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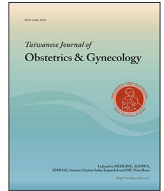
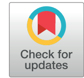


Original Article

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



Pi-Lin Sung [a, b, c], Kuo-Chang Wen [a, b, c], Huann-Cheng Horng [a, b, d], Chia-Ming Chang [a, b], Yi-Jen Chen [a, b, c], Wen-Ling Lee [e, f, **], Peng-Hui Wang [a, b, c, g, *]

[a] *Department of Obstetrics and Gynecology, Taipei Veterans General Hospital, Taipei, Taiwan*
[b] *Institute of Clinical Medicine, National Yang-Ming University School of Medicine, Taipei, Taiwan*
[c] *Department of Obstetrics and Gynecology, National Yang-Ming University, Taipei, Taiwan*
[d] *Institute of BioMedical Informatics, National Yang-Ming University, Taipei, Taiwan*
[e] *Department of Medicine, Cheng-Hsin General Hospital, Taipei, Taiwan*
[f] *Department of Nursing, Oriental Institute of Technology, New Taipei City, Taiwan*
[g] *Department of Medical Research, China Medical University Hospital, Taichung, Taiwan*

A R T I C L E I N F O

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A B S T R A C T

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.
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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-

* Corresponding author. Department of Obstetrics and Gynecology, National Yang-Ming University, Taipei Veterans General Hospital, 201, Section 2, Shih-Pai Road, Taipei, 112, Taiwan. Fax: +886 2 5570 2788.
** Corresponding author. Department of Medicine, Cheng-Hsin General Hospital, 45 Cheng-Hsin Street, Taipei, 112, Taiwan.
E-mail addresses: johnweiwang@gmail.com (W.-L. Lee), phwang@vghtpe.gov.tw, phwang@ym.edu.tw, pongpongwang@gmail.com (P.-H. Wang).

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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 upregulation 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 nonspecific 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 (2×10^6) 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 6×10^5 cells/well and incubated overnight for culturing

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