Original Article

SLC1A3 knockdown in inhibiting the proliferation, apoptosis resistance, and migration of ovarian cancer cells

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Background: Ovarian cancer accounts for 3% of all malignancies in women and kills about 140,000 women worldwide each year, representing the fifth leading cause of cancer-related death in women. At diagnosis, 70% of patients with ovarian cancer are already at stage III or IV disease, with a 5-year survival rate of less than 45%. Studies have found that solute carrier family 1 member 3 (*SLC1A3*) is highly expressed in various cancers and is associated with the poor prognosis of these cancers. However, the role of *SLC1A3* in ovarian cancer remains unknown. The purpose of this study was to investigate the role of the *SLC1A3* gene in the proliferation, apoptosis, migration, and outcomes of ovarian cancer.

Methods: The expression level of *SLC1A3* was measured via quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR). Knockdown experiments were performed with small interfering RNA targeting *SLC1A3* in ovarian cancer cells. After the knockdown of *SLC1A3*, proliferation was evaluated with Cell Counting Kit 8 (CCK8) assay, apoptosis was measured by flow cytometry, and migration was evaluated via wound-healing assay. Kaplan-Meier method was used to analyze the effect of *SLC1A3* expression on the prognosis of patients with ovarian cancer.

Results: High expression of *SLC1A3* was associated with poor prognosis in ovarian cancer patients, and the expression of *SLC1A3* in ovarian cancer cells was higher than that in ovarian epithelial cells. *In vitro* experiments demonstrated that knockdown of *SLC1A3* restrained the proliferation activity of ovarian cancer cells, enhanced cell apoptosis, and inhibited cell migration.

Conclusions: High expression of *SLC1A3* is linked to poor prognosis in ovarian cancer patients. *SLC1A3* activity impedes apoptosis while enhancing the proliferation and migration of ovarian cancer cells, suggesting its potential as a therapeutic target for drug development.

Keywords: Ovarian cancer; solute carrier family 1 member 3 (*SLC1A3*); proliferation; apoptosis; migration

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Introduction

Ovarian cancer constitutes merely 3% of malignancies among women and yet annually, approximately 140,000 women worldwide succumb to this disease. It ranks as the fifth principal cause of cancer-related death in women, following lung cancer, breast cancer, colorectal cancer, and pancreatic cancers (1). Due to the fact that the early manifestations of ovarian cancer are not conspicuous, 70% of patients are already at stage III or IV by the time of diagnosis, and the 5-year survival rate is lower than 45% (2). Hence, there is an acute need to formulate new molecular targets in order to increase the survival rate of patients with ovarian cancer.

Solute carrier family 1 member 3 (*SLC1A3*), a large protein coding gene located in the short arm of chromosome 5, encodes SLC1A3, an aspartate/glutamate transporter. In the absence of glutamine, *SLC1A3* can use aspartate to provide energy to cells and promote the conversion of glutamate, glutamine, and nucleotides. Studies have found that *SLC1A3* is highly expressed in glioma, chondrosarcoma, and is closely related to the poor prognosis of these cancers (3-6). However, the role of *SLC1A3* in ovarian cancer remains unknown.

In this study, we used quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) and bioinformatics to discover *SLC1A3* to be highly expressed in ovarian cancer tissues and closely associated with the unfavorable prognosis of ovarian cancer. Subsequently, *SLC1A3* was knocked down in ovarian cancer cells using siRNA, and the function of *SLC1A3* as an oncogene in ovarian cancer was assessed by detecting the cell proliferation activity, migration capability and cell apoptosis of ovarian cancer cells. We present this article in accordance with the MDAR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1909/rc).

Highlight box

Key findings

- High expression of solute carrier family 1 member 3 (*SLC1A3*) is linked to a poor prognosis of ovarian cancer.
- *SLC1A3* promotes proliferation and migration, and hinders apoptosis based on knockdown experiments in ovarian cancer cells.

What is known and what is new?

- While *SLC1A3* expression has been associated with poor prognosis in various cancers, its impact in ovarian cancer is unknown. These results indicate that high expression of *SLC1A3* is linked to poor prognosis of ovarian cancer, and the expression of *SLC1A3* in ovarian cancer cells is higher than that in ovarian epithelial cells.
- *In vitro* experiments with of ovarian cancer cells demonstrated that *SLC1A3* knockdown restrained proliferation, enhanced cell apoptosis, and inhibited cell migration.

What is the implication, and what should change now?

- This result confirmed that *SLC1A3* can regulate the proliferation, migration, and apoptosis of ovarian cancer cells, suggesting that *SLC1A3* may serve as a crucial therapeutic target in ovarian cancer. The mechanisms of *SLC1A3* in the regulation of various malignant biological behaviors in ovarian cancer cells should be investigated in future studies.

Methods

Cell culture

Human ovarian cancer cell lines (A2780, ES2, and OVCAR3) and the immortalized ovarian epithelial cell line IOSE80 were procured from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). They were cultured in RPMI-1640 medium (Servicebio, Wuhan, China) supplemented with 10% fetal calf serum at 37 ℃ in an incubator with 5% CO_2.

Cell transfection with small interfering RNA (siRNA)

In accordance with the manufacturer's instructions (7), Lipofectamine 2000 (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) and siRNA (60 nM) were separately mixed with RPMI-1640 medium and incubated at room temperature for 5 minutes. Subsequently, the two solutions were mixed and incubated at room temperature for 15 minutes. The mixture was added to a six-well plate and cultivated for 48 hours. The synthesis of siRNA was performed by Shanghai Ribo Medical Technology Co., Ltd. (Shanghai, China). The siRNA sequences used are listed in *Table 1*.

qRT-PCR

Cellular RNA was extracted by means of TRIzol and subsequently reverse transcribed into complement DNA. In line with the manufacturer's instructions (8), qRT-PCR detection was carried out using the SYBR Green qPCR kit (Servicebio Technology Co., Ltd., Wuhan, China). GAPDH was employed as an internal reference. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative messenger RNA (mRNA) expression. The primer sequences are listed in *Table 2*.

Table 1 siRNA sequences

siRNAs	siRNA sequences
siNC	5'-TTCTCCGAACGTGTCACGT-3'
siRNA-1	5'-GGTGAGTCATTTGATTAAA-3'
siRNA-2	5'-GCTTGTTTGGAGCAATATA-3'

siRNA, small interfering RNA; siNC, small interfering negative control.

Table 2 Primer sequences

Gene	Primer sequences
SLC1A3	F: 5'-GGTTGCTGCAAGCACTCATCAC-3'
	R: 5'-CACGCCATTGTTCTCTTCCAGG-3'
GAPDH	F: 5'-GTCTCCTCTGACTTCAACAGCG-3'
	R: 5'-ACCACCCTGTTGCTGTAGCCAA-3'

F, forward primer; R, reverse primer.

Cell Counting Kit 8 (CCK8)

The cells were sown into 96-well plates at a density of 5,000 cells per well. At 24, 48, and 72 hours subsequent to inoculation, 10 µL of CCK8 reagent was added to each well. After incubation at 37 ℃ for 2 hours, the absorbance of each well was gauged at 450 nm.

Colony formation assay

The cells were planted into a six-well plate at a density of 1,000 cells per well, and following culturing for 14 days, the plate was rinsed with phosphate-buffered saline (PBS) fixed with methanol and stained with crystal violet. Colonies consisting of more than 50 cells were recorded.

Cell apoptosis assay

When the cell density reached 50%, siRNA or small interfering negative control (siNC) was transfected. After 48 hours, the cells were collected, washed with PBS, and resuspended in 195 µL of buffer; subsequently, 5 µL of annexin-V phycoerythrin (MedChem Express, Monmouth, NJ, USA) was added and incubated in the dark for 10 minutes, and then the fluorescence intensity was detected with a flow cytometer (FACSCalibur, BD, Franklin Lakes, NJ, USA).

Wound-healing assay

After the cells reached full confluence, a 200-µL pipette tip was used to create a linear wound by gently scraping the central part of the cell monolayer. The area was carefully cleared to create a consistent scratch. The cells were then washed with PBS to remove any debris or detached cells. Images of the scratch area were captured using an inverted microscope to document the initial wound width. The cells were subsequently cultured in serum-free medium for 24 hours to allow for migration. After 24 hours of incubation, the width of the wound was measured again using the inverted microscope to assess cell migration and closure.

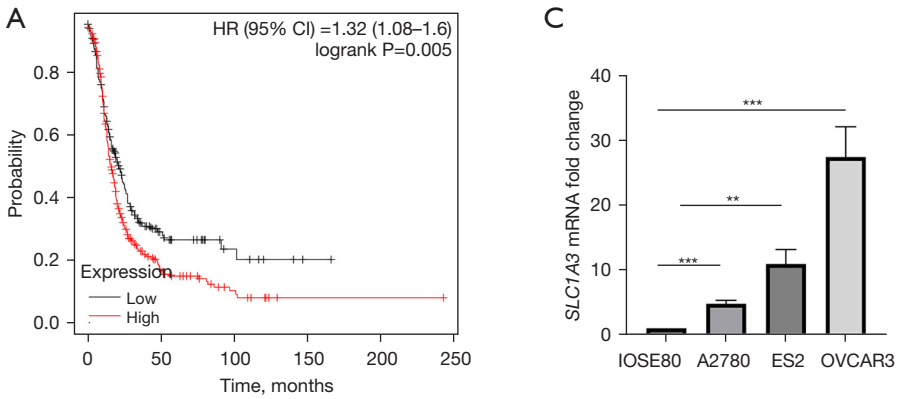
Statistical analysis

All data in this study were analyzed with GraphPad software (GraphPad Software, La Jolla, CA, USA), and the data are presented as the mean ± standard deviation. Comparisons between two independent samples were conducted using the least significant difference *t*-test. Survival analysis of ovarian cancer in GEPIA2 (cancer-pku.cn) was carried out using Kaplan-Meier analysis, and survival was compared via the log-rank test. P<0.05 was regarded as statistically significant.

Results

High expression of SLC1A3 was closely associated with poor prognosis in patients with ovarian cancer

We analyzed the prognostic impact of *SLC1A3* expression on ovarian cancer via the Kaplan-Meier plotter (http://kmplot.com/analysis/index.php?p=background). The outcomes indicated that patients with high *SLC1A3* expression had inferior overall survival [hazard ratio (HR) =1.32; log-rank P=0.005] (*Figure 1A*). Furthermore, the median survival time of patients with high *SLC1A3* expression was 16 months, while the median survival time of patients with low *SLC1A3* expression was 21 months (*Figure 1B*). Additionally, we detected the mRNA expression levels of *SLC1A3* in ovarian epithelial cells and three types of ovarian cancer cells and discovered that the expression levels of *SLC1A3* in several ovarian cancer cells were higher than those in ovarian epithelial cells (*Figure 1C*). In conclusion, the high expression of *SLC1A3* was associated with cancer as compared to normal ovarian epithelial cells, and associated with a poorer prognosis among patients with ovarian cancer.



B

Median survival

Low expression cohort (months)	High expression cohort (months)
21	15.87

Figure 1 A high expression of *SLC1A3* was closely associated with the poor prognosis of ovarian cancer. (A) OS survival curves of patients with ovarian cancer with a high or low expression of *SLC1A3*. HR is 1.32 (95% CI: 1.08–1.6). (B) Median survival of patients with ovarian cancer with a high or low expression of *SLC1A3*. (C) *SLC1A3* mRNA levels in ovarian epithelial and ovarian cancer cells according to data analyzed via *t*-test. **, P=0.006; ***, P<0.001. HR, hazard ratio; CI, confidential interval; mRNA, messenger RNA; OS, overall survival.

Knockdown of SLC1A3 inhibited the proliferation of ovarian cancer cells

In order to investigate the impact of SLC1A3 in the malignant biological behavior of ovarian cancer cells, we selected OVCAR3 cells with the highest *SLC1A3* expression and used siRNA to knock down the expression level of *SLC1A3* in the cells. As shown in *Figure 2A*, two siRNAs with different sequences both decreased the expression of *SLC1A3* mRNA by more than 50%. The CCK8 results indicated that knocking down *SLC1A3* inhibited the proliferation activity of ovarian cancer cells (*Figure 2B*). Colony formation experiments demonstrated that after knockdown of *SLC1A3*, the colony formation ability of ovarian cancer cell OVCAR3 cells was significantly reduced (*Figure 2C*,*2D*). These results suggest that knocking down *SLC1A3* inhibits the proliferation of ovarian cancer cells.

Knockdown of SLC1A3 promoted apoptosis in ovarian cancer cells

In order to determine whether *SLC1A3* affects the apoptosis of ovarian cancer cells, we used flow cytometry to detect the percentage of apoptotic cells. As shown in *Figure 3*, the apoptosis rate in the control group was only 5.43%. After knockdown of *SLC1A3*, the apoptosis percentage of ovarian cancer cells significantly increased to 18.0% with siRNA-1 (P<0.001) and 15.7% with siRNA-2 (P<0.001). This suggests an anti-apoptotic role of *SLC1A3* in ovarian cancer cells.

Knockdown of SLC1A3 inhibited ovarian cancer cell migration

Migration ability of ovarian cancer cells was assessed through wound-healing assay: when the cells covered the culture plate, we scratched the central area of cells and obtained images to record the width of the scratch at this time. To avoid the influence of proliferation, we used serum-free medium culturing for 24 hours and recorded the scratch width again. As shown in *Figure 4*, compared with the siNC group, the *SLC1A3*-knockdown group exhibited reduced migration ability, suggesting that *SLC1A3* promotes cell migration.

Discussion

Ovarian cancer is among the common malignancies in women and due to its insidious onset and poor prognosis, it is often referred to as the “silent killer” (9). However, the

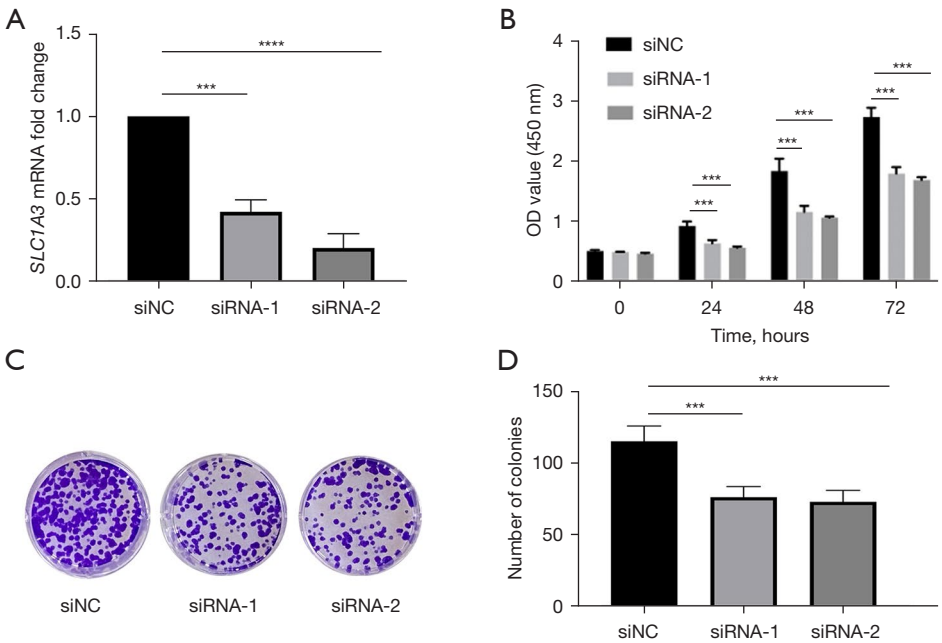


Figure 2 Knockdown of *SLC1A3* inhibited the proliferation of ovarian cancer cells. (A) Validation of *SLC1A3* knockdown in ovarian cancer cells according to quantitative reverse-transcription polymerase chain reaction. (B) Cell Counting Kit 8 assay assessment of cell proliferation after *SLC1A3* knockdown. (C,D) Colony formation assay assessment (stained with crystal violet) of the colony-forming ability of cells after knockdown of *SLC1A3*. ***, P<0.001; ****, P<0.0001. siNC, small interfering negative control; siRNA, small interfering RNA; mRNA, messenger RNA; OD, optical density.

specific molecular mechanisms underlying ovarian cancer are not completely understood. and recent therapeutic advancements had led to incremental gains. Therefore, identifying intervention targets to improve treatment and prognosis is necessary. The rapid development of siRNA screening technology coupled with bioinformatics tools have allowed the discovery of several genes that regulate cancer at the genetic level (10-15). In *Figure 1A*, Kaplan-Meier analysis indicated that the survival rate of patients with ovarian cancer and a high expression of *SLC1A3* was lower than that of those with a low expression of *SLC1A3*. The patients with high *SLC1A3* expression had a median survival of only 16 months, while those with low expression had a median survival of 21 months.

One of the primary characteristics that distinguish cancer cells from normal cells is metabolic reprogramming (16-18). The aberrant expression of enzymes in metabolic pathways activates cellular metabolism, providing energy for cancer cells (19,20). *SLC1A3* is a member of the glutamate transporter protein family and has been found to be overexpressed in several types of cancer (4-6). In thyroid cancer, *SLC1A3* has been shown to promote the self-renewal capacity of $CD133^+$ thyroid cancer cells (21).

In our study, we used siRNA technology to downregulate *SLC1A3* expression in ovarian cancer cells, the effect of *SLC1A3* down-regulation was verified by qRT-PCR, and conducted a series of functional assays, which demonstrated that *SLC1A3* knockdown inhibited the proliferative capacity, colony formation ability, and migration ability of ovarian cancer cells and induced their apoptosis. Hence, *SLC1A3* plays an oncogenic role in ovarian cancer.

However, this study was limited in that it did not deeply examine the mechanisms by which *SLC1A3* regulates various malignant biological behaviors of ovarian cancer cells. And Kaplan-Meier cannot handle multiple covariates that affect the lifetime. It can only deal with grouping variables, and km assumes that the lifetimes between individuals are independent of each other, whereas in reality there may be dependencies between individuals. The role of covariates is not taken into account. We plan to further investigate this aspect in our future research.

This study reveals the role of SLC1A3 in the biological behavior of ovarian cancer cells, providing preliminary evidence for its potential as a biomarker in ovarian cancer.

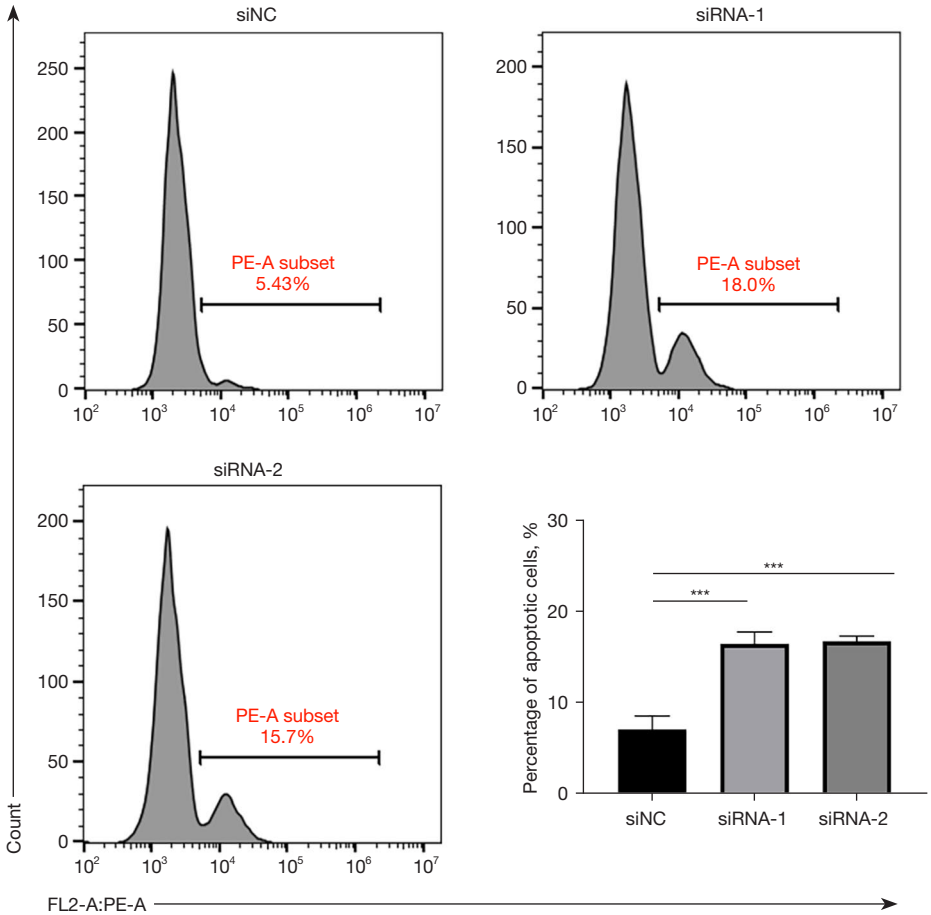


Figure 3 Knockdown of *SLC1A3* promoted apoptosis in ovarian cancer cells. Percentage of apoptotic cells detected by flow cytometry after knockdown of *SLC1A3*. ***, P<0.001. siNC, small interfering negative control; siRNA, small interfering RNA; PE-A, phycoerythrin area.

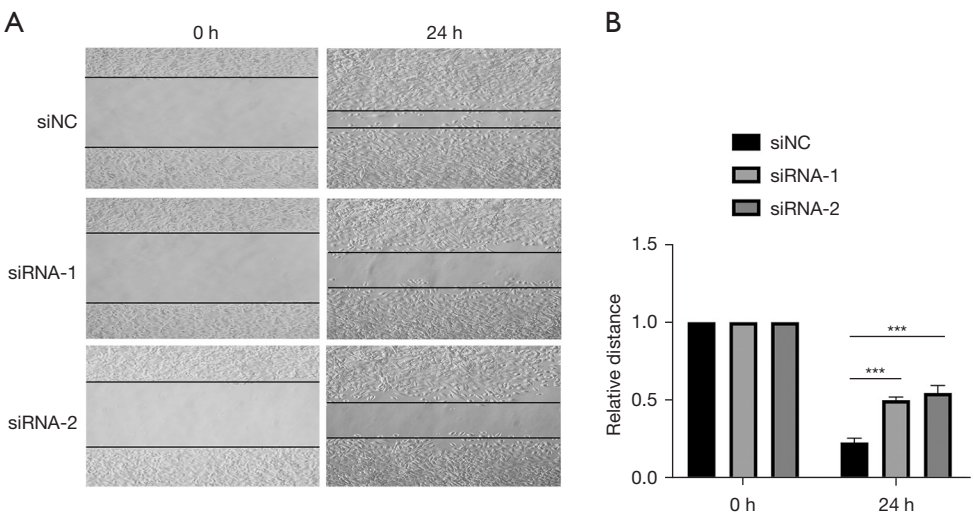


Figure 4 Knockdown of *SLC1A3* inhibited ovarian cancer cell migration. (A,B) The migration ability of cells was tested via wound-healing assay (200× magnification). ***, P<0.001. siNC, small interfering negative control; siRNA, small interfering RNA.

Previous research has shown that SLC1A3 is associated with invasiveness and metastasis in certain cancers, and its expression level may reflect the degree of cancer progression. Therefore, SLC1A3 holds potential as a stratification marker in ovarian cancer patients, helping to distinguish high-risk from low-risk patients.

Furthermore, the expression of SLC1A3 may be closely related to local regional metastasis. In ovarian cancer, metastatic spread is a major factor contributing to poor prognosis, and whether SLC1A3 can serve as a molecular marker for predicting local regional metastasis still requires further validation. Future studies will require larger clinical cohorts, combined with patient tissue samples, to analyze SLC1A3 expression levels and further explore its correlation with clinical staging, tissue differentiation, metastasis, and prognosis, in order to assess its clinical application value.

Conclusions

In conclusion, high expression of *SLC1A3* is linked to poor prognosis in ovarian cancer patients. *SLC1A3* activity impedes apoptosis while enhancing the proliferation and migration of ovarian cancer cells, suggesting its potential as a therapeutic target for drug development. Altogether, SLC1A3 may serve as a potential therapeutic target in ovarian cancer.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1909/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1909/dss

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1909/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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