein backbone had no inhibitory effect. Moreover, antibodies against sialyl Lewis A (sLeα) and sialyl Lewis X (sLeβ) had no effect on adhesion.

Sialidase treatment was used to confirm that the sialyl-T glycans on MUC1 contribute to adhesion. Interestingly, sialidase treatment of S2-013.MUC1F cells had no effect on adhesion when the adhesion assay was done at 37°C (Fig. 4C). However, when done at 4°C, sialidase treatment of S2-013.MUC1F inhibited adhesion (Fig. 4D). We postulated that sialidase treatment of S2-013.MUC1F cells inhibited adhesion only at 4°C because of the inhibition of molecular trafficking within the cell at this temperature. Treatment of S2-013.MUC1F cells with neuraminidase affects only cell surface–associated molecules, and would not act on MUC1 that was being actively processed in the Golgi during de novo synthesis (40). After sialidase treatment, but during the cell adhesion experiment, newly synthesized (sialylated) MUC1 could move to the cell surface and bind to MAG. To test this hypothesis, sodium...
azide was included in the experiment to inhibit intracellular molecular transport. In accordance with the hypothesis, S2-013.MUC1F cells treated with sialidase in the presence of sodium azide partially inhibited adhesion to rSchwann-LMAG cells (Supplemental Data Fig. S1).

The sialyl-T glycans of MUC1 are primarily located on the tandem repeat region of MUC1. Therefore, cells expressing a construct of MUC1 lacking the tandem repeat (ΔTR) were predicted to show reduced adhesion as compared with cells expressing full-length MUC1. Consistent with this prediction, S2-013.MUC1F.ΔTR cells bound fewer rSchwann-LMAG cells compared with S2-013.MUC1F cells (Supplemental Data Fig. S2).

**MUC1 and MAG are expressed in pancreatic cancer perineural invasion and tumor nerve involvement.** Immunohistochemistry was done on paraffin sections of pathologically confirmed PDAC (n = 9). MAG was expressed in all nerves (9/9) found in PDAC (Fig. 5A), Positive staining was seen as punctuate brown coloration of the nerves. MAG was expressed in examples of PDAC PNI (Fig. 5B) and tumor nerve involvement (Fig. 5C). In PDAC PNI, the tumor cells cross the perineurium and are in intimate association with the myelin (Fig. 5B). By contrast, in PDAC tumor nerve involvement, the pancreatic tumor cells touch but do not cross the perineurium (Fig. 5C). MUC1 was expressed throughout all examined tumor glands, PDAC PNI, and tumor nerve involvement (Fig. 5D).

**Discussion**

MAG, a member of the siglec family of proteins (27), is expressed in Schwann cells, the myelinating cells of the peripheral nervous system (29). MAG normally functions as an adhesion molecule to promote axon-myelin adhesion (30). During the progression of pancreatic ductal cancer, tumor cells consistently invade into the nerves, becoming intimately associated with the myelin (3). Pancreatic tumor cells express MUC1 (41), which usually contains sialylated core I O-glycans (15). Immunohistochemical analysis of PDAC exhibiting perineural invasion confirmed that both MUC1 and MAG were expressed in these lesions (Fig. 5), which extends the previous finding of PMP22/Gas3 complexes at these sites (42).

We report that MAG binds to the pancreatic tumor cell lines S2-013 and S2-013.MUC1F, and that this binding is sialidase-sensitive. Furthermore, MAG is physically associated with MUC1 in pancreatic tumor cells. These findings extend previous work defining sialoglycoproteins that interact with MAG, including fibronectin (43) and Nogo-66 receptor homologue NgR2 (44).

Mucins, and specifically MUC1, are natural and likely counter-receptors for MAG. A hallmark of mucins is their tandem repeat domain, consisting of highly similar repeated protein motifs that are rich in serine, threonine, and proline (7). MUC1’s tandem repeat domain can be densely O-glycosylated. Variable glycosylation of MUC1 and other cell surface–associated mucins occurs by a number of mechanisms, including incomplete glycosylation as a consequence of overexpression, and repeated endocytosis cell surface forms to the trans-Golgi network, where vacant Ser/Thr residues are extended as core I O-glycans and sialylated (45, 46). These sialylated core I O-glycans, including sialyl T, are preferred ligands for MAG (33). The stoichiometric power (7) of densely packed sialyl-T’s on the MUC1 tandem repeat is believed to create a locally high concentration of preferred ligands for MAG, which may also contribute to its binding properties. For example, a single MUC1 core protein that contains 60 tandem repeats, each of which has five potential sites of O-glycosylation, could contain 300 oligosaccharide side chains. Therefore, 1 nmol/L of this mucin protein could present an effective concentration of 150 nmol/L if 50% of the O-glycosylation sites are occupied by sialyl-T structures.

Because both MUC1 and MAG are known to mediate heterotypic adhesion, we hypothesized that interactions between these molecules would facilitate tumor cell/Schwann cell adhesion. In agreement with this hypothesis, increasing levels of MAG
correlated with increased levels of adhesion, and overexpression of MUC1 enhanced the adhesion of tumor cells to Schwann cell monolayers. Anti-MAG antibody clone S13 specifically blocked this heterotypic adhesion. Antibody 513 recognizes a conformationally sensitive epitope within the lectin domain of MAG (28, 47). Blocking MAG did not completely inhibit adhesion, suggesting that other receptor/ligand interactions contribute to overall cellular adhesion. A lack of inhibition with antibody B27.29 suggested that the tandem repeat protein backbone of MUC1 did not mediate heterotypic adhesion. This is important because the interaction between MUC1 and intercellular adhesion molecule-1 is mediated by the tandem repeat protein backbone (22).

In contrast, preincubation of S2-013.MUC1F cells with 19-SLE, an antibody that cross-reacts with sialyl-T antigens,3 partially inhibited adhesion. In agreement with this finding, treatment of tumor cells with sialidase inhibited adhesion at 4°C in the presence of sodium azide (to inhibit the turnover of disialylated forms at the cell surface).

Expression of a construct of MUC1 lacking the tandem repeat (MUC1,ΔTR) in S2-013 cells diminished adhesion to rSchwann-LMAG cells compared with cells expressing full-length MUC1 (Supplemental Data Fig. S2). This suggests that the sialyl-T structures to which MAG bind on MUC1 are present in the highly O-glycosylated tandem repeat region.

Preincubation of S2-013.MUC1F cells with anti-sialyl Lewis A or X antibodies had no effect on adhesion. This is not surprising because Schwann cells typically do not express E-selectin (a receptor for sialyl Lewis A and X; ref. 48). Analyses of other adenocarcinomas that show perineural invasion have suggested adhesion roles for bystin (49) and neural cell adhesion molecules (6, 50).

We propose that during PDAC PNI, cancer cells that overexpress MUC1 with sialyl-T O-glycans have a selective adhesive advantage once they have invaded the endoneurium of nerves in which MAG is expressed. This adhesive advantage would contribute to the capacity of pancreatic cancer cells to survive and proliferate within the nerves. Similarly, in prostate cancer PNI, adhesive events have been proposed as a critical first step for prostate cancer cells to avoid apoptosis within the nerves before they divide and proliferate (49).

The interaction between MUC1 and MAG may not be exclusive for PDAC PNI because MUC1 is expressed in other cancers that invade perineurally, including prostate (51), salivary (52), and breast (53) carcinomas. Furthermore, breast cancers can metastasize to the brain (54), where MAG is abundantly expressed (55).

MUC1 interactions with intercellular adhesion molecule-1 during cell adhesion induces a calcium flux as a consequence of signaling through the cytoplasmic tail (56). MUC1’s cytoplasmic tail is involved in multiple signal transduction pathways, which includes nuclear trafficking and colocalization with β-catenin (7, 57). Therefore, it is possible that MAG bound to MUC1 may induce signal transduction in pancreatic tumor cells, a subject area that should be explored in future studies.

In summary, our results show that MUC1 expressed by pancreatic tumor cells is an excellent counter-receptor for MAG, due in part to the stoichiometric power of tandemly repeated sialylated-T antigens. Our results suggest that interactions between MUC1 and MAG contribute to the adhesion between pancreatic tumor cells and Schwann cells during PDAC PNI. Further definition of the molecular events that occur during PDAC PNI will provide leads to develop more effective treatment modalities for pancreatic cancer pain and recurrence.

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