Downregulation of sialyl-transferases and their role in malignant meningioma cells

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Abstract. Meningiomas are the most common primary intracranial tumors and are often curable with gross resection. However, surgery is not entirely effective, as recurrences are reported more frequently in patients with meningiomas with higher-grade tumors, despite the extent of surgical resection. Therefore, elucidating tumor biology at the molecular level is needed. Aberrant sialylation, resulting from altered expression of sialyl-transferases (STs), plays an important role in cancer development and progression. However, the role of altered sialylation in meningioma progression remains unclear. In the present study, downregulation of β-galactoside α2,3-ST (ST3Gals) and β-galactoside α2,6-ST (ST6Gals) genes was found in malignant meningioma tissues from four different Gene Expression Omnibus (GEO) datasets (GEO entries: GSE16581, GSE43290, GSE74385 and GSE136661). Moreover, suppression of sialylation using a pan-sialylation inhibitor (3Fax-peracetyl-Neu5Ac, 3Fax) reduced the activity of STs in a malignant meningioma cell line, leading to an increase in cell migration and invasion capacities. Further investigation of epithelial-mesenchymal transition (EMT) markers, AKT, and ERK signaling in the 3Fax-treated cell lines revealed that high expression of EMT-related transcription factors (*Snail*) and EMT-related proteins (*MMP9*) were regulated via phosphorylation of AKT and ERK. Therefore, the present findings suggested that suppression of sialylation by 3Fax in malignant meningioma increases migration and invasion abilities by enhancing the EMT process.

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Introduction

Meningiomas are the most common primary intracranial tumors, originating from arachnoid cap cells (1) and classified into WHO Grades 1, 2 and 3, based on various histological and anaplastic features, number of mitotic figures, invasive growth patterns and genetic characteristics (2). While most cases are benign Grade 1 tumors (80.5%), Grades 2 and 3 comprise 17.7 and 1.7%, respectively, and exhibit more aggressive behavior (3). Distant metastasis occurs in only 0.18% of cases, typically to the lungs, bones, spinal cord and liver, and is associated after the primary tumor has been diagnosed (93%). However, ~6% of metastatic meningiomas are found simultaneously with the primary tumor at the time of diagnosis. Metastatic meningiomas are associated with male sex, large tumor size and WHO Grade 3 (4). The first-line treatment for all meningiomas is surgical resection (5); however, surgery is not entirely effective, as recurrences are reported more frequently in patients with higher-grade tumors, even after extensive surgical resection (6). Consequently, these tumors are more likely to grow steadily and recur. Therefore, treatments for high-grade meningiomas are still limited and are being developed to better manage the morbidity and mortality of patients with meningiomas (7).

One emerging area of interest in understanding the aggressive behavior of such tumors is the study of sialylation, a post-translational modification known to influence cancer progression. Sialylation, the addition of sialic acids to proteins and lipids, is a critical post-translational modification that influences cell signaling, recognition and immune responses. Sialylation occurs via three prominent linkages: α2,3, α2,6 and α2,8, which are catalyzed by a group of sialyl-transferases (STs). This process plays a crucial role in various biological functions and cellular activities, such as cell-cell communication, cellular recognition and cell adhesion, and affects the circulating half-lives of numerous glycoproteins (8). Aberrant sialylation resulting from altered expression of ST genes has been implicated in cancer progression and development (9). Alterations in this process can lead to changes in

protein structure or impair their function, thereby promoting oncogenic activities, such as increased cell proliferation and survival. In particular, aberrant sialylation, increased tumor growth, and metastasis have been widely described (10). For instance, the addition of α2,6-linked sialic acid to β1 integrin increases its affinity for collagen I, thereby enhancing tumor cell migration and invasion (11). Similarly, α2,3-sialylation of CD44 enhances its interaction with hyaluronic acid (12), which in turn promote further cancer cell migration and metastasis. While the role of sialylation in the progression of various cancers has been extensively studied, its specific impact on the progression and aggressiveness of meningiomas remains poorly understood.

The presence of sialylated tumor-associated carbohydrate antigens, such as sialyl Tn (STn), is frequently linked to poor clinical outcomes due to their role in promoting tumor invasiveness (13). Additionally, a previous study by the authors found that patients with meningiomas exhibit altered expression of glycosyl-transferase enzymes (14). The present study aims to investigate the expression patterns of STs specifically β-galactoside α2,3 and α2,6 ST family members in different grades of meningiomas and examine how altering sialylation affects the migratory and invasive capabilities of meningioma cells.

Materials and methods

Gene expression analysis by Gene Expression Omnibus (GEO) datasets. The gene expression levels of β-galactoside α2,3 and α2,6 ST family members in meningiomas were retrieved from GEO database (https://www.ncbi.nlm.nih.gov/), including entries GSE16581, GSE74385, GSE136661 and GSE43290. Information on each GEO entry is presented in Table I. GEO2R is an interactive web tool that enables users to compare two or more groups of Samples in a GEO series to identify genes that are differentially expressed across experimental conditions. Thus, all expression data from each GEO entry were used in GEO2R to identify genes that were differentially expressed among the WHO Grade 1, 2 and 3 meningiomas. All expression data were transformed to log2 for analysis. The differential expression of ST genes across WHO grades 1, 2 and 3 meningiomas was analyzed using GraphPad Prism® (version 8.0; GraphPad Software, Inc.; Dotmatics). Statistical significance was set at $P<0.05$.

Cell culture and 3Fax treatment. The human HKBMM cell line (Meningioma WHO grade 3) was provided by Associate Professor Norie Araki (Kumamoto University, Kumamoto, Japan). The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; cat. no. 121000-046; MilliporeSigma) supplemented with 10% heat-inactivated fetal bovine serum (FBS; cat. no. A5256701; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin (cat. no. 15140-122; Thermo Fisher Scientific, Inc.). The cells were cultured in a humidified incubator at 37˚C under 5% CO_2. For 3Fax treatments, the HKBMM cell line, at a cell density of $2x10^4$ cells, was seeded into a 24-well plate and cultured overnight in DMEM containing 10% FBS. After overnight seeding, the culture supernatant was replaced with a new medium containing 500 μM 3Fax-peracetyl-Neu5Ac (3Fax; cat. no. 566224; Merck KGaA) or DMSO (cat. no. 04707516001; DMSO; Carlo Erba™) as a control, and the cells were incubated for five days. The expression of sialic acids using *Maackia amurensis* lectin II (MAL II) and *Sambucus nigra* lectin (SNA; cat. no. PK-4000; Vector Laboratories Inc.) staining was performed on both treated and control cells, and the results were analyzed using an Attune™ NxT Acoustic Focusing Cytometer (Thermo Fisher Scientific, Inc.). FACS data were analyzed using Flowing software version 2.5.1 (Turku Bioscience). Sialic acid expression was measured before subsequent experiments. The experiment was performed in three replicates.

Lectin staining and flow cytometry analysis. 3Fax-treated cells and control cells were trypsinized using 0.25% trypsin-EDTA (cat. no. 25200-072; Gibco; Thermo fisher Scientific, Inc.) and centrifuged at 400 x g for 3 min at 4˚C. The cell pellets were washed by resuspending in 1X PBS. The cells were fixed using 4% paraformaldehyde in 1X PBS for 15 min at room temperature. The fixed cells were then blocked for non-specific binding with 2% BSA (HIMedia Laboratories, LLC) in 1X PBS for 30 min on ice. Next, the cells were stained with SNA and MAL II biotinylated lectin for 1 h on ice. After staining, the stained cells were incubated with streptavidin-Alexa Fluor™ 488 conjugate (cat. no. S11223; Thermo Fisher Scientific, Inc.) for 30 min on ice. The cells were then resuspended in 2% FACS buffer and analyzed using an Attune™ NxT Acoustic Focusing Cytometer. Forward and side scatter were used to select the population. The BL1 channel was used to detect the expression of SNA and MAL II positivity, and a histogram represented the positive expression. The FACS data were analyzed using the Flowing software version 2.5.1 (Turku Bioscience). The experiment was performed in three replicates.

Cell proliferation by WST-8 viability assay. 3Fax-treated cells and control cells were trypsinized, seeded at a density of 1,000 cells per well in a 96-well plate and incubated overnight. Cell proliferation was measured daily using the Cell Counting Kit-8 (WST-8; cat. no. ab228554; Abcam) at 10 μl per well for 7 days. Briefly, the cells were incubated with the WST-8 solution for 3 h at 37˚C under 5% CO_2, and cell viability was assessed by measuring the absorbance at 460 nm using a TECAN Infinite 200 Pro microplate reader (Tecan Group, Ltd.). The experiment was performed in three replicates.

Cell proliferation and survival by colony formation assay. 3Fax-treated cells and control cells were trypsinized, seeded at a density of 500 cells in a 100-mm culture dish and incubated overnight. The cells were cultured in complete DMEM containing 10% FBS and incubated at 37˚C under 5% CO_2 for 14 days. The cells were then washed with 1X PBS, fixed with absolute methanol for 30 min at -4˚C, and stained with 5% crystal violet for 30 min at room temperature (RT). Subsequently, the stained cells were washed with tap water, and images of colony formation were captured under a stereomicroscope. Only colonies with >50 cells were included in the count. The experiment was performed in three replicates.

Wound healing assay. The cells were seeded at a density of $2x10^4$ cells in a 24-well plate and cultured overnight in DMEM containing 10% FBS. After seeding for 24 h, the culture

Table I. GEO entries used in the present study.

GEO entry	Platform	Array type	N	Mean age	WHO grade 1	2	3
GSE43290	GPL96	Affymetrix Human Genome U133A Array https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE43290	47	61.7	33	12	2
GSE16581	GPL570	Affymetrix Human Genome U133 Plus 2.0 Array https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE16581	66	63.2	43	19	6
GSE74385	GPL10658	Illumina HumanHT-12 V4.0 expression beadchip https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE74385	53	N/A	17	8	28
GSE136661	GPL20301	Illumina HiSeq 4000 (Homo sapiens) https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE136661	160	N/A	121	32	7

GEO, Gene Expression Omnibus.

supernatant was replaced with a new medium containing 500 μM of 3Fax or DMSO and incubated for 5 days to reach 90% confluence. The 3Fax-treated and control cells were scratched using 200-μl pipette tips to create a cell-free area. The medium was then replaced with serum-free medium. The cells were further incubated, and wound closure in the cell-free area was assessed at 18 and 24 h. The cell-free area was captured using an inverted light microscope, and the images were analyzed using ImageJ software (version 1.54 g; National Institutes of Health). Wound closure was calculated using the following formula: (area of original wound-area of wound during healing)/area of original wound. The experiment was performed in three replicates.

Transwell migration and invasion assay. The Transwell migration assay utilized 6.5 mm inserts with 8.0-μM polycarbonate membranes (cat. no. 3422; Costar® Transwell®; Corning Inc.) in 24-well plates. In the Transwell invasion assay, Matrigel (cat. no. 354234; Corning Inc.) was thawed, and 100 μl of a 6 mg/ml Matrigel matrix was added to 6.5-mm inserts with 8.0-μM polycarbonate membranes. Matrigel was spread evenly using a pipette tip and incubated at 37˚C for 2 h to allow Matrigel to solidify. Subsequently, 3Fax-treated and control cells at a density of $3x10^4$ cells were suspended in 200 μl of serum-free medium and seeded into the upper chamber. Meanwhile, 600 μl of DMEM containing 10% FBS was added to the lower chamber. The cells were then incubated for 18 and 24 h. The cells that had crossed the membrane were fixed with 10% trichloroacetic acid at 4˚C overnight and stained using the Sulforhodamine B colorimetric assay according to a previous study (15) for visualization. Images were captured using an inverted light microscope at x10 magnification. The number of migratory cells was counted and analyzed using ImageJ software (version 1.54 g; National Institutes of Health). The experiment was performed in three replicates.

RNA extraction and reverse transcription-quantitative PCR (RT-qPCR). 3Fax-treated and control cells were extracted from total RNA using TRIzol reagent (cat. no. 15596026; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The quantity and purity of RNA were measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Inc.). RNA purity was indicated via the A260/A280 nm ratio >1.8. RNA quality was assessed using agarose gel electrophoresis. Total RNA was then reverse transcribed into cDNA using the SensiFAST cDNA Synthesis Kit (cat. no. 65054; Meridian Bioscience) according to the manufacturer's protocol. qPCR was performed on the generated cDNA to evaluate the expression levels of epithelial-mesenchymal transition (EMT)-related transcription factors (TFs) and EMT-related proteins using iTag Universal SYBR Green Supermix (cat. no. 1725121; Bio-Rad Laboratories, Inc.). The thermocycling conditions were performed by initial denaturation at 95˚C for 5 min, followed by 40 cycles of denaturation at 95˚C for 10 sec, annealing at 55-63˚C for 10 sec and a final extension at 72˚C for 10 sec. The primers used and annealing temperatures for all investigated genes in this experiment are shown in Table II. The beta-actin gene was used as an internal control to normalize gene expression, and the relative mRNA expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method (16). The experiment was performed in three replicates.

SDS-PAGE and western blot analysis. 3Fax-treated cells and control cells were collected for preparation of whole-cell lysate using protein lysis buffer containing 10% TritonX, protease inhibitor cocktail (Roche Diagnostics GmbH) and Tris-lysis buffer pH 7.5. The protein concentrations of all samples were determined using the Pierce BCA Protein Assay Kit (cat. no. 2322, Thermo Fisher Scientific, Inc.). Total proteins (50 μg) were subjected to 10% SDS-polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane (cat. no. 1060003; MilliporeSigma). Non-specific binding was blocked using 5% BSA at RT for 1 h. The membrane was probed with a specific primary antibody overnight at 18˚C. The primary antibodies used were matrix metalloproteinase 9 (MMP 9; cat. no. 3852), *Snail* (cat. no. 3879), Akt (cat. no. 4685), phosphorylated (p-)Akt (cat. no. 4060), Erk (cat. no. 4695) and p-Erk (cat. no. 9101); all antibodies were diluted at 1:1,000 and purchased from Cell Signaling Technology, Inc. Simultaneously, β-actin (cat. no. sc-47778) was obtained from Santa Cruz Biotechnology, Inc. After incubation, secondary antibodies were added at a dilution

Table II. Primer sequences used in expression of EMT markers.

Assay	Gene name	Primer sequence (5'-3')	Annealing temperature (˚C)
EMT-related transcription factors	*Slug*	F: GCC TCC AAA AAG CCA AAC TAC R: GAG GAT CTC TGG TTG TGG TAT GAC	62
	ZEB1	F: ACC TCT TCA CAG GTT GCT CCT R: AGT GCA GGA GCT GAG AGT CA	62
	Snail	F: TTC TCA CTG CCA TGG AAT TCC R: GCA GAG GAC ACA GAA CCA GAA	62
EMT-related proteins	*CDH1*	F: TAC GCC TGG GAC TCC ACC TA R: CCA GAA ACG GAG GCC TGA T	62
	CDH2	F: ATC CTG CTT ATC CTT GTG CTG R: GTC CTG GTC TTC TTC TCC TCC	60
	MMP2	F: CGT CTG TCC CAG GAT GAC ATC R: ATG TCA GGA GAG GCC CCA TA	63
	MMP7	F: CAGATGTGGAGTGCCAGATG R: TGTCAGCAGTTCCCCATACA	60
	MMP9	F: TTC CAG TAC CGA GAG AAA GCC TAT R: GGT CAC GTA GCC CAC TTG GT	62
Internal control	*ACTB*	F: GAT CAG CAA GCA GGA GTA TGA CG R: AAG GGT GTA ACG CAA CTA AGT CAT AG	55-63

EMT, epithelial-mesenchymal transition; F, forward; R, reverse; *Slug*, Snail family transcriptional repressor 2; *ZEB1*, zinc finger E-box-binding homeobox 1; *Snail*, Snail family transcriptional repressor 1; *CDH1*, E-cadherin; *CDH2*, N-cadherin; *MMP*, matrix metallopeptidase; *ACTB*, beta-actin.

of 1:2,000 and incubated for 1 h at RT. Anti-mouse IgG, HRP-linked antibody (cat. no. 7074) and anti-rabbit IgG, HRP-linked antibody (cat. no. 7076) were purchased from Cell Signaling Technology, Inc. Immunodetection using a chemiluminescent HRP substrate was performed to visualize the target protein signals using ImageQuant™ LAS 500 (Cytiva). The density bands of each target protein were captured using ImageJ software (version 1.54 g; National Institutes of Health). The experiment was performed in three replicates.

Statistical analysis. Data were analyzed using GraphPad Prism® (version 8.0; GraphPad Software, Inc.; Dotmatics). One-way ANOVA followed by Bonferroni's multiple comparisons test was used to determine the differential expression of ST genes across WHO Grade 1, 2 and 3 meningiomas. Differences between the two groups were further analyzed using unpaired t-tests. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Downregulation of ST genes is associated with the progression of meningiomas. To address the significance of ST in meningioma development and progression, the gene expression of β-galactoside α2,3 and α2,6 STs (ST3Gals and ST6Gals), including *ST3Gal1, 2, 3, 4,* and *5,* and *ST6Gal1* and *2,* was obtained from four GEO datasets: GSE16581, GSE43290, GSE74385 and GSE136661. The results demonstrated that downregulation of the *ST3Gal2, ST3Gal3, ST3Gal4, ST3Gal5* and *ST6Gal1* genes was observed across four datasets and was associated with WHO Grade 3 meningiomas. Additionally, decreased expression of ST3Gal3 was commonly observed in two datasets (GSE74385 and GSE136661), whereas decreased expression of *ST3Gal5* was observed in three datasets, including GSE16581, GSE43290 and GSE136661. Moreover, the expression of *ST6Gal1* was significantly lower in WHO Grade 3 meningiomas than in WHO Grade 1 meningiomas in GSE16581, GSE43290 and GSE74385 (Fig. 1). These findings suggested that the downregulation of these STs may play a crucial role in meningioma progression.

Suppression of ST activity does not affect meningioma cell proliferation. To investigate the role of ST in meningioma development and progression, a malignant meningioma cell line, HKBMM, was selected for functional studies. Talabnin *et al* (14) demonstrated the high expression of sialic acids and ST genes in HKBMM cells. HKBMM cells were treated with a ST inhibitor (3Fax) or DMSO for 5 days. The cell viability test demonstrated that 3Fax at 500 μM was not toxic to HKBMM cells (Fig. 2A) and was therefore selected for subsequent experiments. Surface sialic acid expression was measured using MAL II to detect α2,3-sialylated glycans and SNA to detect α2,6-sialylated glycans. The results demonstrated that the staining intensities of both α2,3- and α2,6-sialylated glycans were significantly reduced in 3Fax-treated cells, indicating successful inhibition of sialylation (Fig. 2B). It was then investigated whether the suppression of sialylation affects the proliferation of meningioma cells. 3Fax-treated cells and control cells were used to monitor cell proliferation via cell proliferation and colony formation assays. The results showed

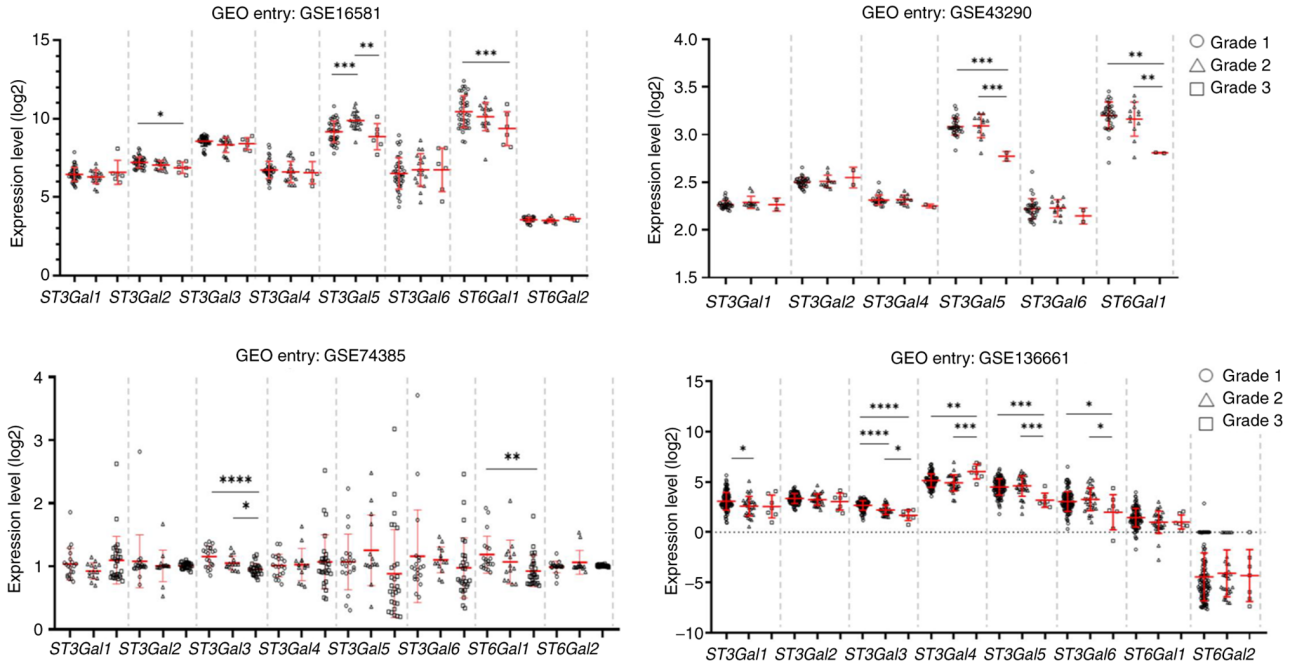

Figure 1. Expression profiles of β-galactoside α2,3 and α2,6 sialyl-transferase family in meningiomas. Expression data obtained from four GEO entries: (A) GSE16581, (B) GSE43290, (C) GSE74385 and (D) GSE136661. Values expressed as the mean ± standard deviation. *P≤0.05, **P≤0.01, ***P≤0.001 and ****P≤0.0001 vs. WHO Grade 1. GEO, Gene Expression Omnibus.

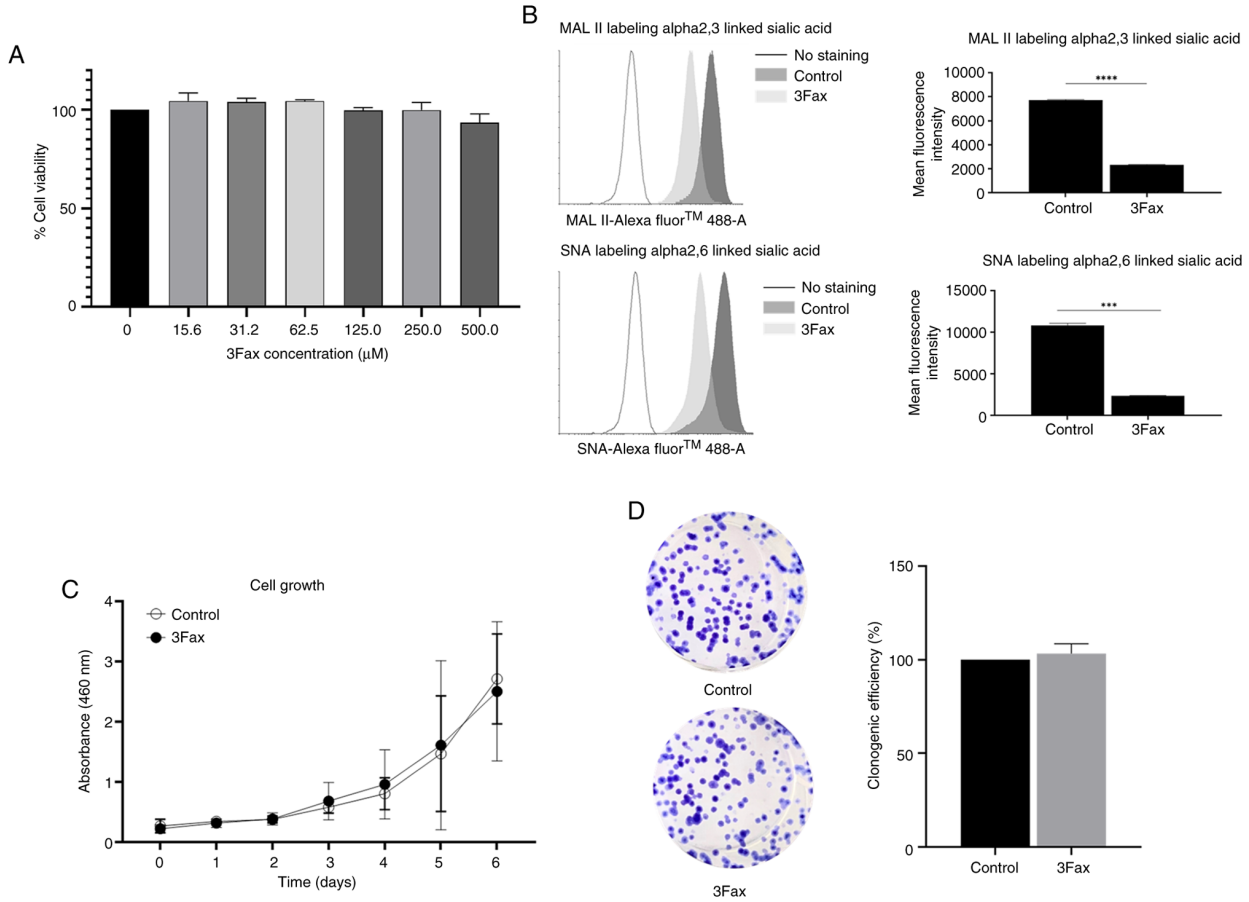

Figure 2. Suppression of sialylation does not affect the proliferation of meningioma cells. HKBMM cells were treated with various concentrations of 3Fax (0, 15.6, 31.2, 62.5, 125.0, 250.0, 500.0 μM) for 5 days. (A) Cytotoxic effect of 3Fax was assessed using the WST-8 viability. (B) Histogram plots and mean fluorescence intensity from lectin flow cytometry showed decreased expression of α2,3-linked MAL II and α2,6-linked SNA lectin sialic acids in 3Fax-treated cells (500 μM) compared with DMSO-treated control cells. (C and D) Cell proliferation and survival were assessed using the (C) WST-8 viability and (D) colony formation assays. Values are expressed as the mean ± standard deviation from three independent experiments. ***P≤0.001 and ****P≤0.0001 vs. control. MAL II, *Maackia amurensis* lectin II; SNA, *Sambucus nigra* lectin.

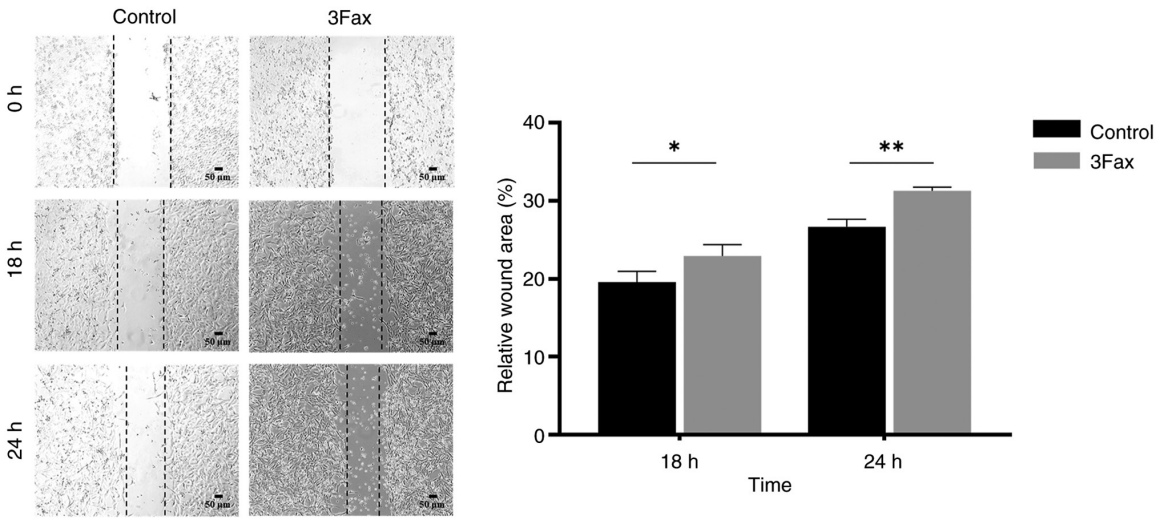

Figure 3. Suppression of sialylation promotes meningioma cell migration. HKBMM cells were treated with 3Fax at 500 μM or DMSO for 5 days. Migration ability was assessed using a wound-healing assay. Representative wound healing images of 3Fax-treated cells compared with control cells at 0, 18 and 24 h were selected from three independent experiments. Values are expressed as the mean ± standard deviation. *P≤0.05 and **P≤0.01 vs. control. Scale bar, 50 μm.

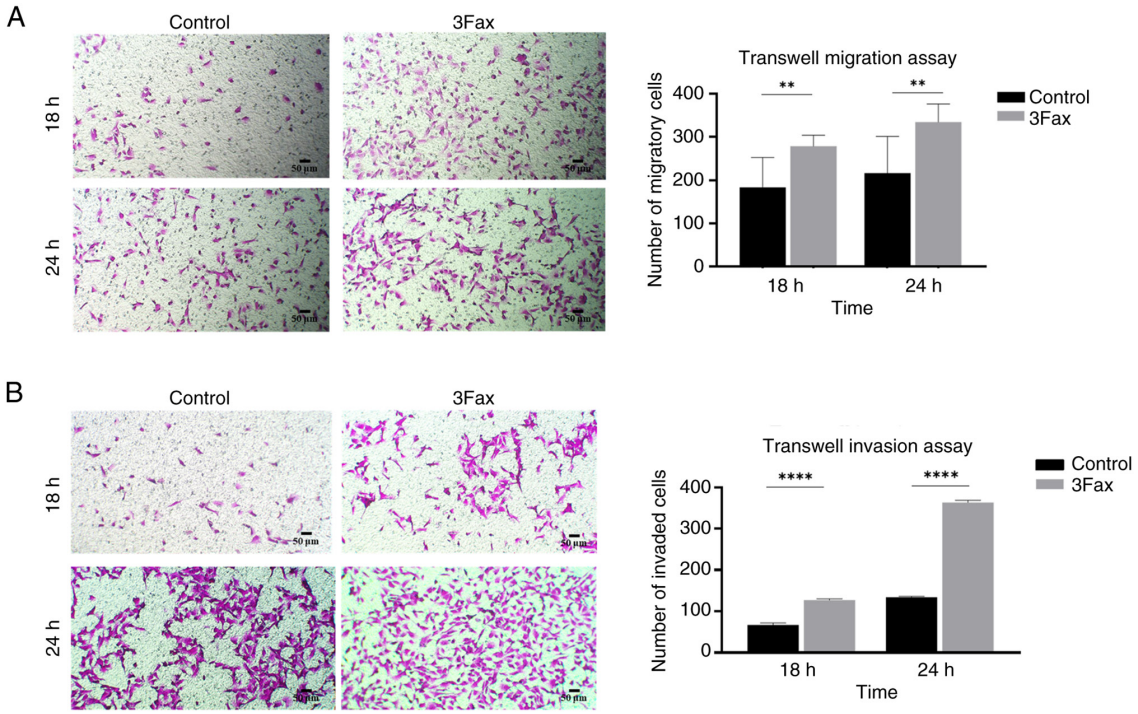

Figure 4. Suppression of sialylation induces an aggressive meningioma cell phenotype. HKBMM cells were treated with 3Fax at 500 μM or DMSO for 5 days. Cell migration and invasion were assessed using Transwell assays. (A and B) Representative migratory cells and invasive cells of 3Fax-treated cells compared with control cells at 18 and 24 h were selected from three independent experiments. Values are expressed as the mean ± standard deviation. **P≤0.01 and ****P≤0.0001 vs. control. Scale bar, 50 μm.

no significant difference in cell proliferation and survival (Fig. 2C and D) between the 3Fax-treated and control cells.

Suppression of ST activity promotes migration and invasion abilities in the malignant meningioma cells. To determine whether the suppression of sialylation affects the migration and invasion of meningioma cells, wound healing and Transwell migration or invasion assays were performed. The wound healing assay demonstrated that the 3Fax-treated cells had the ability to close the wound faster than the control cells at both 18 h (% wound area: 19.6+1.0% vs. 22.9+1.2%, P<0.05) and 24 h (% wound area: 26.7+0.8% vs. 31.3+0.4%, P<0.01) (Fig. 3). These findings are consistent with the results of the Transwell migration assay at 18 h (number of migratory cells: 183+64 vs. 278+23, P<0.01) and 24 h (number of migratory cells: 216+79 vs. 334+39, P<0.01) (Fig. 4A). Additionally, the Transwell invasion assay showed that 3Fax-treated cells had a greater ability to invade the extracellular matrix than control cells in a time-dependent manner (number of invasive cells at 18 h: 66+4 vs. 127+2 and at 24 h: 134+1 vs. 363+4,

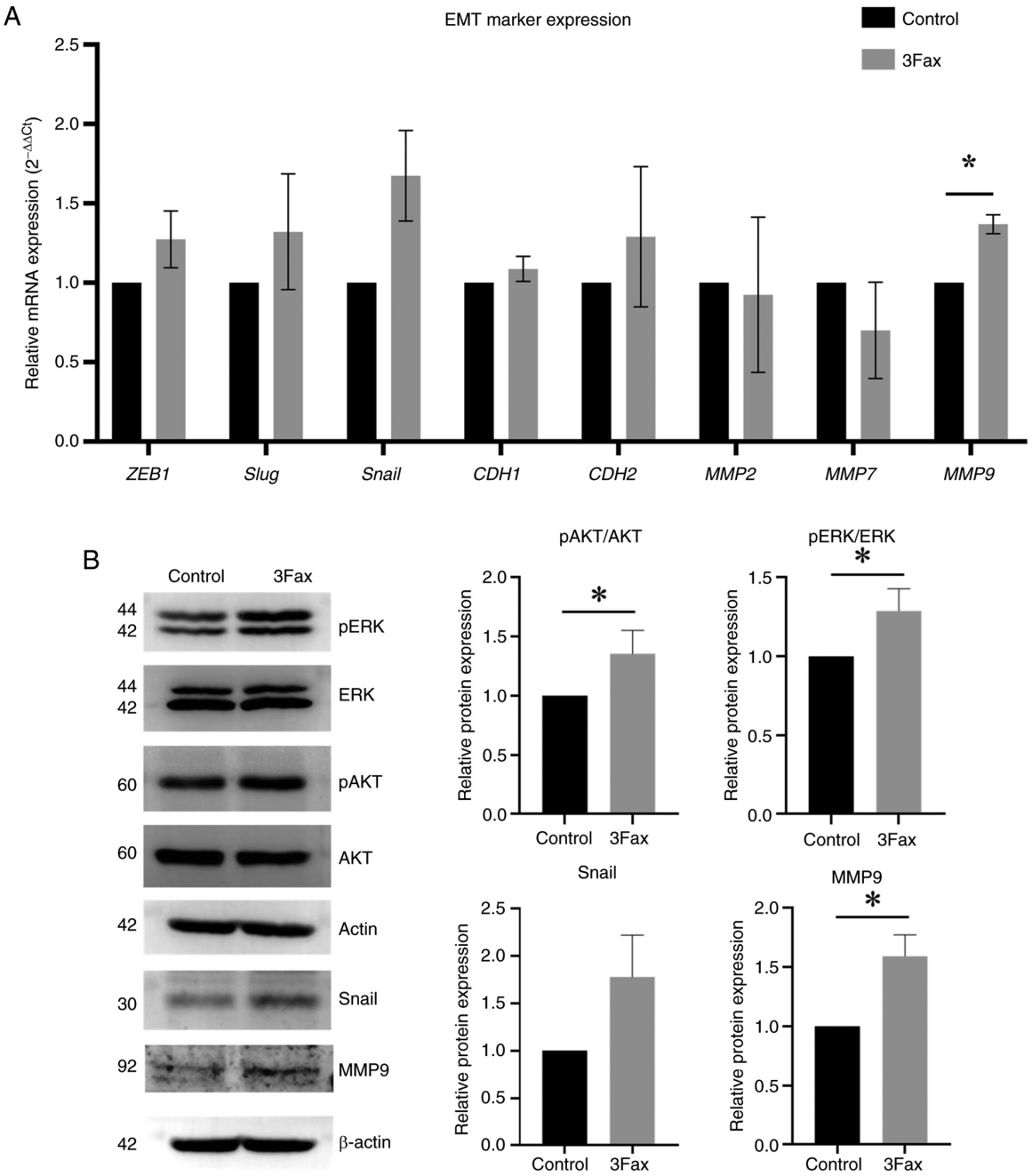

Figure 5. Suppression of sialylation induces expression of *Snail* and *MMP9* via the activation of AKT and ERK signaling in meningiomas. HKBMM cells were treated with 3Fax at 500 μM or DMSO for 5 days. (A) mRNA expression levels of EMT-related transcription factors and EMT-related proteins were determined via reverse transcription-quantitative PCR. (B) Protein expression levels of MMP9, Snail, AKT and ERK were determined via western blotting. Values are expressed as the mean ± standard deviation from three independent experiments. *P≤0.05 vs. control. MMP, matrix metalloproteinase; EMT, epithelial-mesenchymal transition; CDH1, E-cadherin; CDH2, N-cadherin; p-, phosphorylated.

P<0.0001) (Fig. 4B). These findings suggested that the suppression of sialylation enhances the aggressive characteristics of malignant meningioma cells.

Suppression of ST activity promotes the progression of meningiomas via EMT and the activation of ERK/AKT pathways. The present study aimed to investigate whether the suppression of sialylation enhances the aggressive characteristics of malignant meningioma cells via EMT. The expression levels of EMT-related TFs (*ZEB1*, *Snail* and *Slug*) and EMT-related proteins (N-cadherin, E-cadherin and *MMP2, 7 and 9* were determined in 3Fax-treated and control cells. The results demonstrated that the expression of EMT-related TFs (*Snail*: 1.7-fold change, P<0.05) and EMT-related proteins (*MMP9*, 1.4-fold change) were increased in 3Fax-treated cells compared with the control (Fig. 5A). Western blot analysis demonstrated an increase in the expression of MMP9 (1.6-fold change, P<0,05), Snail (1.8-fold change), phosphorylation of AKT (1.4-fold change, P<0.05) and ERK (1.3-fold change, P<0.05) in 3Fax-treated cells (Fig. 5B). These findings suggested that the suppression of ST activity promotes cell migration and invasion by

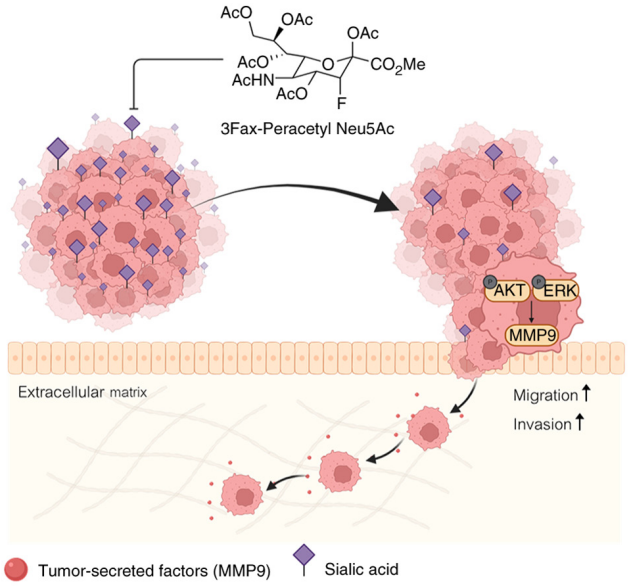

Figure 6. Alteration of sialylation affects the migratory and invasive capabilities of meningioma cells. MMP, matrix metalloproteinase. Created with BioRender.com.

enhancing the EMT process and activating the AKT/ERK pathways (Fig. 6).

Discussion

Aberrant sialylation is associated with cancer progression by promoting tumor growth and metastasis (17), facilitating immune escape (17), conferring resistance to apoptosis (18) and enhancing tumor angiogenesis (19). In the present study, the differential expression of β-galactoside α2,3 and α2,6 STs (ST3Gals and ST6Gals) was demonstrated in WHO Grade 1, 2 and 3 meningiomas using four different GEO datasets. The role of sialylation in meningioma progression was further investigated by chemically suppressing ST activity in malignant meningioma cells. It was found that sialylation suppression plays a crucial role in promoting the migration and invasion of malignant meningioma cells.

In the GEO datasets (GSE16581, GSE43290, GSE74385 and GSE136661), the downregulation of *ST3Gal5* and *ST6Gal1* was commonly observed across all four datasets and was associated with high-grade meningiomas. These results are consistent with studies in which downregulation of *ST6Gal1* is associated with metastasis in liver cancer and cholangiocarcinoma (20,21). Nevertheless, the upregulation of STs is commonly observed in multiple cancer types and is associated with tumor progression (22). The role of ST in meningioma development and progression has not yet been reported. In the present study, suppression of ST activity by a pan-sialylation inhibitor (3Fax-peracetyl-Neu5Ac, 3Fax) did not affect the proliferation or survival of malignant meningioma cells. Several studies have demonstrated that aberrant ST expression is primarily associated with invasion, metastasis and chemoresistance (22). In breast cancer, ST3Gal3 has been found to induce invasiveness and metastasis by regulating the integrinB1/MMP2/9 axis, and ST6Gal1 can promote the invasiveness of breast cancer cells through TGF-β signaling (23,24).

By contrast, suppression of sialylation promoted meningioma cell migration and invasion. A similar observation was made in colon cancer, where the depletion of ST6Gal1 enhanced the migration and invasion of colon cancer cells (25). Additionally, ST6Gal1 silencing promoted liver metastasis *in vivo* by reducing the cellular pool of the metastasis suppressor KAI1 (26). Furthermore, downregulation of ST6Gal1 decreased α2,6 sialylation on MCAM, enhanced the interaction between MCAM and galectin-3, leading to the dimerization of MCAM on the cell surface and subsequently promoting liver cancer metastasis (20). Moreover, it was demonstrated that suppression of sialylation in meningioma cells (HKBMM) induced EMT by increasing the expression of Snail and *MMP9*. The present findings suggested that sialylation by ST serves a protective function during meningioma progression. However, this mechanism could be considered cell type-specific because our explorations were performed using only HKBMM cells. Therefore, further investigation is required in other meningioma cell lines to confirm this.

EMT mechanisms are involved, including cytoskeletal reorganization, altered expression of adhesion molecules, and degradation of the basement membrane through the activation of MMP2 and MMP9 (27). In multiple carcinomas, activation of EMT results in a significant increase in cancer cell proliferation and extravasation, enabling the expansion of metastasis (28). In addition, MMP9 has been observed in various malignancies, and overexpression of MMP9 is positively correlated with poor prognosis and metastasis in patients with cancer. MMP9 can interact with and proteolyze cell surface proteins (CD44, E-cadherin, and α/β integrins) to promote cell migration and invasion. Various downstream pathways, including the PI3K/AKT and MAPK/ERK pathways, as well as various TFs, such as NF-κB, SP1, AP1 and *Snail*, appear to play a significant role in MMP9 expression and secretion (29,30). In the present study, it was demonstrated that suppression of ST activity markedly induced AKT and ERK phosphorylation. Taken together, the current findings from *in vitro* experiments suggest that the suppression of ST activity by a pan-sialylation inhibitor (3Fax) enhances meningioma progression through the AKT/ERK pathways. However, further *in vivo* studies are required to establish the role of STs in meningioma progression. Notably, the role of ST in meningiomas was demonstrated using a pan-sialylation inhibitor. Therefore, further studies using gene-specific knockdown or Clustered Regularly Interspaced Short Palindromic Repeats techniques are required to identify which specific ST plays a critical role in the progression of meningioma and to investigate the underlying mechanism of the candidate ST through the interaction with glycoprotein partners such as MCAM and CD44.

In summary, differential expression of STs was observed across the three grades of meningioma, suggesting downregulation of ST gene expression in malignant meningiomas. Additionally, suppression of sialylation using a ST inhibitor significantly enhanced meningioma cell migration and invasion by inducing the EMT process. These findings suggest that sialylation plays an important role in meningioma progression. Therefore, the sialylation status of meningioma tissues, as determined by MAL II and SNA staining,

could be a promising biomarker for assessing meningioma progression.

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Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

Authors' contributions

CT and KT acquired funding, designed the study and revised the manuscript. PJ and CT performed the biological experiments and analyzed the data. PJ and CT confirm the authenticity of all the raw data. KT, PA and PK analyzed and interpretated the data. PJ drafted the manuscript. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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