Downregulating SynCAM and MPP6 expression is associated with ovarian cancer progression

FEIXUE XU[1*], XIAOQIANG SI[2*], JINGRAN DU[1], FEIHUA XU[3], AIHONG YANG[1], CAIXIA ZHANG[1], XIUCAI ZHANG[1] and YONGXIU YANG[1]

[1]Department of Gynecology, The First Hospital of Lanzhou University; [2]Department of Plastic Surgery, The Gansu Provincial Hospital; [3]Department of Labor and Environmental Health, The School of Public Health of Lanzhou University, Lanzhou, Gansu 730000, P.R. China

Received July 30, 2018; Accepted May 17, 2019

DOI: 10.3892/ol.2019.10542

Abstract. Synaptic cell adhesion molecules (SynCAMs) are single transmembrane proteins that belong to the immunoglobulin superfamily of cell adhesion molecules. In the present study, a decrease in SynCAM levels in ovarian tumor tissues compared with normal tissues is reported; the downregulation was accompanied by the grade malignancy. The observations suggested that SynCAM may be essential for important novel functions in ovarian cancer. Further experiments showed that low SynCAM expression inhibited membrane palmitoylated protein 6 (MPP6) expression, a member of the palmitoylated membrane protein subfamily of peripheral membrane-associated guanylate kinases. In addition, low levels of MPP6 in ovarian tumor tissues correlated with shorter patient survival. A SynCAM-regulated pathway may provide molecular targets for the treatment of ovarian cancer and novel biomarkers to be used in clinical diagnosis.

Introduction

Ovarian cancer (OC) is the most lethal gynecological malignancy (1). Surgical resection remains the primary option for patients with OC (2). High mortality rates (5-year survival rate, ~30%) of OC are based on late diagnosis, distant metastasis within 5 years after surgical treatment and chemotherapeutic resistance (3,4). Patients with different pathological stages of OC remain a considerable prognostic challenge (5). In a clinical setting, same stage tumors can lead to different outcomes (6). While mucin 16, cell surface associated and WAP four-disulfide core domain 2 levels exhibited great potential in the detection of high-grade serous ovarian carcinoma at later stages, the levels are not sensitive enough for early stage detection of this disease (7). Consequently, further understanding of the molecular mechanisms associated with the progression of OC is required.

Various cell adhesion molecules are located at synapses, but only few are considered synaptic cell adhesion molecules (8). Synaptic cell adhesion molecules (SynCAMs/CADMs) are a subfamily of the immunoglobulin superfamily of cell adhesion molecules (8). SynCAMs are single transmembrane proteins that were discovered in the central nervous system, due to their ability to induce synapse formation (9,10). SynCAMs are true synaptic cell adhesion molecules and are crucial for synapse formation and plasticity (11,12). SynCAM in the cytoplasmic domain contains the binding motifs that connect to actin fibers (8). SynCAMs are involved in synapse formation, neuronal connectivity, myelination and cerebellum morphogenesis (13-15). Furthermore, it was suggested that SynCAMs may contribute to autism spectrum disorder (16), glioma generation, non-small cell lung cancer and hepatocarcinogenesis (17,18). However, the prognostic importance of SynCAM expression and the associated underlying mechanisms has not fully been elucidated.

A biomarker can be defined as any measurable characteristic that provides an indication of the biological state of a patient (19). In the present study, SynCAM expression was investigated in 74 patients with OC using immunohistochemistry. In addition, membrane palmitoylated protein 6 (MPP6), a member of the palmitoylated membrane protein subfamily of the peripheral membrane-associated guanylate kinases (MAGUK), levels were investigated in OC cells.

Materials and methods

Patients and tissue samples. The inclusion criteria were as follows: i) Patients agreed to surgical resection or chemotherapy; and ii) the all ovarian tumor was primary. The exclusion criteria were as follows: i) Patients that had refused any surgical

Correspondence to: Professor Feixue Xu or Professor Yongxiu Yang, Department of Gynecology, The First Hospital of Lanzhou University, 1 Donggang West Road, Lanzhou, Gansu 730000, P.R. China
E-mail: xfx.sxq@163.com
E-mail: yongxiuyang@163.com

*Contributed equally

Key words: synaptic cell adhesion molecules, ovarian cancer, membrane palmitoylated protein 6 MPP6, biomarker, molecular targets

resection or chemotherapy; ii) ovarian metastatic tumor; and iii) there was no sufficient tissue specimen for the immunohistochemical and western blot analyses. A total of 74 OC tissue specimens were derived from patients in The First Hospital of Lanzhou University (Lanzhou, China) between January 2011 and December 2012. None of the patients with OC had received chemo or radiotherapy prior to surgery. All patients underwent radical resection and were regularly followed up. The median age was 53 years (range, 17-75 years). The median follow-up time was 33 months (range, 6-60 months). The study was approved by the Ethics Committee of the First Hospital of Lanzhou University (Lanzhou, China) and written informed consent was obtained from the patients or their families. Borderline ovarian tumors (BOT; n=24) and benign ovarian tumor tissues (BEOT; n=34) were obtained from patients with ovarian tumors. Parts of the tumor specimens were frozen in liquid nitrogen after collection and stored at -80˚C until use and other parts were formalin-fixed for 1 week at room temperature and paraffin-embedded.

Immunohistochemical staining. The specimens were cut into 4 μm sections. The deparaffinized specimens were washed twice with distilled water, 5 min at a time. Citrate buffer (pH 6.0) was used in antigen retrieval at 121˚C for 20 min. After retrieval, the tissue sections were cooled for 20 min at room temperature. Finally, 0.01 mol/l PBS buffer was used to wash the sections twice for 5 min each time, and distilled water was then used to wash the sections three times for 3 min each time. To block endogenous peroxidase activity, 3% hydrogen peroxide was used for 15 min at 37˚C. The slides were blocked with 10% normal goat serum (OriGene Technologies, Inc.) in PBS for 30 min at room temperature, and further incubated with rabbit anti-human primary monoclonal antibodies against SynCAM (1:500; cat. no. GR3184359-5; Abcam) and MPP6 (1:500; cat. no. 5324; Signalway Antibody LLC) at 4˚C overnight. The following day the slides were incubated with ultraView universal HRP Multimer secondary antibody (1:1,000; cat. no. TA130015; anti-rabbit IgG Detection System; OriGene Technologies, Inc.) for 30 min at 37˚C to assess protein expression, according to the manufacturer's protocol. Hematoxylin was used to stain cell nuclei for 1-2 min at 37˚C. PBS was used as a negative control.

The tissue sections were assessed under a light microscope (Olympus Corporation) at x10 magnification using the Allred scoring system (20). Brown-yellow staining was considered as positive protein expression. For the semi-quantitative evaluation of protein levels in the tissues, an immunoreactivity-scoring system was used as previously described (21). The staining intensity was graded (0, no stain; 1+, weak stain; 2+, moderate stain; and 3+, strong stain). High expression of SynCAM and MPP6 was defined as detectable immunoreactions in cytoplasm and membranes with scores ≥2.

Western blot analysis. Ovarian tumor tissues were homogenized in cold NP40 buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 1% NP-40; Beyotime Institute of Biotechnology), sonicated (20 KHz, 300 W, 5 min) on ice and lysates were centrifuged at 10,000 x g for 15 min at 4˚C to collect the supernatant. The protein concentration was determined using a bicinchoninic acid assay (Beijing Solarbio Science & Technology Co., Ltd.). A total of 30 μg of protein per lane were separated on 10% SDS-PAGE gels and transferred to polyvinylidene fluoride membranes (OriGene Technologies, Inc.). Membranes were incubated with primary antibodies for SynCAM (dilution, 1:1,000; cat. no. GR3184359-5; Abcam), MPP6 (dilution, 1:1,000; cat. no. 5324; Signalway Antibody LLC) and β-actin (dilution, 1:5,000; cat. no. 14395-1-AP; Proteintech Group, Inc.) for 24 h at 4˚C following a blocking step with 5% fat-free milk in TBST for 2 h at room temperature. Membranes were then incubated with secondary goat anti-rabbit IgG H&L antibody (1:1,000; cat. no. TA100015; OriGene Technologies, Inc.) for 1 h at room temperature. Immunoreactive bands were visualized using enhanced chemiluminescence (Thermo Fisher Scientific, Inc.). Densitometry of the bands was performed using Quantity One software version 4.5.5 (Bio-Rad Laboratories, Inc.).

Statistical analysis. All data are presented as the mean ± standard deviation. Student's t-test was used for statistical analysis between two groups. $P<0.05$ considered to indicate a statistically significant difference. Positive expression rates and clinicopathological factors in the low and high SynCAM expression patients were compared using a χ^2 test with a threshold value of $P<0.05$. Survival curves were plotted using the Kaplan-Meier method and were compared using the log-rank test. One-way ANOVA followed by Tukey post-hoc test were used to analysis of variance between groups. All statistical analyses were performed using the SPSS statistical software package (version 19.0; IBM, Corp.).

Results

Clinicopathological characteristics. Demographic, clinical and histopathological variables are presented in Table I. The current study included 74 patients with OC. The median age was 53 years (range, 17-75 years) and the cohort comprised 16 (21.62%) cases diagnosed at T1 and 12 (16.22%), 40 (54.05%) and 6 (8.11%) cases diagnosed at stages T2, T3 and T4 (TNM staging system) (22), respectively. The median follow-up time was 33 months (range, 6-60 months).

Association between SynCAM expression and clinicopathological characteristics. In the present study, immunohistochemical analysis of 132 human ovarian tumor specimens was used evaluate SynCAM protein expression. SynCAMs exhibited cytolymph or cytoplasmic expression in ovarian tumor specimen. Representative images demonstrating SynCAM expression by staining BEOT, BOT and OC specimen were indicated (Fig. 1). Increased SynCAM expression was detected in BEOT (Fig. 1A) and BOT (Fig. 1B) compared with OC tissues (Fig. 1C). A total of 28/34 BEOT specimens exhibited positive SynCAM expression, representing a positive expression rate of 82.35% and 19/24 BOT cases exhibited positive SynCAM expression, representing a positive expression rate of 79.17%. Out of 74 OC specimen only 30 cases exhibited positive SynCAM expression, representing a positive expression rate of 40.54%. The majority of OC tissues exhibited no evidence for SynCAM staining. The positive expression rate was significantly increased in BEOT and BOT compared with OC ($P<0.0001$; Fig. 1D). The results

Table I. Association between SynCAM expression and clinicopathological characteristics in patients with ovarian cancer.

Variables	Number (%)	SynCAM expression (n=74)		P-value
		Low expression	High expression	
Age (years)				0.7886
<60	48 (64.86)	28	20	
≥60	26 (35.14)	16	10	
Tumor size (cm)				0.0308[a]
<8	50 (67.60)	34	16	
≥8	24 (32.40)	10	14	
T stage				0.1772
T1	16 (21.62)	10	6	
T2	12 (16.22)	10	2	
T3	40 (54.05)	22	18	
T4	6 (8.11)	2	4	
N stage				0.2509
N0	50 (67.57)	32	18	
N1	24 (32.43)	12	12	
M stage				0.5639
M0	66 (89.19)	40	26	
M1	8 (10.81)	4	4	
Differentiation level of tumor cells				<0.0001[a]
Low	18 (24.32)	2	16	
High	56 (75.68)	42	14	

[a]P<0.01. SynCAM, synaptic cell adhesion molecules; T, tumor; N, node; M, metastasis.

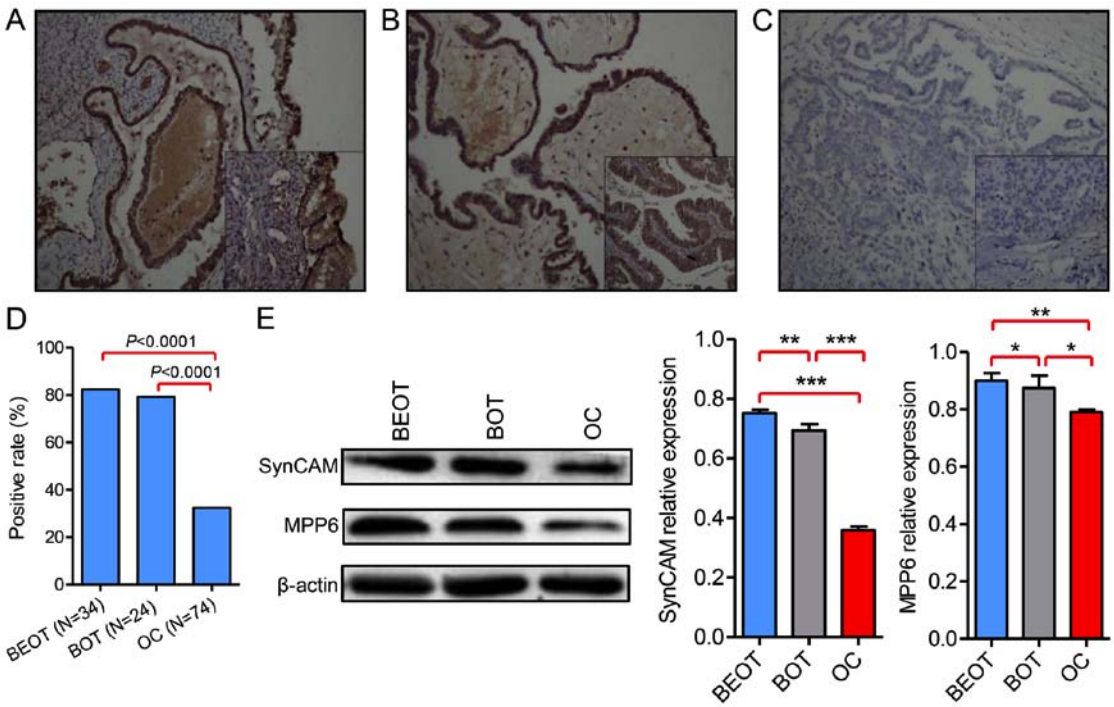

Figure 1. SynCAM expression in ovarian tumor tissues. Representative images of SynCAM expression in (A) BEOT, (B) BOT and (C) OC specimen using immunohistochemical analysis (magnification, x200 and x400 for inset images). SynCAM exhibited cytoplasmic expression. (D) Positive expression rates of SynCAM were significantly decreased in OC compared with BEOT and BOT specimen. (E) The protein expression level of SynCAM was highest in BEOT specimens and significantly decreased in BOT and OC specimen; MPP6 expression was significantly downregulated in OC compared with BEOT and BOT and accompanied by the grade malignancy. *P<0.05, **P<0.01 and ***P<0.001. BEOT, benign ovarian tumor; BOT, borderline ovarian tumors; OC, ovarian cancer; SynCAM, synaptic cell adhesion molecules; MPP6, membrane palmitoylated protein 6.

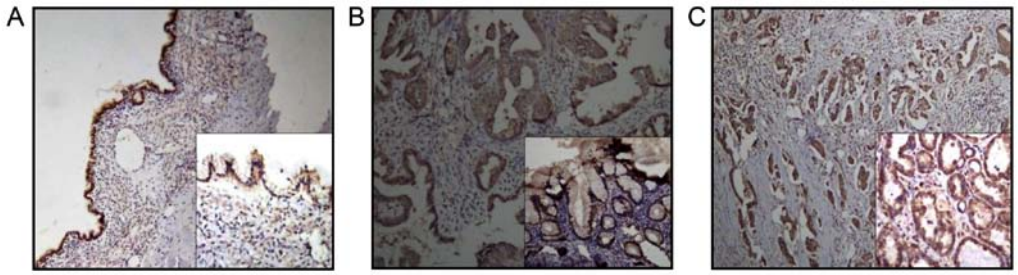

Figure 2. MPP6 expression in ovarian tumor tissues. MPP6 expression in ovarian tumor specimens of (A) BEOT, (B) BOT and (C) OC (magnification, x200 and x400 for inset images). BEOT, benign ovarian tumor; BOT, borderline ovarian tumors; OC, ovarian cancer; MPP6, membrane palmitoylated protein 6.

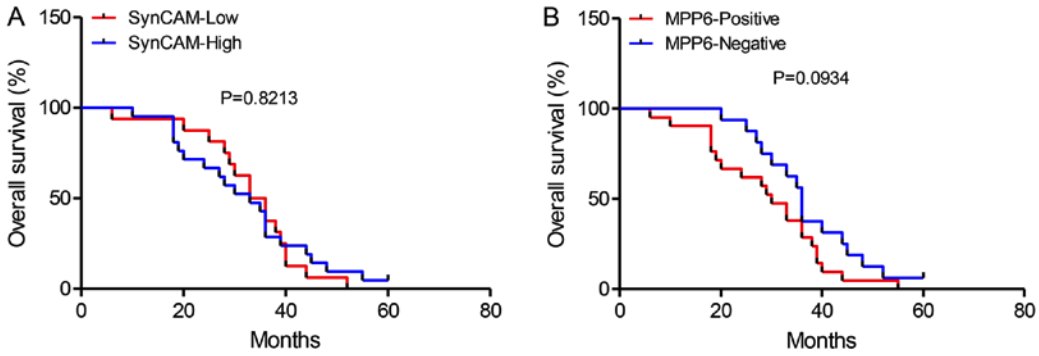

Figure 3. Overall survival time curves of patients with ovarian cancer. Kaplan-Meier analysis according to (A) SynCAM and (B) MPP6 expression. SynCAM, synaptic cell adhesion molecules; MPP6, membrane palmitoylated protein 6.

suggested that SynCAM downregulation occurs in human ovarian tumor tissues. To further investigate the expression pattern of SynCAM in ovarian tumor tissues, SynCAM expression was examined by western blot analysis. The results showed that SynCAM levels were significantly decreased in OC and BOT compared with BEOT (P<0.001; Fig. 1E). Results demonstrated that SynCAM expression was decreased in all ovarian tumor tissues compared with normal tissues (Fig. 1E). The data suggested that SynCAM may function as a tumor suppressor in ovarian tumor tissues. SynCAM expression and the clinicopathological characteristics are presented in Table I. SynCAM expression was correlated with the tumor diameter (P=0.0308) and differentiation level of tumor cells (P<0.0001).

MPP6 protein expression is downregulated in OC. Immunohistochemical analysis was used to evaluate MPP6 protein expression in ovarian tumor tissues. The MPP family belongs to the MAGUK family. MPP6 exhibited cytomembrane and cytoplasm expression in ovarian tumor tissues (Fig. 2). Fig. 2A-C are representative microphotographs of immunostained tissue sections of BEOT, BOT and OC, respectively, highlighting MPP6 staining. MPP6 expression in ovarian tumor samples was further determined by western blot analysis. The results suggested that MPP6 expression was significantly downregulated in OC compared with BEOT and BOT specimen (P<0.01 and P<0.05, respectively; Fig. 1E).

Association between survival and expression of SynCAM and MPP6. SynCAM expression was not associated with overall survival (OS; n=74; P=0.8213; Fig. 3A). MPP6 expression was associated with poorer OS time; however not significantly (n=74; P=0.0934; Fig. 3B).

Discussion

Metastasis, recurrence and chemotherapeutic resistance occur in patients with OC following radical resection are leading causes of mortality worldwide (23,24). SynCAMs, members of the immunoglobulin superfamily, encode for membrane glycoproteins and participate in cell adhesion (25). SynCAMs associate with different intracellular binding partners, including proteins of the MAGUK family (26,27). SynCAMs act as tumor suppressors in various types of human cancer including lung, prostate, pancreas and breast cancer, and are preferentially inactivated in invasive cancer (28,29). At present, to the best of our knowledge, little is known about the functional role of SynCAMs in OC. This study investigated the potential role of SynCAMs as tumor suppressors in OC.

SynCAMs are tumor suppressors in non-small cell lung cancer, hepatic cell carcinoma and pancreatic cancer (30,31). To the best of our knowledge, SynCAM expression in OC has not yet been reported. In the present study, it was demonstrated that SynCAM expression was downregulated in human OC tumor tissues compared with normal tissues, using immunohistochemical and western blot analyses. The majority of paraffin-embedded human OC tissues exhibited no evidence for SynCAM staining. The loss of SynCAM expression was correlated with increased tumor size and differentiation levels of tumor cells. SynCAM was expressed at higher levels in normal ovarian tissue compared with OC tissue. These findings demonstrated that SynCAMs may serve a tumor suppressive

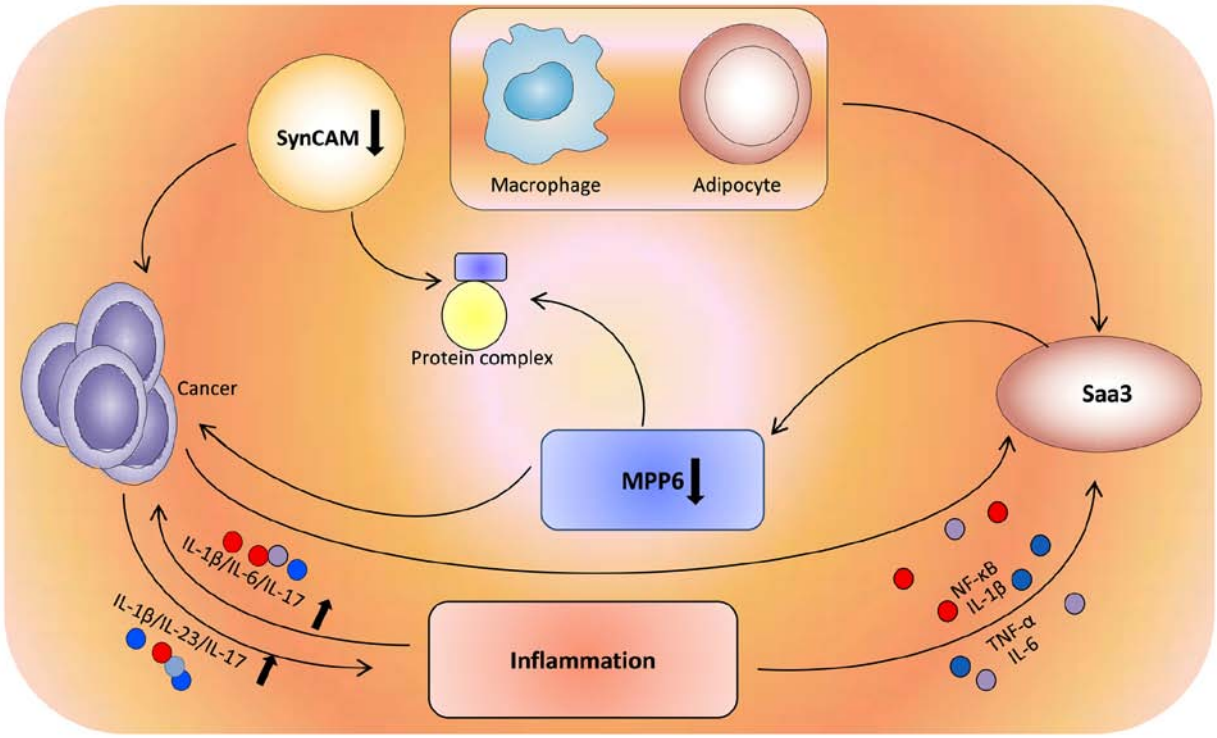

Figure 4. Schematic representation of the mechanism of SynCAM and MPP6 expression downregulation and its association with ovarian cancer progression. SynCAM, synaptic cell adhesion molecules; MPP6, membrane palmitoylated protein 6.

role in human OC. It is suggested that OC cell proliferation may be affected by downregulating SynCAM expression. Loss of SynCAMs expression is observed in non-small cell lung cancer, and could cause morphological transformation leading cancer cells to invasion and/or metastasis (28). The results of the present study are consistent with this finding.

Downregulation of SynCAM expression may further be associated with silenced methylation (32). SynCAM mRNA levels were described to be increased after demethylation in glioblastoma cell lines (32). However, the mechanism of SynCAM inactivation by promoter hypermethylation remains to be elucidated. MPP6, a member of the palmitoylated membrane protein subfamily of peripheral MAGUK, is primarily involved in controlling epithelial cell polarity (33). MPPs further function in tumor suppression and receptor clustering by forming multiprotein complexes containing distinct sets of transmembrane, cytoskeletal and cytoplasmic signaling proteins (34). A direct association between tumor suppression and cell polarity proteins is equivocal in mammals (35). Disruption of cell polarity and function causes abnormalities in vertebrates (35). Similar to E-cadherin, MPP loss can disturb cell-cell junctions and the mechanical integrity of epithelial cells, resulting in defective branching morphogenesis and maintenance of epithelial tubular structures (36-38). Saa3 is a member of the acute-phase serum amyloid A (SAA) apolipoprotein family. Murine Saa3 has been shown to be expressed in macrophages (39) and adipose tissue (40). Accumulation of SAA proteins in the blood is observed during chronic inflammation and cancer (39). Saa3 expression is effectively induced by interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and IL-6 through NF-κB signaling in inflammatory processes (41). Inflammatory diseases greatly increase the risk of cancer, due to elevated expression of inflammatory cytokines, including IL-6, TNF, and IL-1β (42). Stimulating IL-23 and IL-17 production can promote cancer development and progression (42). MPPs can interact with SynCAM and form a protein complex (28). The protumorigenic activity of Saa3 can be regulated by MPP6. MPP6 overexpression is responsible for the loss of the protumorigenic effect of Saa3 in cancer-associated fibroblasts (41) (Fig. 4). In the present study, MPP6 immunolocalization was examined in OC tissues. MPP6 exhibited cytomembrane and cytoplasm staining and expression was significantly downregulated in ovarian tumor tissues compared with normal tissues. SynCAM and MPP6 expression may be used as prognostic factors to predict OS for individual patients with OC. However, SynCAM was determined not to be a prognostic factor and MPP6 expression was not significantly associated with worse OS, which may be due to the small sample size of OC in the present study. Further experiments are necessary to elucidate the potential association between SynCAM and MPP6.

In summary, the data suggested that human OC cells proliferate through downregulation of SynCAM expression. It is suggested that decreased expression of SynCAM was associated with downregulation of the tight junction protein MPP6. Therefore, a SynCAM-regulated pathway may be an important molecular target in the treatment of ovarian cancer and the associated biomarkers may be used in a diagnostic clinical setting.

Acknowledgements

The authors would like to thank the research assistance provided by Gansu Key Laboratory of Gynecologic Oncology (Lanzhou, China).

Funding

The present study was supported by the Talent Training Plan of Gansu Province.

Availability of data and materials

The datasets used or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

FXX and YY conceived and designed the study. Data collection and experiments were performed by FXX, XS, JD and FHX. AY, CZ and XZ analyzed the data. FX and YY wrote the manuscript. All authors have read and approved of the final version of the manuscript.

Ethics approval and consent to participate

The research program used in the study was approved by the Ethics Committee of the First Hospital of Lanzhou University (Lanzhou, China), and written informed consent was obtained from patients or patients' family.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2017. CA Cancer J Clin 67: 7-30, 2017.
2. Jin M, Cai J, Wang X, Zhang T and Zhao Y: Successful maintenance therapy with apatinib inplatinum-resistant advanced ovarian cancer and literature review. Cancer Biol Ther 19: 1088-1092, 2018.
3. Tang G, Guo J, Zhu Y, Huang Z, Liu T, Cai J, Yu L and Wang Z: Metformin inhibits ovarian cancer via decreasing h3k27 trimethylation. Int J Oncol 52: 1899-1911, 2018.
4. Cannistra SA: Cancer of the ovary. N Engl J Med 351: 2519-2529, 2004.
5. Winter WE III, Maxwell GL, Tian C, Carlson JW, Ozols RF, Rose PG, Markman M, Armstrong DK, Muggia F and McGuire WP; Gynecologic Oncology Group Study: Prognostic factors for stage III epithelial ovarian cancer: A Gynecologic Oncology Group Study. J Clin Oncol 25: 3621-3627, 2007.
6. Zhuo C, Wu X, Li J, Hu D, Jian J, Chen C, Zheng X and Yang C: Chemokine (C-X-C motif) ligand 1 is associated with tumor progression and poor prognosis in patients with colorectal cancer. Biosci Rep 38: BSR20180580, 2018.
7. Áyen Á, Jiménez Martínez Y Marchal JA and Boulaiz H: Recent progress in gene therapy for ovarian cancer. Int J Mol Sci 19: E1930, 2018.
8. Takai Y, Ikeda W, Ogita H and Rikitake Y: The immunoglobulin-like cell adhesion molecule nectin and its associated protein afadin. Annu Rev Cell Dev Biol 24: 309-342, 2008.
9. Biederer T, Sara Y, Mozhayeva M, Atasoy D, Liu X, Kavalali ET and Südhof TC: SynCAM, a synaptic adhesion molecule that drives synapse assembly. Science 297: 1525-1531, 2002.
10. Gomyo H, Arai Y, Tanigami A, Murakami Y, Hattori M, Hosoda F, Arai K, Aikawa Y, Tsuda H, Hirohashi S, *et al*: A 2-Mb sequenceready contig map and a novel immunoglobulin superfamily gene IGSF4 in the LOH region of chromosome 11q23.2. Genomics 62: 139-146, 1999.
11. Frei JA and Stoeckli ET: SynCAMs-From axon guidance to neurodevelopmental disorders. Mol Cell Neurosci 81: 41-48, 2017.
12. Frei JA, Andermatt I, Gesemann M and Stoeckli ET: The SynCAM synaptic cell adhesion molecules are involved in sensory axon pathfinding by regulating axon-axon contacts. J Cell Sci 127: 5288-5302, 2014.
13. Fowler DK, Peters JH, Williams C and Washbourne P: Redundant postsynaptic functions of SynCAMs 1-3 during synapse formation. Front Mol Neurosci 10: 24, 2017.
14. Stagi M, Fogel AI and Biederer T: SynCAM 1 participates in axo-dendritic contact assembly and shapes neuronal growth cones. Proc Natl Acad Sci USA 107: 7568-7573, 2010.
15. Maurel P, Einheber S, Galinska J, Thaker P, Lam I, Rubin MB, Scherer SS, Murakami Y, Gutmann DH and Salzer JL: Nectin-like proteins mediate axon Schwann cell interactions along the internode and are essential for myelination. J Cell Biol 178: 861-874, 2007.
16. Casey JP, Magalhaes T, Conroy JM, Regan R, Shah N, Anney R, Shields DC, Abrahams BS, Almeida J, Bacchelli E, *et al*: A novel approach of homozygous haplotype sharing identifies candidate genes in autism spectrum disorder. Hum Genet 131: 565-579, 2012.
17. Du H, Li W, Wang Y, Chen S and Zhang Y: Celecoxib induces cell apoptosis coupled with up-regulation of the expression of VEGF by a mechanism involving ER stress in human colorectal cancer cells. Oncol Rep 26: 495-502, 2011.
18. Wakayama Y, Inoue M, Kojima H, Murahashi M, Shibuya S and Oniki H: Localization of sarcoglycan, neuronal nitric oxide synthase, beta-dystroglycan, and dystrophin molecules in normal skeletal myofiber: Triple immunogold labeling electron microscopy. Microsc Res Tech 55: 154-163, 2001.
19. Ahmadzada T, Kao S, Reid G, Boyer M, Mahar A and Cooper WA: An update on predictive biomarkers for treatment selection in non-small cell lung cancer. J Clin Med 7: E153, 2018.
20. Liang C, Wang S, Qin C, Bao M, Cheng G, Liu B, Shao P, Lv Q, Song N, Hua L, *et al*: TRIM36, a novel androgen-responsive gene, enhances anti-androgen efficacy against prostatecancer by inhibiting MAPK/ERK signaling pathways. Cell Death Dis 9: 155, 2018.
21. Liu D, Zhang XX, Li MC, Cao CH, Wan DY, Xi BX, Tan JH, Wang J, Yang ZY, Feng XX, *et al*: C/EBPβ enhances platinum resistance of ovarian cancer cells by reprogramming H3K79 methylation. Nat Commun 9: 1739, 2018.
22. Webber C, Gospodarowicz M, Sobin LH, Wittekind C, Greene FL, Mason MD, Compton C, Brierley J and Groome PA: Improving the TNM classification: Findings from a 10-year continuous literature review. Int J Cancer 135: 371-378, 2014.
23. Gao Y, Foster R, Yang X, Feng Y, Shen JK, Mankin HJ, Hornicek FJ, Amiji MM and Duan Z: Up-regulation of CD44 in the development of metastasis, recurrence and drug resistance of ovarian cancer. Oncotarget 6: 9313-9326, 2015.
24. Vaughan S, Coward JI, Bast RC Jr, Berchuck A, Berek JS, Brenton JD, Coukos G, Crum CC, Drapkin R, Etemadmoghadam D, *et al*: Rethinking ovarian cancer: Recommendations for improving outcomes. Nat Rev Cancer 11: 719-725, 2011.
25. Galuska SP, Rollenhagen M, Kaup M, Eggers K, Oltmann-Norden I, Schiff M, Hartmann M, Weinhold B, Hildebrandt H, Geyer R, *et al*: Synaptic cell adhesion molecule SynCAM 1 is a target for polysialylation in postnatal mouse brain. Proc Natl Acad Sci USA 107: 10250-10255, 2010.
26. Frei JA and Stoeckli ET: SynCAMs extend their functions beyond the synapse. Eur J Neurosci 39: 1752-1760, 2014.
27. Cheadle L and Biederer T: The novel synaptogenic protein Farp1 links postsynaptic cytoskeletal dynamics and transsynaptic organization. J Cell Biol 199: 985-1001, 2012.
28. Sakurai-Yageta M, Masuda M, Tsuboi Y, Ito A and Murakami Y: Tumor suppressor CADM1 is involved in epithelial cell structure. Biochem Biophys Res Commun 390: 977-982, 2009.
29. Kuramochi M, Fukuhara H, Nobukuni T, Kanbe T, Maruyama T, Ghosh HP, Pletcher M, Isomura M, Onizuka M, Kitamura T, *et al*: TSLC1 is a tumor-suppressor gene in human non-small-cell lung cancer. Nat Genet 27: 427-430, 2001.
30. Watabe K, Ito A, Koma YI and Kitamura Y: IGSF4: A new intercellular adhesion molecule that is called by three names, TSLC1, SgIGSF and SynCAM, by virtue of its diverse function. Histol Histopathol 18: 1321-1329, 2003.
31. Tsujiuchi T, Sugata E, Masaoka T, Onishi M, Fujii H, Shimizu K and Honoki K: Expression and DNA methylation patterns of Tslc1 and Dal-1 genes in hepatocellular carcinomas induced by N-nitrosodiethylamine in rats. Cancer Sci 98: 943-948, 2007.

32. Zhang X, Li W, Kang Y, Zhang J and Yuan H: SynCAM, a novel putative tumor suppressor, suppresses growth and invasiveness of glioblastoma. Mol Biol Rep 40: 5469-5475, 2013.
33. Quinn BJ, Welch EJ, Kim AC, Lokuta MA, Huttenlocher A, Khan AA, Kuchay SM and Chishti AH: Erythrocyte scaffolding protein p55/MPP1 functions as an essential regulator of neutrophil polarity. Proc Natl Acad Sci USA 106: 19842-19847, 2009.
34. Tseng TC, Marfatia SM, Bryant PJ, Pack S, Zhuang Z, O'Brien JE, Lin L, Hanada T and Chishti AH: VAM-1: A new member of the MAGUK family binds to human Veli-1 through a conserved domain. Biochim Biophys Acta 1518: 249-259, 2001.
35. Saito Y, Desai RR and Muthuswamy SK: Reinterpreting polarity and cancer: The changing landscape from tumor suppression to tumor promotion. Biochim Biophys Acta Rev Cancer 1869: 103-116, 2018.
36. Mahoney ZX, Sammut B, Xavier RJ, Cunningham J, Go G, Brim KL, Stappenbeck TS, Miner JH and Swat W: Discs-large homolog 1 regulates smooth muscle orientation in the mouse ureter. Proc Natl Acad Sci USA 103: 19872-19877, 2006.
37. Nechiporuk T, Fernandez TE and Vasioukhin V: Failure of epithelial tube maintenance causes hydrocephalus and renal cysts in Dlg5-/- mice. Dev Cell 13: 338-350, 2007.
38. Schneider MR, Dahlhoff M, Horst D, Hirschi B, Trülzsch K, Müller-Hücker J, Vogelmann R, Allgäuer M, Gerhard M, Steininger S, *et al*: A key role for E-cadherin in intestinal homeostasis and Paneth cell maturation. PLoS One 5: e14325, 2010.
39. Ather JL, Ckless K, Martin R, Foley KL, Suratt BT, Boyson JE, Fitzgerald KA, Flavell RA, Eisenbarth SC and Poynter ME: Serum amyloid A activates the NLRP3 inflammasome and promotes Th17 allergic asthma in mice. J Immunol 187: 64-73, 2011.
40. Sommer G, Weise S, Kralisch S, Scherer PE, Lössner U, Blüher M, Stumvoll M and Fasshauer M: The adipokine SAA3 is induced by interleukin-1beta in mouse adipocytes. J Cell Biochem 104: 2241-2247, 2008.
41. Djurec M, Graña O, Lee A, Troulé K, Espinet E, Cabras L, Navas C, Blasco MT, Martín-Díaz L, Burdiel M, *et al*: Saa3 is a key mediator of the protumorigenic properties of cancer-associated fibroblasts in pancreatic tumors. Proc Natl Acad Sci USA 115: E1147-E1156, 2018.
42. Zhong Z, Sanchez-Lopez E and Karin M: Autophagy, inflammation, and immunity: A troika governing cancer and its treatment. Cell 166: 288-298, 2016.