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Original Article

# The role of α2,3-linked sialylation on clear cell type epithelial ovarian cancer

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ABSTRACT

*Objective:* Our previous study has shown that high expression of α2,3-sialytransferase type I was associated with advanced stage serous type epithelial ovarian cancer (EOC). The aim of the current study further attempts to evaluate the altered α 2,3-sialylation on the behavior of clear cell type EOC (C-EOC).
*Materials and methods:* Immunohistochemistry staining, bioinformatics analysis and tissue array were used to disclose the clinical significance of over α2,3-sialylation in C-EOC. An α2,3 sialylation inhibitor, soyasaponin I (SsaI) was used to investigate the behavior change of the C-EOC cell line.
*Results:* We reconfirmed that α2,3-sialylation, instead of α2,6- sialylation, was associated with late-stage C-EOC. Soyasaponin I could inhibit α2,3-sialylation of C-EOC cell lines and increase E-cadherin expression with subsequently suppressing migration of C-EOC cells.
*Conclusions:* The current study demonstrated the important role of α2,3-linked sialylation in C-EOC and targeting of α2,3-linked sialylation might offer as a potential therapeutic strategy in the future.


## Introduction

Epithelial ovarian cancer (EOC), while detected at the late stage, is a leading cause of gynecologic cancer deaths in the United States, and 22,440 new cases were diagnosed in 2017, contributed to 14,080 deaths [1]. This highly lethal disease is also significantly increased in both Taiwan and China [2–4]. The standard therapy for EOC includes an intensive and thorough cytoreductive surgery, before or after paclitaxel-platinum based chemotherapy [5–7]. However, the long-term survival rate is still low. Many targeting therapies and modifications of chemotherapy, including dose, routine, and interval were attempted, which included angiogenesis inhibitors, poly(ADP-ribose) polymerase inhibitors, intraperitoneal route, and hyperthermia have been used in routine clinical practice [8–10], but the effects are still controversial and the results are conflicted. Therefore, the development of novel biomarker therapeutics is urgently in demand for the management of EOC patients [11–13].

Altered sialylation on tumor cell surface proteins along with a marked upregulation of sialyltransferases (STs) activity might be a hallmark of cancer [14,15]. The change of surface sialylation involves tumor proliferation and behavior [16,17]. In humans, sialylation, the transfer of sialic acids (SAs) from GMP–SA to an acceptor carbohydrate, is a process catalyzed by different STs based on their linkage and acceptor molecule, which includes an α2-3- or an α2-6-bond to galactose (Gal), an α2-6-bond to N-acetylgalactosamine (GalNAc) or N-acetylglucosamine (GlcNAc), and an α2-8-bond to another SA, to control the synthesis of specific sialylated structures with unique biological roles [18–22].

Many researchers use maackia amurensis leukoagglutinin (MAL) and maackia amurensis hemagglutinin (MAH, MAL II, MAL-

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2), the seed lectins from maackia amurensis, as glycoanalytical tools to probe biological targets for α2-3-linked SAs [23,24]. MAH is a teramer that is composed of a 33-kDa subunit, and binds preferentially to sialylated O-linked glycans, whereas MAL is a dimer that is composed of a disulfide-containing 70-kDa subunit, and binds preferentially to sialylated N-linked glycans [25–27]. Maackia amurensis agglutinin (MAA) comprises two isolectins: MAL and MAH (MAL-2) [28–30]. Sambucus nigra agglutinin (SNA) is used to detect for α2,6-linked SAs [26,27,30]. The importance of these linked sialylation may not only regulate tumor growth and progression as shown above [16–18,21,22,24], but also immune responses, inflammation, viral infection, and neurological disorders [31–33]. Our previous study showed that the expression of MAA was significantly increased in serous type EOC tissues compared to normal tissues and the mRNA expression levels of α2-3 sialyltransferase type 1 (ST3Gal I) and α2-6 sialyltransferase type 1 (ST6Gal I) were also increased in serous type EOC tissues [34]. Therefore, the increased expression of ST3Gal I may contribute directly to the increased α2,3-linked sialylation in serous type EOC [34]. Recently, we found that ST3Gal I could regulate serous type EOC cell migration and peritoneal dissemination mediated through epidermal growth factor receptor (EGFR) signaling [35]. Since there are at least 4 main subtypes of EOCs, including serous, mucinous, clear cell and endometrioid type, the role of α2,3-linked sialylation on the other subtype EOCs (serous type was excluded) are still worthy of investigation.

In the current study, we investigated the prognostic role of α2,3-linked sialylation in clear cell type EOC (C-EOC) using an ovarian tissue microarray and found that over α2,3-linked sialylation is associated with late-stage C-EOC. Furthermore, we found that an ST inhibitor (soyasaponin I) could influence expression of ST3Gal I and α2,3 sialyltransferase type 4 (ST3Gal IV) genes as well as tumor migration in the C-EOC cell line, which might be due to the up-regulation of E-cadherin. These additional results might further raise the interest in exploring the new therapeutic targeting therapy for clear cell type EOC.

## Materials and methods

### *Cell culture*

The human C-EOC cell line ES-2 was cultured and maintained in McCoy's 5A medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin. A mouse ovarian surface epithelial cell line, MOSEC, was cultured and maintained in RPMI-1640 supplemented with 10% FBS, 1% non-essential amino acids, 1% sodium pyruvate, 1% L-glutamine, 0.1% beta-mercaptoethanol (beta-ME) and 1% penicillin–streptomycin. These cells were incubated at 37 °C in a humidified atmosphere of 5% $CO_2$.

### *Immunohistochemistry (IHC) specimens*

Immunohistochemistry (IHC) specimens were sourced from a commercial tissue array (SUPER BIO CHIPS, Korea) with complete clinical data, including clinical stage, grade and OS, and were available for a CJ2 human ovarian cancer tissue array with 59 specimens. Tissue sections were immersed in a coplin jar filled with diluted Target Retrieval Solution at a 10× concentration (Dako Denmark; Code S1699), heated for 5 min at 121 °C, and cooled down to approximately 85 °C. The endogenous peroxidase activity was quenched with 0.6% hydrogen peroxide/methanol. The sections were incubated with goat serum (BioGenex, San Ramon, CA 94583 USA) for 30 min at room temperature to prevent non-specific binding. Then, the sections were incubated with 20 μg/ml of horseradish peroxidase-conjugated MAA lectins (EY laboratories, Catalog Number: H-7801-1) and SNA (EY Laboratories, Catalog Number: H6802-1) overnight at 4 °C. Staining was visualized with AEC Substrate-Chromogen (DAKO Denmark), and the sections were lightly counterstained with hematoxylin. The negative control sections were treated with diluent instead of horseradish peroxidase-conjugated MAA. Other negative control sections were treated with horseradish peroxidase-conjugated MAA that had been pre-incubated for 60 min at 4 °C with 0.2 mM sialyllactose with a NeuAc 2,3Gal 1,4Glc structure (Funakoshi Co., Tokyo, Japan). For the IHC staining score, the staining of IHC using different antibodies was determined by calculating a total staining score as the product of an intensity score (0–3). The intensity score represented the estimated staining intensity (0, no staining; 1, weak; 2, moderate; and 3, strong). A strong intensity score was defined as a high expression of MAA; other intensity scores indicated low expression.

### *Soyasaponin I (SsaI) preparation*

Soyasaponin I was prepared from a commercial preparation of soybean saponins. The purification process was performed as described in a previous study [36–38]. The purity of the purified soyasaponin I in this study was >99% as determined by an analytical HPLC C18 column (4.6 mm × 250 mm, Phenomenex).

### *Flow cytometry*

The ovarian cancer cell suspension ($2 \times 10^6$) was suspended in 100 μl of FACS staining buffer. The cells were incubated for 30 min at 2–8 °C in the dark after being combined with a fluorescence-conjugated antibody (FITC) to detect MAA expression (EY Laboratories, Catalog Number: F-7801-2). The corresponding isotype staining was also prepared as a control. 200 μl of FACS staining buffer was added to wash the cells, which were then spun down at 1200 rpm for 5 min. The cells were transferred into FACS tubes after re-suspending the stained cells in 200–300 μl of FACS staining buffer. A BD FACSCanto II was used to analyze the expression of MAA in 2 ovarian cancer cell lines after SsaI or DMSO-control treatment for 48 h.

### *Protein isolation and western blot analysis*

The immunoblot analysis of the target proteins was done at the recommended dilution. Briefly, a given type of cells treated with or without Ssa I was grown to 70–80% sub-confluency and treated with lysis buffer containing 1% Triton X-100 in PBS and protease inhibitor mixture tablets (Roche, Barcelona, Spain). 100 μg of total cell lysate were electrophoresed on 10–15% SDS-polyacrylamide gels, depending on the different types of studied proteins, and transferred electrophoretically to Immobilon membranes (Millipore, Bedford, MA). After blocking in blocking solution (5% nonfat dry milk/0.1% Tween-20/PBS), the membranes were incubated overnight with a recommended dilution of primary antibodies. The primary antibodies included MAA (EY Laboratories, Catalog Number: H-7801-1) and MAL-2 (Vector Laboratories, Catalog Number: B-1265). The primary antibodies were washed away in 0.05% Tween-20/PBS, and then, the membranes were incubated with the horseradish peroxidase-conjugated secondary antibody. The proteins were visualized using an enhanced chemiluminescence reagent (Amersham Pharmacia Biotech) followed by exposure to X-ray film.

### *Wound healing assay*

MOSEC and ES2 ovarian cells were seeded into 6-well plates at a density of $6 \times 10^5$ cells/well and incubated overnight for culturing

up to 90–100% confluency. The cell monolayer was drawn across the center of the well using a 1-ml sterile pipette tip in order to produce a wound area. The cells were washed twice with 1xPBS to remove the detached cells and cell debris and then treated with complete medium containing DMSO and 100 μM SsaI for 24 h. Three random fields along the scraped line in each well were photographed using 100× magnification at 0 h, 6 h and 24 h. The images were used to measure and analyze the widths of the cell-migrated gaps.

*Microarray analysis (L1000)*

The microarray experiments were conducted using the L1000 Operating Procedure (L1000 SOP). In brief, a human ovarian cancer cell line, ES-2, was treated with DMSO (no drug treated, as control group) or SsaI (drug treated, as a study group) in YM002 plates. After 6 h of drug treatment, the medium was removed, and lysis buffer was added (contained in the L1000 kit) to the wells for 30 min. After the cell lysis step, the lysate was stored at 80 °C for at least one night before transferring it to the 384-well plate for the L1000 assay, which was processed using the following commercial instruction protocol: http://s3.amazonaws.com/support.lincscloud.org/protocols/data_generation/L1000_SOP.pdf. The gene expression profiles in the control and study groups were detected by L1000 array technology. Up and down probe sets were selected by 2-sample t-test with a p value lower than 0.01 and a fold-change greater than 1.5-fold.

*RNA isolation for cDNA synthesis and real-time quantitative PCR (RT-qPCR)*

The total RNA was isolated from cultured cell lines using TRIzol reagent (Invitrogen). The cells were lysed in TRIzol and centrifuged after adding bromochloropropane (Sigma–Aldrich). The RNAs were precipitated with isopropanol, washed with ethanol to remove impurities, and dissolved in RNAse-free water (Invitrogen). The RNA concentrations were quantified spectrophotometrically. For cDNA synthesis, reverse transcription using SuperScript II™ reverse transcriptase (Introgen) was performed according to the manufacturer's instructions. Using the Roche Online Assay Design Center, specific primers and the associated probes were selected for the genes of interest. The samples were amplified in triplicate using a LightCycler 480 Instrument (Roche). The quantification cycle values (crossing point, Cp), which were defined as the cycle number at which the reporter fluorescence passed a fixed threshold above the baseline, were calculated for each gene. The expression level of the gene of interest in a sample was normalized using the average of the housekeeping genes. Experiments were performed 3 times.

*Thiazolyl blue (MTT) assay*

The cells were seeded at $5 \times 10^3$ in 96-well plates in RPMI 1640 with 5% charcoal-stripped/heat-inactivated FCS, grown for 24 h, then exposed either to DMSO-control (diluted in dimethyl sulfoxide), or 100 μM SsaI (Soyasaponin I, Sigma–Aldrich) for 48 h. Two days later, 200 μl of thiazolyl blue (MTS, 5 mg/ml, Sigma–Aldrich) was added into each well with 1 ml of medium for 4 h at 37 °C. After incubation, 2 ml of 0.04 N HCl in isopropanol was added to each well. After several rounds of pipetting and 5 min of incubation at room temperature, the absorbance was read at a test wavelength of 570 nm. The background absorbance was measured at 600 nm. The results were expressed as the mean ± SD of three separate replicates.

*Statistical analysis*

For the cell invasion assay data, statistical comparisons among groups were performed with a non-paired Student's t-test. Statistical analysis was performed using the SPSS software program. Survival curves of ovarian cancer patients were calculated according to the Kaplan–Meier method. P values were evaluated using the log-rank test for censored survival data. The survival time was censored if the patient was alive at the time of evaluation. The relationship between IHC expression and other clinical or tumor parameters was calculated using the $\chi^2$ test. $P < 0.05$ was considered significant.

## Results

*Over α2,3-linked sialylation was a poor prognostic factor for human clear cell type EOC*

To characterize the role of sialylation in C-EOC, we performed immunohistochemical staining using MAA, which is a NeuNAcα (2–3) Galβ (1–4) GlcNAc/Glc-specific lectin, to detect the extent of the α2,3-linked sialylation in a human clear cell type EOC using tissue array (CJ2 set from SUPER BIO CHIPS, Seoul/South Korea). We found that the intensity of the α2,3-linked sialylation MAA staining (Fig. 1A) positively correlated with the grade of cancer (Fig. 1B) and the overall survival rate (Fig. 1C). The patients with high MAA expression had significantly advanced stage that presented as upper peritoneal seeding and distant metastasis compared with low expression (67% *versus* 43%, respectively; *p* value < 0.01). Moreover, these cases with high MAA as indicated by IHC staining also had a significantly poor overall survival rate. In contrast, α2,6-linked sialylation, represented by SNA, did not have an important impact in the staging and prognosis of EOC (Fig. 1D–F). Taken together, we concluded that α2,3-linked sialylation was associated with worse outcomes of C-EOCs.

*α2,3-sialylation inhibitors suppress α2,3-linked sialylation in clear cell type EOC*

To target α2,3-linked sialylation in clear cell type EOC, we used an ST inhibitor, soyasaponin I (SsaI), to investigate whether SsaI can suppress the α2,3-sialylation in C-EOC. The clear cell type EOC cell line was treated with SsaI to investigate the α2,3-sialylation and was assessed using Western blotting for MAA and MAL-2 for the α-2,3 linkage. The expression of MAA and MAL-2 were decreased after SsaI treatment compared to DMSO control (Fig. 2A–B). The result of flow cytometry (Fig. 2C–D) after SsaI treatment also demonstrated that SsaI could significantly decrease α2,3-sialylation of these C-EOC cells. In summary, SsaI could repress α2,3-sialylayion in C-EOC successfully, similar to melanoma cell line and breast cancer cell line, as shown our previous studies [30,31].

*α 2,3-sialylation inhibitors suppress migration of clear cell type EOC cell line*

Since late-stage C-EOC had higher α2,3-sialylation MAA staining from the IHC results, we further investigated whether SsaI might inhibit the migration ability of these C-EOC cells. Then, we conducted a wound-healing assay in ES-2 ovarian cancer cell lines. The results showed that the inhibition of α2,3-sialylation by SsaI significantly inhibited the motility of C-EOC lines (Fig. 3A–D). MOSEC cells functioned as control (Fig. 3A–D). The suppression of α2,3-sialylation by SsaI treatment did not affect tumor cell growth and proliferation (Fig. 3E–F), suggesting targeting α2,3-sialylation

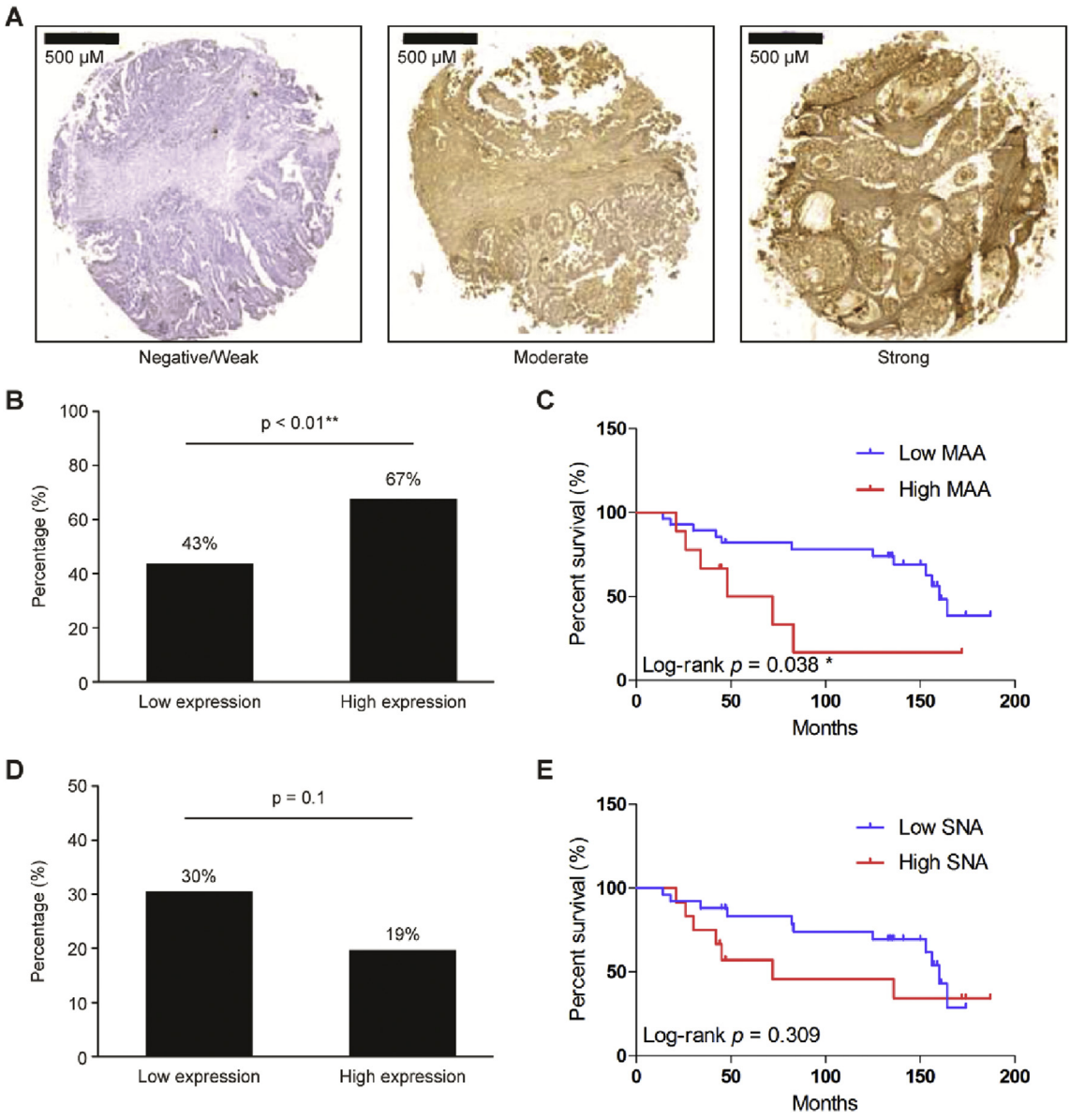


**Fig. 1.** ***Over α2,3-linked sialylation was a poor prognostic factor for human clear cell type epithelial ovarian cancer.*** **(A)** A commercial human ovarian cancer tissue array (Super Bio Chips, CJ2, Korea) was used for immunohistochemical staining, and maackia amurensis agglutinin (MAA) was stained. The intensity score represented the estimated staining: 0, no staining; 1, weak; 2, moderate; 3, strong. Low MAA concentrations included no staining, weak, and moderate; a high MAA concentration was defined as strong staining. Scale bars representing 500 μm were added from an image taken at identical magnification and resolution. **(B)** The percentage of early-stage (FIGO stage I–II) or late-stage (FIGO stage III–IV) disease was assessed in the low- and high-MAA groups; Fisher's exact test was used to analyze the stage percentage (*: $p < 0.05$, **: $p < 0.01$). **(C)** A Kaplan–Meier survival curve was used to analyze the overall survival (OS) in the low- and high-MAA groups. **(D)** The percentage of early-stage (FIGO stage I–II) or late-stage (FIGO stage III–IV) disease was determined in the low- and high-sambucus nigra agglutinin (SNA) groups; Fisher's exact test was used to analyze the stage percentage. (E) A Kaplan–Meier survival curve was used to analyze the OS in the low- and high-SNA groups.

of C-EOC cells only influenced the "behavior" changes of these cancer cell lines.

*Inhibition of α2,3-linked sialylation can up-regulate E-cadherin*

Furthermore, the possible downstream factors associated with behavior change of these cancer cell lines after inhibition of α2,3-sialylation were investigated. The RNA expression of C-EOC was used to analyze the genetic expression. A L1000 microarray study and an *in vitro* study showed the up-regulation of the cell–cell adhesion molecule CDH1 (E-cadherin, left panel of Fig. 4A–B) and the down-regulation of the metastatic molecule CDH2 (N-cadherin, right panel of Fig. 4A–B). It was interesting to find that ST3Gal I and ST3Gal IV were altered by the α2,3-sialylation inhibitors (Fig. 4C, and Table 1). These data support the notion that targeting 2,3-sialylation could reverse the characteristics of epithelial-mesenchymal transformation (EMT) change in the original C-EOC cell lines and subsequently diminish the migration ability *in vitro*.

## Discussion

The current standard therapy for EOC consists of the combination of comprehensive and thorough cytoreductive surgery and platinum-paclitaxel based chemotherapy, with/without targeted therapy [8–11]. The outcome after treatment is still disappointing, because of late diagnosis, wide-intraperitoneal dissemination, and

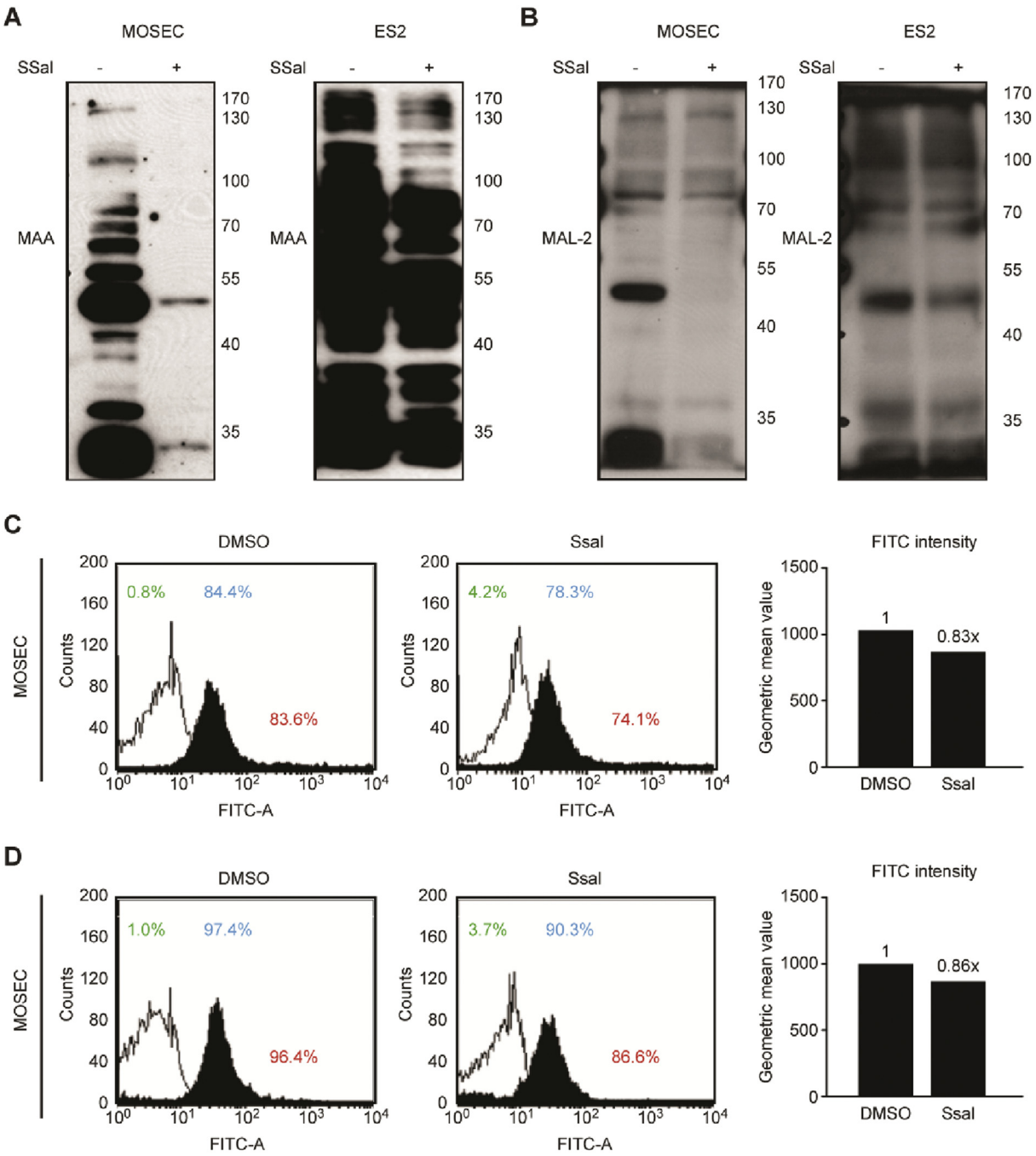


**Fig. 2.** ***α2,3-sialylation inhibitors suppress α2,3-linked sialylation in ovarian cancer.*** **(A–B)** The Western blot of α2,3-linked sialylation, maackia amurensis agglutinin (MAA) and maackia amurensis hemagglutinin (MAL-2) after treatment with soyasaponin I-SsaI (100 μM) or a DMSO control for 72 h. GAPDH was used as a control. **(C–D)** MAA expression in ovarian cancer cells was analyzed by flow cytometry; Maackia amurensis agglutinin type 2 (MAH, MAL-2), specific for α2,3-linked NeuNAc, was used to investigate the changes in the sialylation of glycoconjugates. The isotype control (empty histogram) is shown in green, and the results from the experiment (black histogram) are shown in blue. The value in red was the real increase in the experimental samples (DMSO or soyasaponin I-SsaI). The geometric FITC intensity after treatment with SsaI (100 μM) or a DMSO control is shown; the number above bar represents the relative fold. (For interpretation of the references to color/colour in this figure legend, the reader is referred to the Web version of this article.)

high recurrent rates in these patients [8,39]. In our previous study, we found that the high expression of ST3Gal I was associated with advanced-stage serous type EOCs [34,35]. α2,3-STs, including ST3Gal I-VI, mediate the transfer of SA with an α2,3-linkage to terminal Gal residues. In the current study, we re-confirmed that α2,3-linked sialylation might contribute to peritoneal dissemination (advanced stage or late stage) because over α2,3-sialylation (enhanced MAA staining), instead of α2,6-linked sialylation (SNA staining) was found in late-stage C-EOC.

MAL-I and MAL-II are the two main glycoanalytical tools used to probe biological targets for α2-3-linked SAs [23–30]. MAL-I, which is also called MAL, is most preferably bound to N-linked glycans; MAL-II, which is also called MAH binds preferentially to O-linked glycans [23,24]. In bioinformatics microarray tests, treatment with SsaI could decrease the expression of ST3Gal I and ST3Gal IV significantly compared to other α2,3-, α2,6-, or α2,8- STs (Table 1). α2,3-ST3Gal I has been shown to determine the *O*-linked sialylation in cancer cells, and ST3Gal IV is mainly involved in N-linked

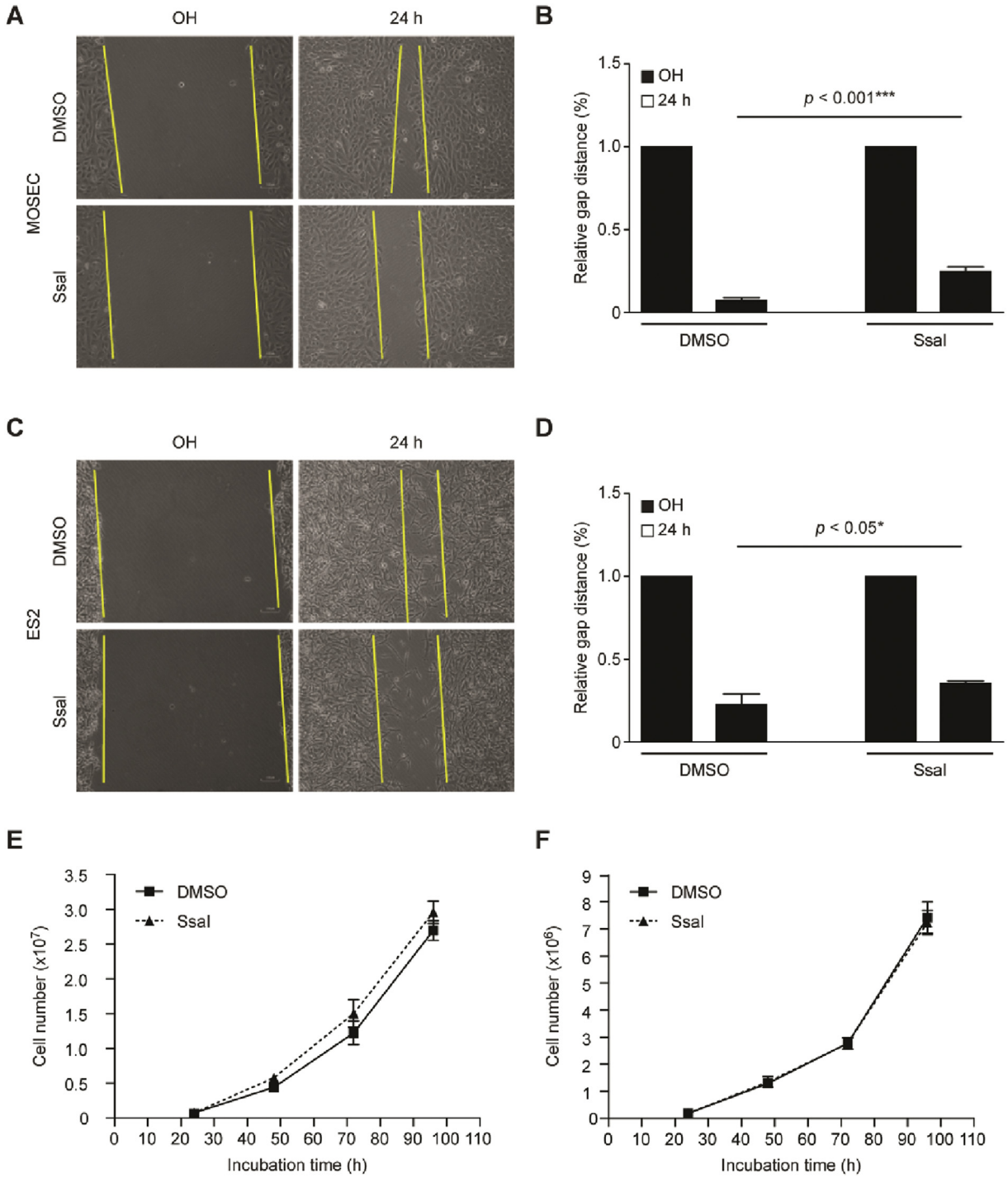


**Fig. 3.** *α 2,3-sialylation inhibitors suppress migration of clear cell type EOC cell line and do not affect the tumor growth.* **(A–D)** Ovarian cancer cells were treated with soyasaponin I-SsaI (100 μM) or DMSO-control for 24 h in a wound-healing assay (low panel). The data shown are the mean ± SD of 3 separate experiments (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$). **(E–F)** The serial cell number was counted for a tumor growth curve in SsaI (100 μM)-treated or DMSO-control cells. The data shown are the mean ± SD of separate samples.

sialylation [23–30]. We found that the expression levels of MAA and MAL-2 were decreased after SsaI treatment compared to the control. Therefore, SsaI *in vitro* predominately inhibited O-linked sialylation (ST3Gal I), instead of N-linked sialylation (ST3Gal IV) [40,41]. Gomes et al. reported that the expression of ST3Gal IV leads to $SLe^x$ antigen expression and induces an increased invasive phenotype through the activation of c-Met, which is associated with Src, FAK and Cdc42, Rac1 and RhoA GTPases activation in gastric cancer [41]. The role of ST3Gal IV and N-linked sialylation in EOC needs further investigation.

The CDH1 gene encodes a transmembrane glycoprotein, epithelial cadherin, which is also known as cadherin 1 or E-cadherin [42]. E-cadherin is known to maintain the integrity and polarity of epithelial cells. It localizes to cell–cell borders and is strongly expressed in adherent junctions. E-cadherin is involved in the invasion and metastasis of a variety of cancers, and the loss or

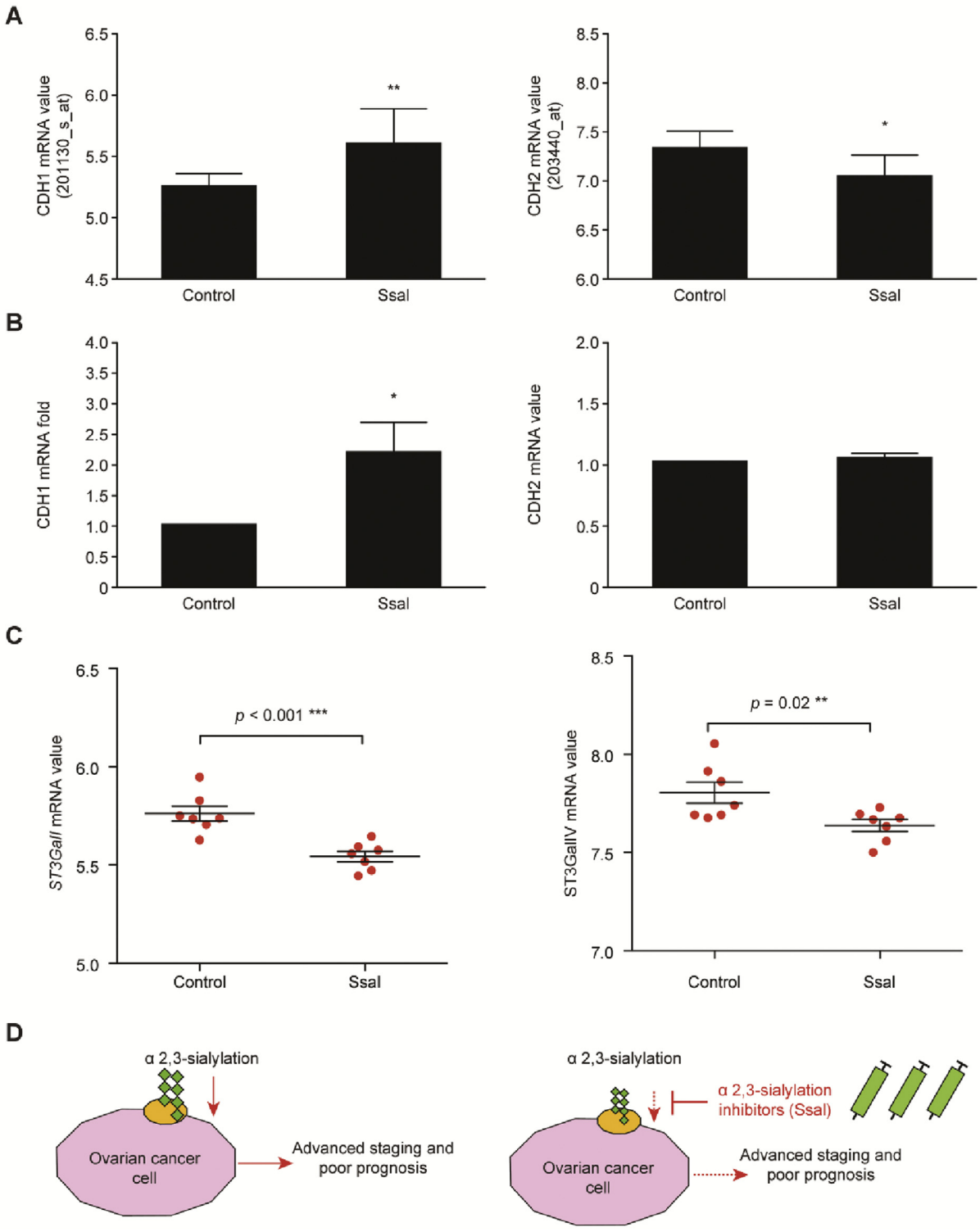


**Fig. 4.** ***Inhibition of α2,3-linked sialylation can up-regulate E-cadherin.*** **(A–B)** The RNA expression of epithelial-mesenchymal transition factors, including E-cadherin and N-cadherin, was investigated after treatment with soyasaponin I (SsaI) or DMSO (*: $p < 0.05$, **: $p < 0.01$). **(C)** The α2,3-linked sialyltransferases in an ovarian cancer cell line (ES2) treated with DMSO (no drug treatment, as a control study) were analyzed using a L1000 RNA microarray. The data shown are the mean ± SD of separate repeat experiments (**: $p < 0.01$, ***: $p < 0.001$). (D) A cartoon figure of the α2,3-sialylation of clear cell type epithelial ovarian cancer and the inhibitor-SsaI.

down-regulation of E-cadherin expression can lead to several pathological oncogenic changes, such as loss of contact inhibition, uncontrolled growth and tumor cell dedifferentiation [43,44]. In the absence of E-cadherin, the connections between cells become loose and disorganized, which promotes invasion [45]. E-cadherin is one of the key molecular markers of the EMT, which is a fundamental biological process associated with cancer cell invasion and metastasis [42,43,45]. Meta-analysis study found the prognostic value of E-cadherin expression in cancers, including breast cancer, head and neck cancer, ovarian cancer, and endometrial cancer

**Table 1**
L1000 mRNA expression value of sialyltransferases (STs).

| Sialyltransferase (ST) α2,3; α2,6; α2,6-NAC; α2,8 | | L1000 mRNA expression value | | |
|---|---|---|---|---|
| | | Control | Soyasaponin I | *P* value |
| α2,3-sialylation | ST3Gal I | 5.761 ± 0.102 | 5.543 ± 0.070 | < 0.001 |
| | ST3Gal II | 4.723 ± 0.104 | 4.842 ± 0.197 | 0.185 |
| | ST3Gal IV | 7.7805 ± 0.143 | 7.637 ± 0.081 | 0.02 |
| | ST3Gal V | 5.802 ± 0.125 | 5.854 ± 0.168 | 0.522 |
| | ST3Gal VI | 4.806 ± 0.077 | 4.853 ± 0.149 | 0.479 |
| α2,6-sialylation | ST6Gal I | 4.909 ± 0.070 | 4.875 ± 0.063 | 0.351 |
| | ST6GalNAC II | 6.086 ± 1.179 | 5.784 ± 0.446 | 0.538 |
| | ST6GalNAC IV | 4.815 ± 0.216 | 4.980 ± 0.259 | 0.219 |
| | ST6GalNAC V | 6.055 ± 0.203 | 5.587 ± 0.460 | 0.03 |
| α2,8-sialylation | ST8SIA I | 5.451 ± 0.199 | 5.513 ± 0.169 | 0.537 |
| | ST8SIA II | 4.902 ± 0.151 | 4.956 ± 0.180 | 0.550 |
| | ST8SIA III | 4.780 ± 0.119 | 4.867 ± 0.343 | 0.538 |
| | ST8SIA IV | 4.562 ± 0.132 | 4.633 ± 0.106 | 0.288 |
| | ST8SIA V | 5.013 ± 0.113 | 5.022 ± 0.159 | 0.905 |

[46–51]. The reduced expression of E-cadherin is significantly correlated with poor prognosis of breast cancer patients (hazard ratio [HR] of disease free survival (DFS): 1.62, 95% confidence interval [CI]: 1.31–1.99; HR of overall survival [OS]: 1.79, 95% CI: 1.41–2.27) [49]. For endometrial cancer, the reduced expression of E-cadherin was associated with worse prognosis (HR of OS: 2.42, 95% CI 1.50–3.89) [51]. For ovarian cancer, the association of between down-regulation of E-cadherin expression and ovarian cancer remains unclear [52]. One report showed that CDH1 promoter methylation (reduced expression of E-cadherin) had an increased risk of ovarian cancer in cancer tissues (odds ratio [OR]: 8.71) in comparison with nonmalignant tissues and in addition, OR value of CDH1 promoter methylation is Asian population subgroup (OR 13.2) was higher than in Caucasian population subgroup (OR 3.84), suggesting the ethnicity difference [52]. The wound assay in Fig. 3 demonstrated that SsaI could reduce the migration of a clear cell type EOC cell line. Global distribution of EOC subtypes seems to be different between Orientals and Caucasian population. A significantly high percentage of endometriosis-associated EOC, which includes clear cell type and endometrioid type, is found in Japan and Taiwan [2,53–57]. Here, increased E-cadherin because of the inhibition of α2,3-sialylation by SsaI might be the reason for the decreased metastasis and invasion in clear cell subtype EOC. The cross-talk between E-cadherin and α2,3- sialylation in clear cell type EOC might be a new therapeutic target for advanced EOC.

The strength of our study is that we assessed the poor prognostic role of α2,3- sialylation in EOC and found that it could serve as a biomarker. In addition, we used clear-cell cell line to investigate the role of α2,3- sialylation on the behavior of EOC, and this subtype EOC is often associated with high percentage of Taiwanese population and also contributes to high possibility of chemotherapy, with subsequent therapeutic failure [58]. The limitation of our study is that the role of α-2,3-sialylation of glycoprotein N-glycans via ST3Gal IV remains unclear. In previous studies with a pancreatic cancer cell model [59,60], ST3Gal III was found to modify the sialylation pattern of E-cadherin and change the morphologic changes in cell–cell contacts with E-cadherin delocalization in ST3Gal III transfected clones, which suggested a possible alteration of the adhesive function in these cells and a contribution to their loss of cell–cell aggregation capacity and higher invasion. The interaction between E-cadherin and α2,3-sialylation in clear cell type EOC is worthy of further investigation.

In summary, α2,3-linked sialylation in clear cell type EOC may be a biomarker, and SsaI, which acts as an ST inhibitor, has a role in clear cell type EOC migration. Our results suggested that targeting α2,3-linked sialylation might be a potential avenue for women with clear-cell type EOC.

## Conflict of interest

The Authors declare that there are no conflicts of interest.

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