Suppression of sialylation increases sensitivity of glioblastoma cells to cisplatin and 5-fluorouracil

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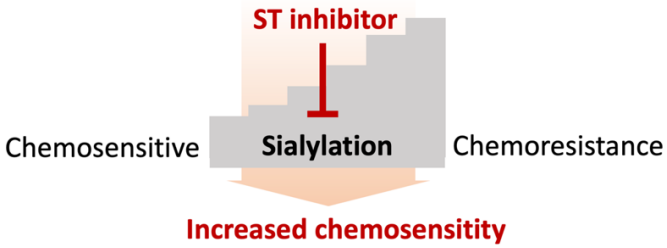
Abstract: *Background and objective*: Aberrant sialylation was found to promote tumorigenesis and progression of many cancer types. We aimed to investigate the involvement of sialylation in chemosensitivity of glioblastoma cells. *Materials and Methods*: Expression of sialylated glycans in glioblastoma cell lines (U373, U251, and U87) was determined by lectin fluorescence using *Maackia amurensis* lectin II (MAL-II) and *Sambucus nigra* agglutinin (SNA). The sensitivity of glioblastoma cells to cisplatin and 5-fluorouracfil (5-FU) was investigated by sulforhodamine B assay, after suppression of sialylation by a pan-sialylation inhibitor (3Fax-peracetyl-Neu5Ac, 3Fax). *Results*: By lectin fluorescence staining, α2,3- and α2,6- sialylated glycans were found to differentially express in U373, U251, and U87 glioblastoma cell lines. Cell viability of U373, U251, and U87 were significantly decreased after combined-treatment of cisplatin or 5-FU with 3Fax, comparing with chemo-drugs or 3Fax alone. This information suggested the involvement of sialylation in chemoresistance of glioblastoma cells. *Conclusions*: Sialylation was demonstrated in our study to play an important role in chemoresistance of glioblastoma cells, suggesting the potential of using sialylation inhibitor as a chemosensitizer for treatment of glioblastoma in the future.

Graphical abstract:



Keywords: gliomas; chemosensitivity; sialyltransferase inhibitor; sialylation

1. Introduction

Sialylation is a terminal glycosylation process of glycoproteins and glycolipids with sialic acid. It has been demonstrated to play important roles in many biological processes and cellular activities. Pattern of sialylation in cells is regulated by the level of CMP-sialic acid and expressions of sialyltransferases (STs) and neuraminidases (NEUs) or sialidases. STs are responsible for adding sialic acid onto oligosaccharide chains of glycoproteins and glycolipids, while the process to remove terminal sialic acid is driven by NEUs.

Aberrant sialylation was found to involve in tumorigenesis and progression of many cancers. Increasing the expression of surface sialylated-glycans was found to associate with the increase of aggressiveness of cancer cells [1]. It has been reported that an increasing of sialylation can promote the chemoresistance of ovarian cancer [2, 3], and cholangiocarcinoma [4]. Sialylation was also found to facilitate the invasion and metastasis in breast cancer [5], gastric cancer [6], and gliomas [7-9].

This study aimed to investigate the involvement of sialylation in therapeutic resistance of glioblastomas. Expression of sialylated glycans in glioblastoma cell lines; including U373, U251 and U87; was determined using lectin fluorescence staining. Role of sialylation in chemosensitivity of glioblastomas was assessed by treatment of the cells with sialyltransferase-inhibitor to suppress the sialylation, followed measuring chemosensitivity against cisplatin and 5-fluorouracil (5-FU).

2. Materials and Methods

2.1 Cell culture and treatment

The glioblastoma cell lines; U87, U251, and U373; were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplement with 10% (v/v) FBS under adherent condition at 37°C with 5% CO_2. At 80% confluence, the cells were passaged using trypsin-EDTA (0.25% w/v). For cell treatments, the cells were seeded in 24-well (2.0×10^4 cells/well) or 96-well plate (2.0×10^3 cells/well) and cultured overnight. After complete incubation, the culture supernatant was replaced by new medium containing 100 μM of 3Fax-peracetyl-Neu5Ac (3Fax, Merck Millipore, Darmstadt, Germany) and incubated for an additional 48-72 h. Cells treated with an equal concentration of dimethyl sulfoxide (DMSO; PanReac AppliChem, Darmstadt, Germany), instead of 3Fax, were used as a control.

2.2 Lectin fluorescence staining

After complete treatments, cells were washed with PBS and fixed with 4% paraformaldehyde for 30 min. Non-specific reaction was blocked using 3% (w/v) bovine serum albumin (BSA). Cells were then probed overnight with biotin-labelled lectins. *Maackia amurensis* lectin-II (MAL-II; Vector Laboratories, Burlingame, CA) and *Sambucus nigra* agglutination (SNA; Vector Laboratories, Burlingame, CA) were used to detect α2,3 sialylated-glycan and α2,6 sialylated-glycan, respectively. After complete incubation, cells were washed and incubated with 1:500 ALEXA®488-conjugated streptavidin (Invitrogen, Carlsbad, CA, USA) at room temperature for 1 h. Nucleus was counter-stained with 1:10,000 Hoechst-33342 (Invitrogen, Carlsbad, CA, USA). Expression of sialylated glycans was observed under fluorescent microscope. Negative control was performed by probing the cells with PBS instead of lectins, other steps were similar to lectin staining.

2.3 Chemosensitivity assay

To determine the involvement of sialylation in chemosensitivity of glioblastomas, after treatment with 100 μM 3Fax for 48 h, cells were undergone treatment with 2 μM and 10 μM of cisplatin (Sigma Aldrich, St. Louis, MO) or 5-fluorouracil (5-FU; Sigma Aldrich, St. Louis, MO), for an additional 48 h. Cell viability was measured at 0 and 48 h after chemo-drug treatments using sulforhodamine B (SRB) assay. Cells treated with the equal DMSO concentration were used as a control.

2.4 Statistical analysis

The data were analyzed in GraphPad Prism® 9.0 software (GraphPad software, Inc., La Jolla, CA). The difference between two dependent groups was analyzed by independent t-test. The $p < 0.05$ were considered as statistically significance.

3. Results

3.1. Sialyltransferase inhibitor, 3Fax, significantly suppressed the expression of sialylated glycan in glioblastoma cells.

Using lectin fluorescence staining, MAL-II binding α2,3- sialylated glycan (MAL-SG) and SNA binding α2,6- sialylated glycan (SNA-SG) were differentially detected in U373, U251, and U87 cell lines (**Figure 1**). Staining intensity of MAL-SG in glioblastoma cell lines was stronger than SNA-SG, we therefore focused on MAL-SG only for the further experiments.

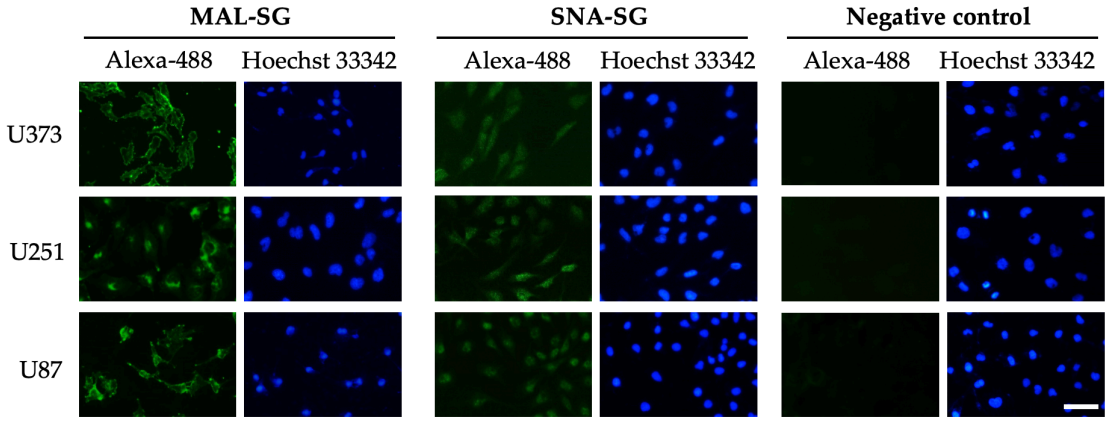


Figure 1. Expression of MAL-SG and SNA-SG in glioblastoma cell lines (U373, U251, and U87) using lectin cytofluorescence staining. The signal of MAL-SG and SNA-SG were presented by ALEXA 488 (green), the nucleus was stained by Hoechst 33342 (blue). Cells incubated with PBS instead of lectin were used as a negative control. (Scale bar = 50 μm)

To investigate the effects of 3Fax on sialylation of glioblastoma, cells (U373, U251, and U87) were treated with 100 μM of 3Fax for 72 h followed by determining the expression of MAL-SG by lectin fluorescence staining. The results showed that 3Fax could significantly suppress the expression of MAL-SG in U373, U251, and U87 glioblastoma cells, as shown in **Figure 2**.

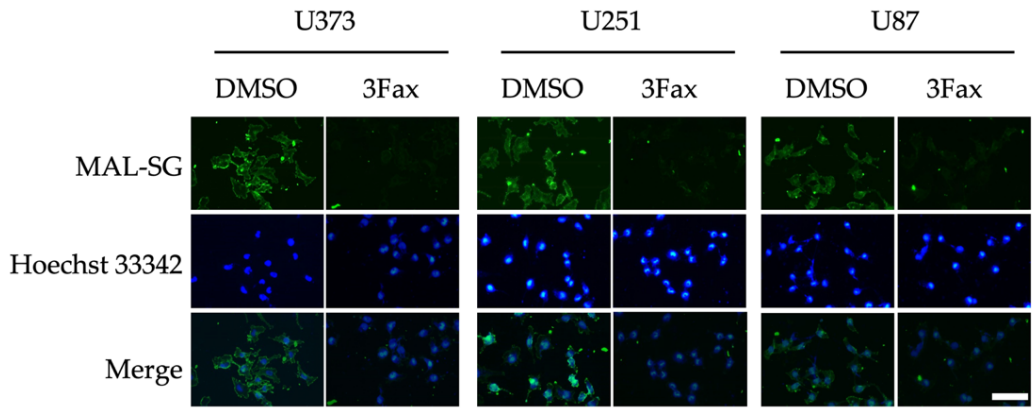


Figure 2. Detection of MAL-SG expression in glioblastoma cell lines (U373, U251, and U87) after treatment with 100 μM of 3Fax for 72h by lectin fluorescence staining. The signal of MAL-SG was presented by ALEXA 488 (green), the nucleus was stained by Hoechst 33342 (blue). (Scale bar = 50 μm)

3.2 Suppression of sialylation could enhance the chemosensitivity of glioblastoma cells.

To explore the involvement of sialylation in chemosensitivity of glioblastomas, cells were pre-treated with 100 μM of 3Fax for 48 h. After complete treatment, the cells were subsequently treated with 2 and 10 μM of cisplatin or 5-FU for an additional 48 h, comparing with DMSO treated control. Our results showed that 3Fax significantly enhances the sensitivity of U373, U251 and U87 glioblastoma cells to chemo-drug treatment (**Figure 3**). Viability of U373, U251, and U87 cells were significantly decreased in the combination of cisplatin or 5-FU with 3Fax, comparing with treatments with either chemo-drugs or 3Fax alone ($p < 0.05$, Student's t-test).

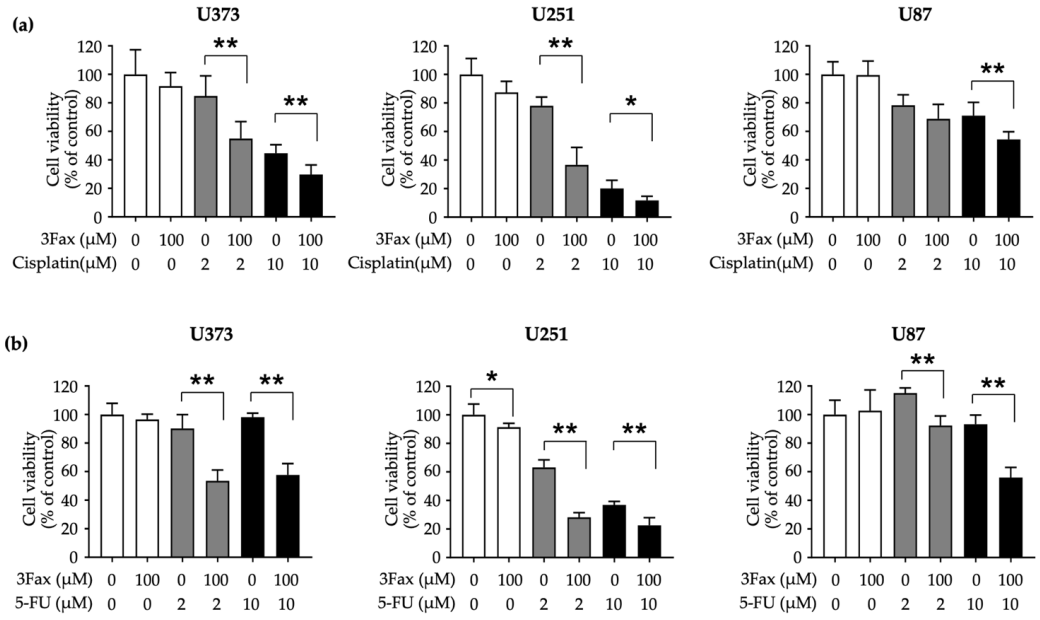


Figure 3. Chemo-sensitizing effect of 3Fax in glioblastoma cells. The U373, U251 and U87 cells were treated with 3Fax for 48 h, followed by the cytotoxicity assay, against 2 and 10 μM of **(a)** cisplatin or **(b)** 5-FU, using SRB assay.

4. Discussion

Aberrant sialylation was found to associate with cancer progression via promoting tumor metastasis [10], and chemo-resistance in many cancer such as breast cancer [5], gastric cancer [6], ovarian cancer [11], prostate cancer [12], and cholangiocarcinoma [4]. Our present study demonstrated that sialylated glycans were differentially expressed in the glioblastoma cell lines, suggesting that the sialylation may play some roles in glioblastomas. To study the roles of sialylation in glioblastomas, we have investigated the effect of sialylation suppression on chemosensitivity of glioblastoma cell lines. Using 3Fax, a pan-sialyltransferase inhibitor [13], we found that sialylation plays an important role in the resistance of glioblastoma cells to cisplatin and 5-FU.

Sialylation was discovered as an important mechanism to promote tumor progression and therapeutic resistance [14-16]. In glioma, sialylation was found to elevate in cancer cells and played important roles in tumor progression [10, 17]. The previous study in glioma tissues using lectin-histochemistry showed that sialylation was enhanced in glioma, compared with normal cells [8]. The study by Chong *et al* showed that the enzyme ST3Gal1 was highly detected in cancer cells and associated with poor prognosis and shorter survival of the patients [17]. This evidence suggested the importance of sialylation and its related enzyme in glioma progression. Our present data added up the significance of sialylation in chemoresistance of glioblastoma cells.

The use of cisplatin and 5-FU is the first base for treatment in cancers [18, 19]. In this study, we have demonstrated the potential of using 3Fax to enhance the sensitivity of glioblastoma cells to cisplatin and 5-FU. Our results showed that inhibition of sialylation by 3Fax could significantly augment the sensitivity of glioblastoma cell lines to cisplatin and 5-FU, suggesting the role of sialylation in chemoresistance of glioblastoma. Sialylation was found in other cancers to promote the chemoresistance [2-4, 20], we have added up here the information on role sialylation in chemoresistance in glioblastoma. This finding suggested the potential of using cisplatin and 5-FU in combination with 3Fax to improve the treatment of glioblastoma. In addition, the chemosensitizing effect of 3Fax or other sialylation inhibitors against temozolomide, a standard chemotherapy for glioblastoma, should be further elucidated. Taking all these together, sialylation inhibitor(s) are potentially used as a chemosensitizer in the new therapeutic strategy for glioblastoma treatment.

5. Conclusions

Our present study demonstrated the possible role of sialylation in resistance of glioblastoma to cisplatin and 5-FU. Suppression of sialylation by sialyltransferase inhibitor could significantly enhance the sensitivity of glioblastoma cells to chemotherapeutic drugs. The information obtained in our study suggested the potential of using sialyltransferase inhibitor, at least 3Fax, to sensitize the chemotherapy of glioblastoma in the future.

Author Contributions: Conceptualization, A.S. and N.A.; methodology, S.P. and N.A.; software, S.L.; validation, O.P. and S.L.; formal analysis, S.P.; investigation, O.P. and A.S.; resources, N.A.; data curation, S.L.; writing—original draft preparation, S.P.; writing—review and editing, A.S.; visualization, S.P.; project administration, S.L.; funding acquisition, A.S. and S.P. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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