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Antisense lncRNA As-SLC7A11 suppresses epithelial ovarian cancer progression mainly by targeting SLC7A11

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Antisense lncRNAs play a key role in the progression of multiple cancers. Thus, it is important to elucidate the function and mechanism of antisense lncRNAs, which may play a role in the treatment of epithelial ovarian cancer (EOC). In the current study, for the first time, we showed that the level of As-SLC7A11 was markedly reduced in EOC cancer tissues and cell lines compared with those of normal control. Further study showed that silencing of As-SLC7A11 could enhance ovarian cancer cell migration. In comparison, overexpression of As-SLC7A11 markedly induced ovarian cancer cell apoptosis. These data demonstrate the tumor suppressor role of As-SLC7A11 in ovarian cancer malignancies. We also demonstrated that overexpression of As-SLC7A11 could significantly suppress the expression of SLC7A11, indicating a negative correlation between As-SLC7A11 and SLC7A11 in ovarian cancer cells. In summary, we first showed that reduction of As-SLC7A11 level prompted ovarian cancer cell migration mainly by suppressing the expression of SLC7A11.

1. Introduction

Ovarian cancer is one of the most prevalent gynecological cancers in the world (McGuire et al. 2002; Li et al. 2015). Among the ovarian malignancies, epithelial ovarian cancer (EOC) accounts for highest mortality rate (Gislefoss et al. 2015). Although the overall surgical technologies and adjuvant chemotherapy have made great progress, the 5-year survival rate for EOC patients is still at 30% (Seebacher et al. 2017). Therefore, it is of great importance to explore the molecular mechanisms leading to EOC and develop novel therapeutic strategies for the disease.

SLC7A11 is a cystine/glutamate transporter (Drayton et al. 2014). It maintains the glutathione status in cells through providing cysteine, which is a key rate-limiting amino acid for the antioxidant peptide synthesis (Huang et al. 2005). In multiple cancers, SLC7A11 is enhanced thereby suppressing oxidative damage and protecting the cancer cells from apoptosis (Liu et al. 2011; Galvan et al. 2016). In EOC, abnormal expression of SLC7A11 has been widely reported. However, whether SLC7A11 is regulated by long non-coding RNAs (lncRNAs) has never been explored.

LncRNAs are RNA molecules longer than 200 nucleotides without protein coding potential (Chen et al. 2016; Wu et al. 2016; Zhang et al. 2016). In the human gene, the total number of lncRNAs is greater than that of protein-coding genes. The wide involvement of lncRNAs was reported in various cellular biological functions, including cell division, apoptosis, autophagy (Chai et al. 2016; Zhou et al. 2016). According to the chromosomal locations, lncRNAs are divided into several groups: antisense, intronic, bidirectional, intergenic, and overlapping lncRNAs (Zhou et al. 2016). The antisense lncRNA is transcribed from the opposite direction either within the gene or at the end of its sense transcript. Genome-wide analysis indicates that over 20% of human genes are modulated by sense–antisense (S/AS) pairs (Alcid and Tsukiyama 2016; Zong et al. 2016). Then, to biological functions as well as the functioning mechanisms of antisense lncRNAs is of great clinical significance.

In the current study, we first identified an antisense lncRNA, SLC7A11, in EOC cancer tissues and cells. Our data indicated that reduction of As-SLC7A11 level prompted the malignancies of EOC mainly through suppressing the expression of SLC7A11, indicating a potential novel therapeutic target in EOC cancer cells.

2. Investigations and results

2.1. Decreased level of As-SLC7A11 in EOC cancer tissues and cell lines

We first explored the expression of As-SLC7A11 and found it significantly reduced in EOC cancer tissues compared with that of normal control (Fig. 1A). In contrast, the mRNA level of SLC7A11 was significantly increased in the EOC cancer tissues (Fig. 1B). Moreover, we found that the level of As-SLC7A11 was significantly reduced in OVCA433, OVCA429, and TOV112D cells than that of HOSE cells (Fig. 1C). Real time PCR analysis showed that the mRNA level of SLC7A11 was significantly increased in OVCA433, OVCA429, and TOV112D cells compared to that of HOSE cells (Fig. 1D).

2.2. Knockdown of As-SLC7A11 expression enhanced OVCA433 and OVCA429 cell migration and invasion

Then, we analyzed whether As-SLC7A11 could affect OVCA433 and OVCA429 cell migration and invasion. Firstly, we selected the specific siRNAs targeting As-SLC7A11 in OVCA433 and OVCA429 cells (Fig. 2A and 2B). As shown in Fig. 2C, inhibition of As-SLC7A11 significantly enhanced OVCA433 and OVCA429 cell migration. In addition, cell invasion capacity was also significantly enhanced in OVCA433 and OVCA429 cells transfected siRNA targeting SLC7A11 compared with that of negative control (Fig. 2D).

2.3. Overexpression of As-SLC7A11 induced ovarian cancer cell apoptosis

Next, we investigated whether As-SLC7A11 could induce ovarian cancer cell apoptosis. Transfection with Ad-As-SLC7A11 could markedly enhance the lncRNA level of As-SLC7A11 in OVCA433 ovarian cancer cells (Fig. 3A). Flow cytometry analysis indicated that upregulation of As-SLC7A11 significantly induced OVCA433

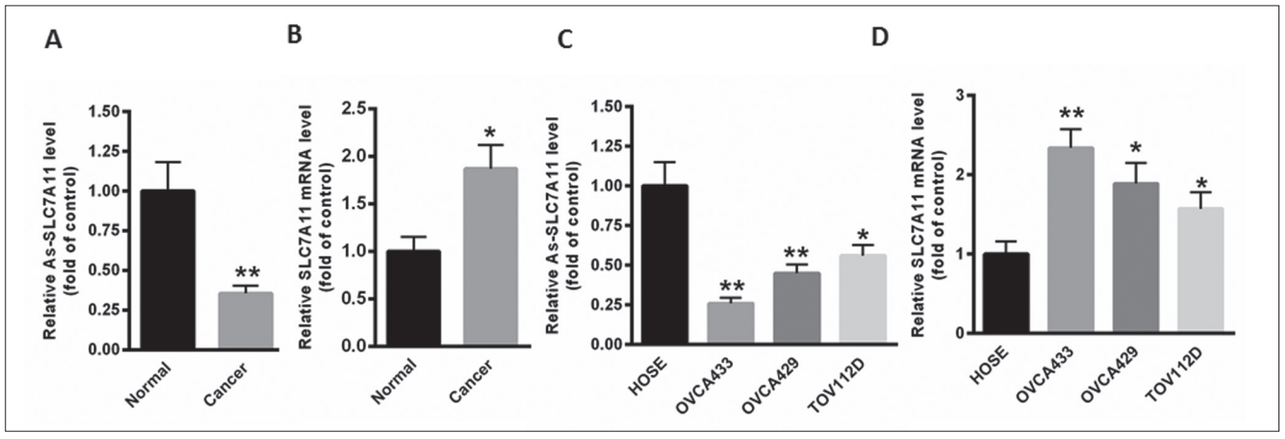

Fig. 1: LncRNA As-SLC7A11 was decreased in the EOC cancer tissues and cells compared with that of normal control. (A) Real time PCR analysis of the As-SLC7A11. (B) Real time PCR analysis of SLC7A11 mRNA in the EOC tissues. (C) The level of As-SLC7A11 was reduced in OVCA433, OVCA429, and TOV112D cells than that of HOSE cells. (D) Real time PCR analysis showed that the mRNA level of SLC7A11 was significantly increased OVCA433, OVCA429, and TOV112D cells than that of HOSE cells. *p<0.05, **p<0.01, vs. control.

cancer cell apoptosis (Fig. 3B). Furthermore, TUNEL staining showed increased numbers of apoptotic cells after transfection with Ad-As-SLC7A11 (Fig. 3C). These data indicated the tumor suppressor role of As-SLC7A11.

2.4. As-SLC7A11 could suppress the expression of SLC7A11 in ovarian cancer cells

Finally, we tried to explore the possible mechanism in which As-SLC7A11 is involved in the progression of EOC. As shown in Fig. 4A, upregulation of As-SLC7A11 significantly suppressed the expression of SLC7A11. In contrast, silencing of As-SLC7A11 markedly increased the level of SLC7A11 (Fig. 4A). Furthermore, we explored the role of SLC7A11 in OVCA433 cancer cell migration and apoptosis. We found that knockdown of SLC7A11 significantly suppressed cell migration in OVCA433 cancer cells (Fig. 4B), and silencing of SLC7A11 markedly induced cancer cell apoptosis (Fig. 4C), indicating an oncogenic role of SLC7A11 in ovarian cancer cells.

3. Discussion

Ovarian cancer is one of the most common cancer among females in the world (Jemal et al. 2011). Currently, the treatment outcome for ovarian cancer is still not satisfactory, especially considering advanced-stage tumors. It is indicated that tumor metastasis is the major reason of all cancer-related deaths (Gupta and Massague 2006). Therefore, to develop novel treatment methods for ovarian cancer is important.

As a cystine/glutamate transporter, SLC7A11 is involved in the production of reactive oxygen species (ROS) production within cancer cells (Galvan et al. 2016). Previous studies have shown oxidative stimuli could enhance the expression of SLC7A11 in cancer cells and increased SLC7A11 could eliminate ROS production thereby improving cancer cell viability and migration (Chang et al. 2016; Sehm et al. 2016)

LncRNAs are long non-coding RNA molecules that longer than 200 nucleotides and recent studies have shown the wide involvement of lncRNAs in various human diseases (Mizrahi et al. 2010; Zhu et al. 2015). During the progression of gynecological cancer, several lncRNAs have been suggested, including HOTAIR, MALAT-1, H19, and LSINCT5 (Silva et al. 2011; Wu et al. 2016; Zheng et al. 2016; Zhou et al. 2016). However, no studies have been performed regarding the possible role of lncRNAs in the regulation of SLC7A11.

In the current study, we first showed that the level of As-SLC7A11 was markedly reduced in EOC cancer tissues and cell lines compared with those of normal control. Further study showed that silencing of As-SLC7A11 could enhance ovarian cancer cell migration. In comparison, overexpression of As-SLC7A11 markedly induced ovarian cancer cell apoptosis. These data showed the tumor suppressor role of As-SLC7A11 in ovarian cancer malignancies. Further study demonstrated that overexpression of As-SLC7A11 could significantly suppress the expression of SLC7A11, demonstrating a negative correlation between As-SLC7A11 and SLC7A11 in ovarian cancer cells.

In summary, for the first time, we showed the expression of SLC7A11 was modulated by an antisense RNA, As-SLC7A11. During the progression of EOC, reduced As-SLC7A11 prompted ovarian cancer cell migration mainly by suppressing the expression of SLC7A11.

4. Experimental

4.1. Tissue samples

A total of 30 fresh ovarian cancer tissues and 30 non-cancerous ovarian tissues were collected from September 2014 to November 2015 in the Second Affiliated Hospital of Zhengzhou University, China. Written informed consent was obtained from all participants involved in this study. The study was performed in accordance with the Declaration of Helsinki and approved by the Second Affiliated Hospital of Zhengzhou University, China.

4.2. Cell culture

The human ovarian cancer cell lines OVCA433, OVCA429, and TOV112D were purchased from the Committee of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Normal human ovarian surface epithelial (HOSE) cell line was purchased from ScienCell Research Laboratories (San Diego, CA). All cell lines were cultured in Dulbecco's Modified Eagle's Medium supplemented with 10 % fetal bovine serum (FBS) (v/v), streptomycin (100 mg/ml) and penicillin (100 IU/ml) at 37 °C in a humidified atmosphere containing 5 % CO_2.

4.3. Transient transfection

Cells were seeded at 10^6 cells/well in the 6-well plates. Meanwhile, the siRNAs targeting As-SLC7A11 or negative control (Genepharma) were mixed with HiperFect transfection reagent (QIAGEN) and incubated at room temperature for 10 min. Then, the complex was added in to the culture medium for 48 h.

4.4. RNA extraction and real-time PCR

The total RNA from cultured cells was isolated with TriZol (Invitrogen) according to the manufacturer's instructions. The total RNA was reverse transcribed into complementary DNA (cDNA) with TaqMan RNA Reverse Transcription Kit (Applied Biosystems). A quantitative real-time PCR assay was performed using SYBR Green Supermix (Bio-Rad) in a BIO-RAD iCycleriQ real-time PCR detection system as previously described (Guo et al. 2014).

4.5. Protein extraction and Western blot analysis

Proteins samples were extracted in RIPA buffer (1% TritonX-100, 15 mmol/L NaCl, 5 mmol/L EDTA, and 1 0mmol/L Tris-HCl (pH 7.0) (Solarbio, China) supplemented

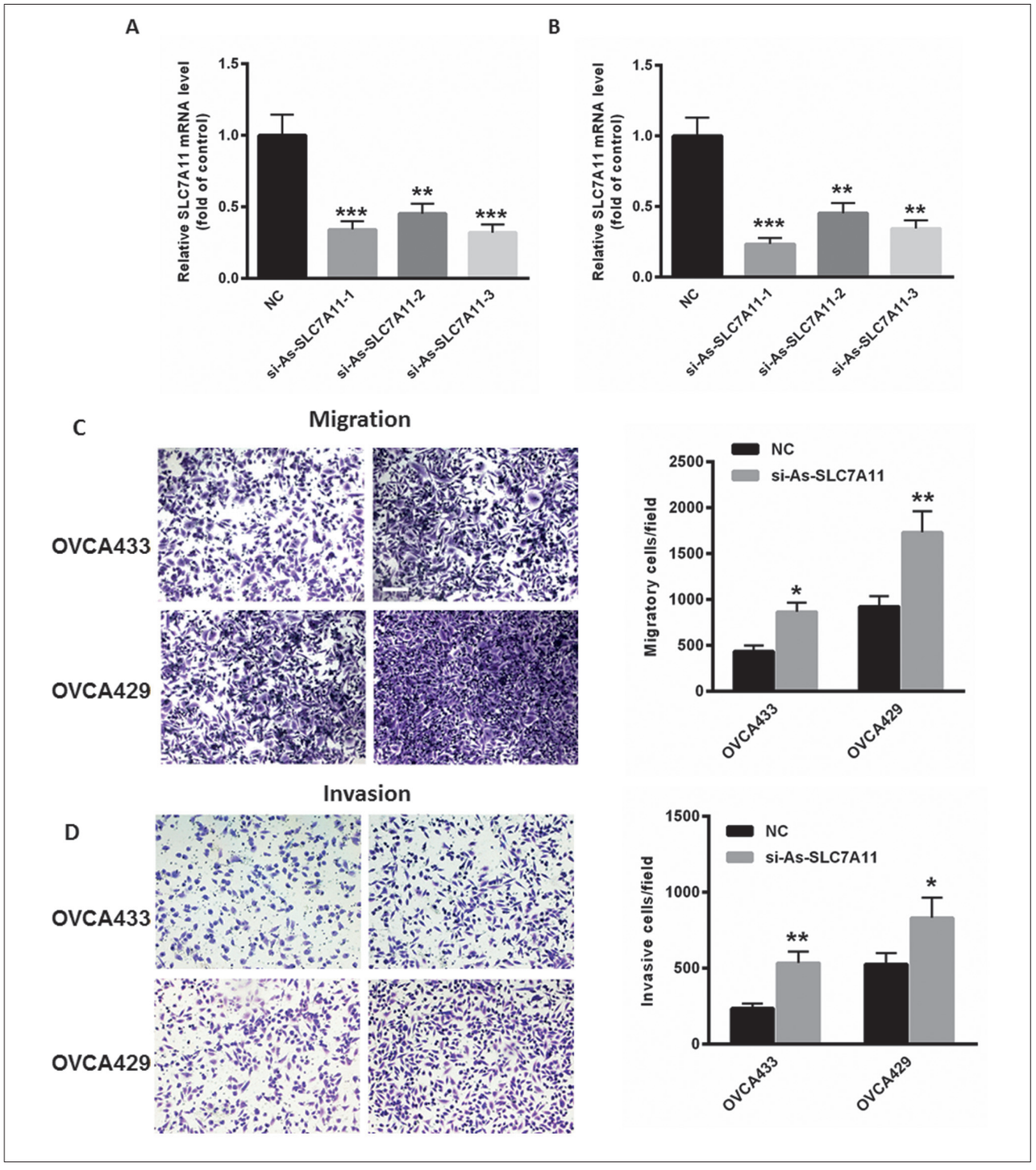

Fig. 2: Reduced As-SLC7A11 expression enhanced OVCA433 and OVCA429 cell migration and invasion. Real time PCR analysis of As-SLC7A11 level in OVCA433 (A) and OVCA429 (B) cells. (C) The inhibition of As-SLC7A11 significantly enhanced OVCA433 and OVCA429 cell migration. (D) Cell invasion capacity was also significantly enhanced in OVCA433 and OVCA429 cells compared with that of normal control. *p<0.05, **p<0.01, vs. control.

with a protease and phosphatase inhibitor cocktail (Sigma) and then separated by 10% SDS-PAGE, followed by electrophoretic transfer to a PVDF membrane. After soaking with 8% milk in PBST (pH 7.5) for 2 h at room temperature, the membranes were incubated with the following primary antibodies: anti-c-Met, anti-SLC7A11 and anti-GAPDH (Cell signaling). Immunodetection was performed by enhanced chemiluminsscence detection system (Millipore) according to the manufacturer's instructions. The house-keeping gene GAPDH was used as the internal control.

4.6. Apoptosis assay

Firstly, the cells were washed with PBS for three times. To determine cell apoptosis, an Annexin-V FITC–PI Apoptosis Kit (Invitrogen, Carlsbad, CA) was used. Briefly, the cells were washed with 1 × Annexin-V binding buffer (10 mM HEPES/NaOH, pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$) at a concentration of 2-3 × 10^6 cells/mL. Then, the annexin-v FITC and propidium iodide buffer was added followed by incubation at room temperature for 15 min. After treatment, the cells were filtered by a 300 mesh filter and analyzed by flow cytometry (Becton Dickinson, Franklin Lakes, NJ) within 1 h of staining.

4.7. TUNEL

Nuclear fragmentation was detected by TUNEL staining with an apoptosis detection kit (R&D Systems) according to the manufacturer's protocol.

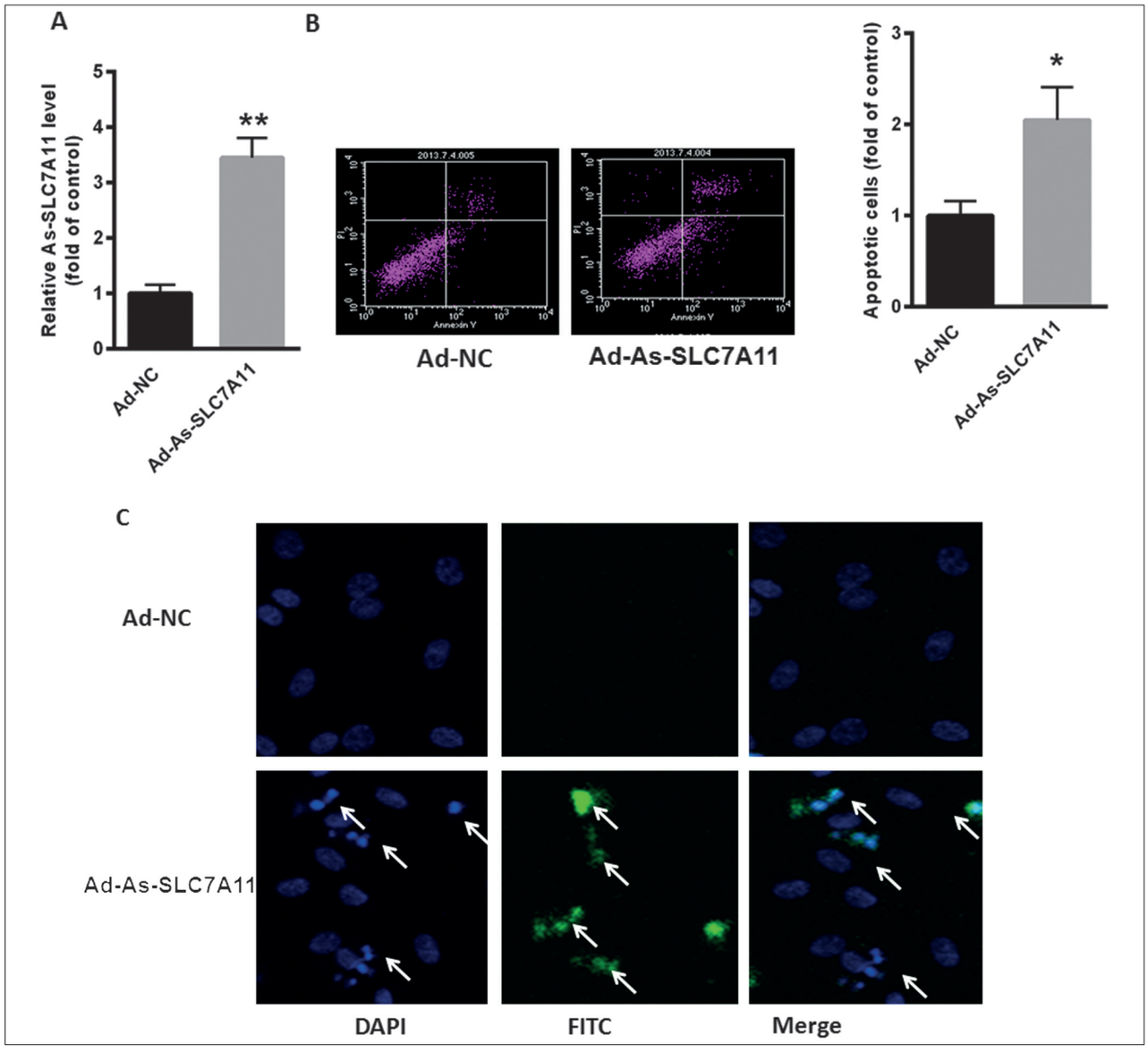

Fig. 3: Overexpression of As-SLC7A11 induced ovarian cancer cell apoptosis. (A) Real time PCR analysis of As-SLC7A11 after transfection with Ad-As-SLC7A11 in OVCA433 cancer cells. (B) Flow cytometry analysis. (C) TUNEL staining. *p<0.05, **p<0.01, vs. control.

4.8. Invasion and motility assays

Firstly, cells were seeded in the top chamber of each insert at 1.0×10^5 cells/well (BD Biosciences, San Jose, CA, USA) with 8.0-mm pores for motility assay. And for the invasion assays, 2.0×10^5 cells were cultured in a chamber (BD Biosciences) pre-coated with 0.2% Matrigel (Collaborative Research, Boston MA, USA) at 37 °C. As a chemoattractant, 10% fetal bovine serum was added to the culture medium in the lower chamber. After 24 h, the cells remained in the upper compartment were removed by cotton swabs, and those invaded through the membrane were stained with a dye solution containing 20% methanol and 0.1% crystal violet. The cells were then imaged under a light microscope (Olympus) and ten individual fields were counted per insert. The results are presented as an average of three separate experiments.

4.9. Statistical analysis

Data were presented as mean±SD from three independent experiments or five mice. Statistical analysis was carried out with Student's t test. $P < 0.05$ was considered as statistically significant difference.

Conflicts of interest: None declared.

References

Alcid, EA, Tsukiyama T (2016) Expansion of antisense lncRNA transcriptomes in budding yeast species since the loss of RNAi. Nat Struct Mol Biol 23: 450-455.

Chai Y, Liu J, Zhang Z, Liu L (2016) HuR-regulated lncRNA NEAT1 stability in tumorigenesis and progression of ovarian cancer. Cancer Med 5: 1588-1598.

Chang EY, Chang YC, Shun CT, Tien YW, Tsai SH, Hee SW, Chen IJ, Chuang LM (2016) Inhibition of prostaglandin reductase 2, a putative oncogene overexpressed in human pancreatic adenocarcinoma, induces oxidative stress-mediated cell death involving xCT and CTH gene expressions through 15-keto-PGE2. PLoS One 11: e0147390.

Chen ZJ, Zhang Z, Xie BB, Zhang HY (2016) Clinical significance of up-regulated lncRNA NEAT1 in prognosis of ovarian cancer. Eur Rev Med Pharmacol Sci 20: 3373-3377.

Drayton RM, Dudziec E, Peter S, Bertz S, Hartmann A, Bryant HE, Catto JW (2014) Reduced expression of miRNA-27a modulates cisplatin resistance in bladder cancer by targeting the cystine/glutamate exchanger SLC7A11. Clin Cancer Res 20: 1990-2000.

Galvan I, Inacio A, Romero-Haro AA, Alonso-Alvarez C (2016) Adaptive down-regulation of pheomelanin-related Slc7a11 gene expression by environmentally induced oxidative stress. Mol Ecol 26: 849-858.

Gislefoss RE, Langseth H, Bolstad N, Nustad K, Morkrid L (2015) HE4 as an early detection biomarker of epithelial ovarian cancer: investigations in prediagnostic specimens from the Janus serumbank. Int J Gynecol Cancer 25: 1608-1615.

Guo J, Li M, Meng X, Sui J, Dou L, Tang W, Huang X, Man Y, Wang S, Li J (2014) MiR-291b-3p induces apoptosis in liver cell line NCTC1469 by reducing the level of RNA-binding protein HuR. Cell Physiol Biochem 33: 810-822.

Gupta GP, Massague J (2006) Cancer metastasis: building a framework. Cell 127: 679-695.

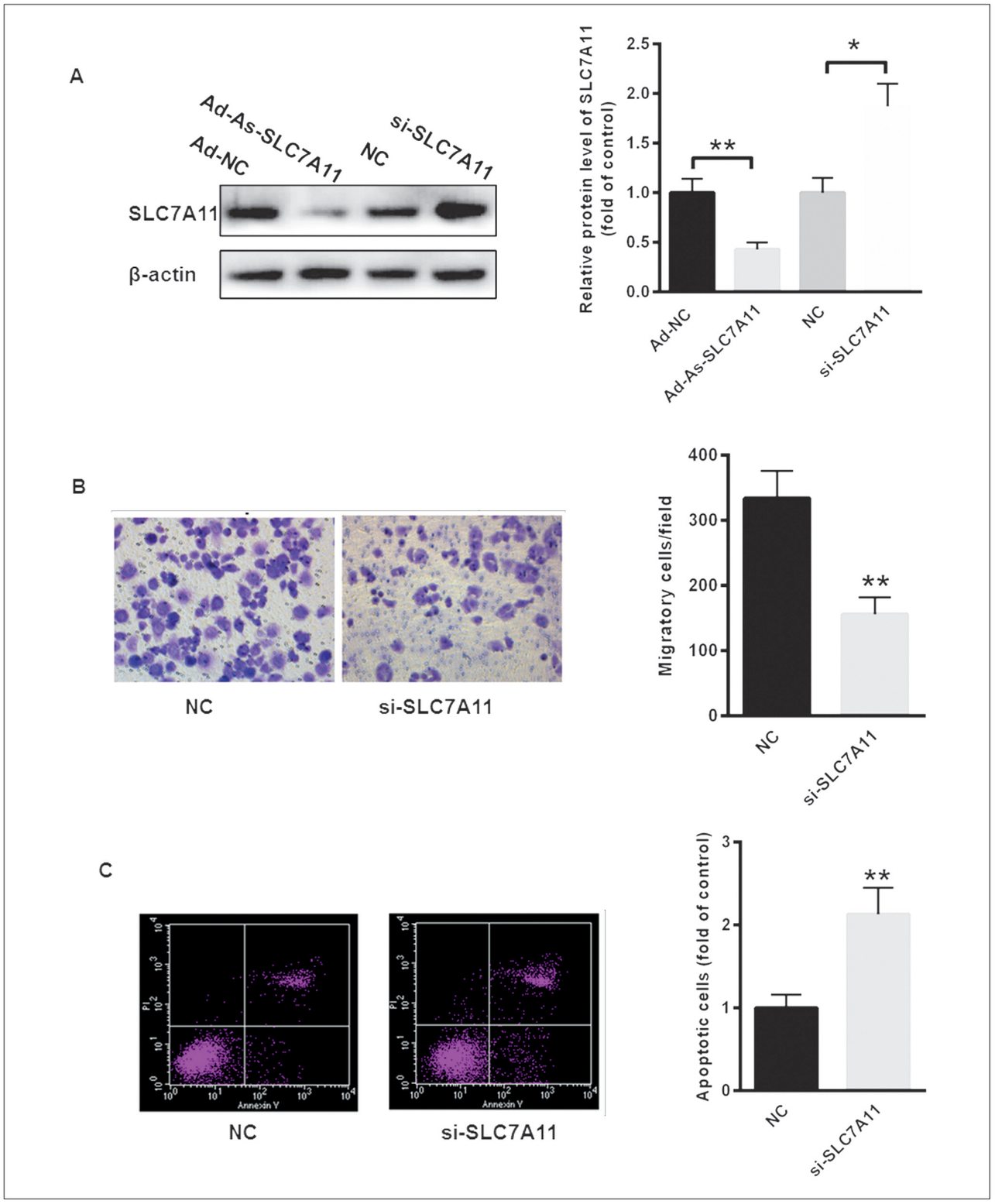

Fig. 4: As-SLC7A11 could suppress the expression of SLC7A11 in ovarian cancer cells. (A) Overexpression of As-SLC7A11 significantly suppressed the expression of SLC7A11, while silencing of As-SLC7A11 markedly increased the level of SLC7A11. (B) Knockdown of SLC7A11 significantly suppressed cell migration in OVCA433 cancer cells. (C) Silencing of SLC7A11 markedly induced cancer cell apoptosis. *p<0.05, **p<0.01, vs. control.

Huang Y, Dai Z, Barbacioru C, Sadee W (2005) Cystine-glutamate transporter SLC7A11 in cancer chemosensitivity and chemoresistance. Cancer Res 65: 7446-7454.

Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2011) Global cancer statistics. CA Cancer J Clin 61: 69-90.

Li H, Zhang H, Zhao S, Shi Y, Yao J, Zhang Y, Guo H, Liu X (2015) Overexpression of MACC1 and the association with hepatocyte growth factor/c-Met in epithelial ovarian cancer. Oncol Lett 9: 1989-1996.

Liu XX, Li XJ, Zhang B, Liang YJ, Zhou CX, Cao DX, He M, Chen GQ, He JR, Zhao Q (2011) MicroRNA-26b is underexpressed in human breast cancer and induces cell apoptosis by targeting SLC7A11. FEBS Lett 585: 1363-1367.

McGuire V, Jesser CA, Whittemore AS (2002) Survival among U.S. women with invasive epithelial ovarian cancer. Gynecol Oncol 84: 399-403.

Mizrahi A, Czerniak A, Ohana P, Amiur S, Gallula J, Matouk I, Abu-Lail R, Birman T, Hochberg A, Levy T (2010) Treatment of ovarian cancer ascites by intra-peritoneal injection of diphtheria toxin A chain-H19 vector: a case report. J Med Case Rep 4: 228.

Seebacher V, Reinthaller A, Koelbl H, Concin N, Nehoda R, Polterauer A (2017) The impact of the duration of adjuvant chemotherapy on survival in patients with epithelial ovarian cancer - a retrospective study. PLoS One 12: e0169272.

Sehm T, Rauh M, Wiendieck K, Buchfelder M, Eyupoglu IY, Savaskan NE (2016) Temozolomide toxicity operates in a xCT/SLC7a11 dependent manner and is fostered by ferroptosis. Oncotarget 7: 74630-74647.

Silva JM, Boczek NJ, Berres MW, Ma X, Smith DI (2011) LSINCT5 is over expressed in breast and ovarian cancer and affects cellular proliferation. RNA Biol 8: 496-505.

Wu H, Shang X, Shi Y, Yang Z, Zhao J, Yang M, Li Y, Xu S (2016) Genetic variants of lncRNA HOTAIR and risk of epithelial ovarian cancer among Chinese women. Oncotarget 7: 41047-41052.

Zhang Z, Cheng J, Wu Y, Qiu J, Sun Y, Tong X (2016) LncRNA HOTAIR controls the expression of Rab22a by sponging miR-373 in ovarian cancer. Mol Med Rep 14: 2465-2472.

Zheng ZG, Xu H, Suo SS, Xu XL, Ni MW, Gu LH, Chen W, Wang LY, Zhao Y, Tian B, Hua YJ (2016) The essential role of H19 contributing to cisplatin resistance by regulating glutathione metabolism in high-grade serous ovarian cancer. Sci Rep 6: 26093.

Zhou M, Sun Y, Sun Y, Xu W, Zhang Z, Zhao H, Zhong Z, Sun J (2016) Comprehensive analysis of lncRNA expression profiles reveals a novel lncRNA signature to discriminate nonequivalent outcomes in patients with ovarian cancer. Oncotarget 7: 32433-32448.

Zhou M, Wang X, Shi H, Cheng L, Wang Z, Zhao H, Yang L, Sun J (2016) Characterization of long non-coding RNA-associated ceRNA network to reveal potential prognostic lncRNA biomarkers in human ovarian cancer. Oncotarget 7: 12598-12611.

Zhou Y, Xu X, Lv H, Wen Q, Li J, Tan L, Li J, Sheng X (2016) The long noncoding RNA MALAT-1 Is highly expressed in ovarian cancer and induces cell growth and migration. PLoS One 11: e0155250.

Zhu Z, Song L, He J, Sun Y, Liu X, Zou X (2015) Ectopic expressed long non-coding RNA H19 contributes to malignant cell behavior of ovarian cancer. Int J Clin Exp Pathol 8: 10082-10091.

Zong X, Nakagawa S, Freier SM, Fei J, Ha T, Prasanth SG, Prasanth KV (2016) Natural antisense RNA promotes 3' end processing and maturation of MALAT1 lncRNA. Nucleic Acids Res 44: 2898-2908.