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Long non-coding RNA DSCAM-AS1 indicates a poor prognosis and modulates cell proliferation, migration and invasion in ovarian cancer via upregulating SOX4

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Abstract. – OBJECTIVE: Recent studies have revealed that long noncoding RNAs (lncRNAs) play a crucial role in tumor progression. Ovarian cancer is a common type of fatal gynecological cancer worldwide. This study aims to investigate how lncRNADSCAM-AS1 functions in the progression of ovarian cancer.

PATIENTS AND METHODS: DSCAM-AS1 expression of both ovarian cancer cells and 56 paired of tissue samples was detected by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). Moreover, the function of DSCAM-AS1 was identified via transwell assay, wound healing assay, colony formation assay and proliferation assay *in vitro*. The underlying mechanism was explored through qRT-PCR and Western blot assay.

RESULTS: DSCAM-AS1 expression was remarkably upregulated in tumor tissues compared with that in the adjacent normal tissues. Besides, ovarian cancer proliferation, migration and invasion were promoted after overexpressing DSCAM-AS1 *in vitro*. Moreover, after overexpression of DSCAM-AS1, SOX4 was upregulated at mRNA and protein level in vitro. Furthermore, the expression of SOX4 in tumor tissues was positively correlated with the expression of DSCAM-AS1.

CONCLUSIONS: The above results suggested that DSCAM-AS1 can promote cell migration, invasion and proliferation in ovarian cancer by upregulating SOX4, which may offer a new therapeutic indicator for patients with ovarian cancer.

Words: long noncoding RNA, DSCAM-AS1, Ovarian cancer, SOX4.

Introduction

Ovarian cancer, one of the fatal gynecologic malignancies and the fifth leading culprit of death in cancer, killed nearly 14,100 women in America in 2017. Besides, there were another 22,500 new patients diagnosed with ovarian cancer. However, most of the cases are diagnosed at late stage due to atypical and insignificant symptoms at the early stage. Moreover, the high rate of therapy resistance and metastasis occurs in almost 80% of cases contributes to the high mortality of ovarian cancer[2,3]. This situation underscores the urgency of early detection of these patients with ovarian cancer and the establishment of new therapeutic methods for successful intervention.

Long non-coding RNAs (lncRNAs) are known as a class of non-coding transcripts. Recent studies have revealed that lncRNAs are emerging with a variety of heterogeneous molecular actions, including the complicated gene-regulation networks in tumorigenesis. For example, the overexpression of lncRNA CCAT2 promoted the proliferation and metastasis in intrahepatic cholangiocarcinoma, and lead to a poor prognosis of the patients[4]. LncRNA FAL1 was found to be a potential oncogene for colon cancer by enhancing the proliferation and inhibiting the apoptosis of colon cancer cells[5]. LncRNA SOCS2-AS1 acted as an oncogene in the development of castration-resistant prostate cancer by inhibiting cell apoptosis[6]. By targeting MUC2 and regulating miR-34c, lncRNA AF147447 represses cancer proliferation and invasion in *Helicobacter pylori*-related gastric cancer[7]. However, it remains unexplored how lncRNADSCAM-AS1 functions in the proliferation of ovarian cancer.

The current study revealed that lncRNA DSCAM-AS1 expression is significantly upregulated in ovarian cancer samples. Moreover, the *in vitro* experiment showed that DSCAM-AS1 facilitates the migration, invasion and proliferation of ovarian cancer cells. Furthermore, the authors discovered that lncRNA DSCAM-AS1 exerts its effect on ovarian cancer by regulating SOX4.

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Patients and Methods

Cell Culture

A2780, TOV112D, HO-8910, OVCAR-3 and SKOV3 ovarian cancer cell lines, a normal ovarian cell (ISOE80) and 293T cell (Chinese Type Culture Collection, Chinese Academy of Sciences, Shanghai, China) were used in this study. The culture medium consisted of penicillin (Dulbecco's Modified Eagle's Medium – DMEM; Hyclone, South Logan, UT, USA) and 10% fetal bovine serum (FBS; Life Technologies, Gaithersburg, MD, USA). Besides, cells were cultured in a humidified incubator, which contained 5% CO_2 and was set at 37°C.

Clinical Samples

Human tissues came from 56 patients with ovarian cancer who underwent surgery at the Weihai Municipal Hospital. All tissues were kept at -80°C. Each patient signed the informed consent before the surgery. This study was approved by the Ethics Committee of the Weihai Municipal Hospital.

Cell Transfection

Lentiviral virus targeting DSCAM-AS1 [illegible] compounded before the study, and pLenti [illegible] EGFP-F2A-Puro vector (Biosettia Inc., San Diego, CA, USA) was then used for cloning. S[illegible] followed by these viruses, the empty vector (control) and the DSCAM-AS1 lenti[illegible] DSCAM-AS1) packaged in 293T cells[illegible]

RNA Extraction and Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Total RNA [illegible] ained from [illegible] ples with TRIzol reagent (Invitrogen, Carlsbad, CA, USA). It was reverse transcribed to complementary deoxyribose nucleic acids (cDNAs) using Reverse Transcription [illegible] TaKaRa Biotechnology Co., Ltd., Dalian, China). The performance of qRT-PCR was conducted on [illegible] system (Applied Biosystems, Foster City, CA, USA). An [illegible] BR Green Real Time-PCR [illegible] pplied [illegible] wing are the primers used for qR[illegible]CR: D[illegible]M-AS1, forwards 5'-CCTATC-C[illegible]CTCTAAGAA-3' and reverse 5'-ACTTCT-[illegible]AA[illegible]GTGCTG-3'; SOX4, forward 5'-CTT-[illegible]ACATGATTAGCTGGCATGATT-3' and reverse [illegible]CTGTGCAATATGCCGTGTAGA-3'; glyceraldehyde 3-phosphate dehydrogenase (GAPDH), forward 5'-CCAAAATCAGATGGGGCAATGCTGG-3' and reverse 5'-TGATGGCATGGACTGTGGTCATTCA-3'. Thermal cycle was as follows: 95°C for 30 sec, 95°C for 5 sec for 40 cycles, 60°C for 35 sec.

Colony Formation Assay

After culturing with FBS for 14 days in a six-well plate, all the cells were fixed with methanol and stained with 0.1% crystal violet (Solarbio, Beijing, China). Meanwhile, a number of colonies were counted for comparison. Each assay was independently repeated in triplicate.

Cell Proliferation Assay

Since following the protocol (Dojindo Molecular Technologies, Inc., Kumamoto, Japan), the cell proliferation of these treated cells in 96-well plates was monitored every 24 h by Cell Counting Kit-8 (CCK-8) assay. A spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was utilized to measure the absorbance at 450 nm.

Wound healing Assay

After being transferred into 6-well plates, cells were cultured in DMEM medium overnight. After scratched with a plastic tip, cells were cultured in serum-free DMEM. Wound closure was visualized at different time points. Each assay was independently [illegible]ated in triplicate.

Matrigel Assay

10^4 cells in 200 µL of serum-free DMEM were transformed to the top chamber of an 8 µm pore size insert (Millipore, Billerica, MA, USA) with or without Matrigel (50 µg; BD Biosciences, Franklin Lakes, NJ, USA). The bottom chamber was added with DMEM and FBS 48 h later, a cotton swab was used to wipe the top surface of chambers and immersed for 10 min with precooling methanol. The followings were stained in crystal violet for 30 min. The count for the invasion proceeded in three fields per membrane.

Western Blot Analysis

Radioimmunoprecipitation assay (RIPA; Beyotime, Shanghai, China) reagent was utilized to extract protein from cells. Bicinchoninic acid (BCA) protein assay kit (TaKaRa, Dalian, China) was chosen to quantify the protein concentrations. The target proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Before incubated with antibodies, they were removed to polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). Cell Signaling Technology (CST, Danvers, MA, USA) provided us rabbit anti-GAPDH and rabbit anti-SOX4, as well as the goat anti-rabbit secondary antibody. The chemiluminescent film was applied for assessment of protein expression with Image J software (Bethesda, MD, USA).

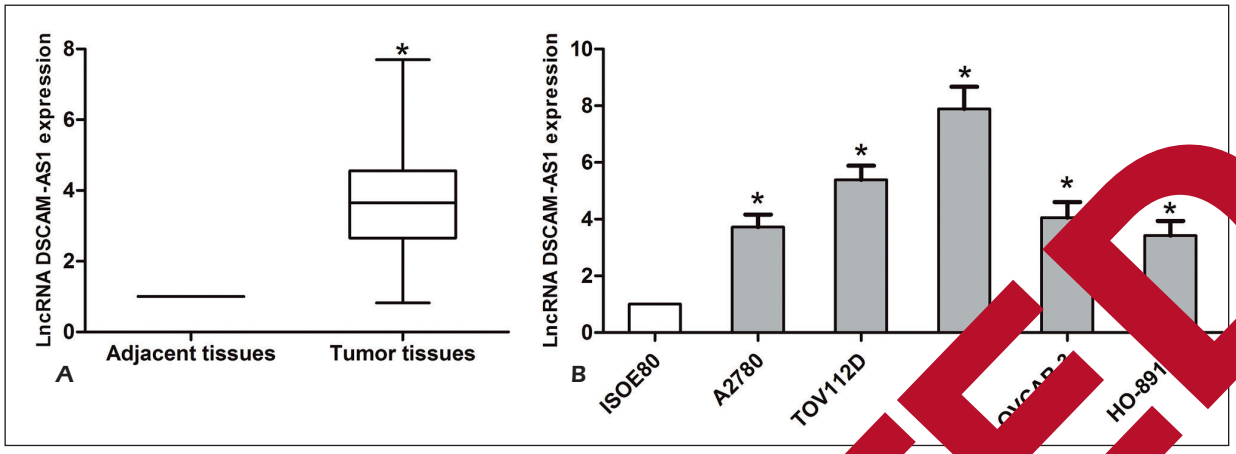

Figure 1. Expression levels of DSCAM-AS1 were increased in ovarian cancer tissu[illegible] cell lines. [illegible]CAM-AS1 expression was significantly increased in the ovarian cancer tissues compared with adjac[illegible]ues. **B**, Expres[illegible] of DSCAM-AS1 relative to GAPDH were determined in the human ovarian cancer cell lines [illegible]arian cell (IS[illegible]) by qRT-PCR. Data are presented as the mean ± standard error of the mean. * $p<0.05$.

Statistical Analysis

The statistical analysis was performed via Statistical Product and Service Solutions 17.0 (SPSS Inc., Chicago, IL, USA). Chi-square test, Student's *t*-test and Kaplan-Meier method were selected when appropriate. The results were [illegible]sented by mean ± SD (standard deviation). [illegible] was considered statistically significant.

Results

Expression Level of D[illegible]M-AS1 in Tissues and Cells o[illegible]ria

First, qRT-PCR [illegible] to detect DSCAM-AS1 exp[illegible]n in 56 pa[illegible]' tissues and 5 ovarian canc[illegible]As a result, [illegible]AM-AS1 was significan[illegible]more [illegible]gulated in t[illegible]mor tissue samples th[illegible]that in the [illegible]cent tissues (Figure 1A). Co[illegible]ed with the exp[illegible]n in normal ovarian c[illegible]SOE80, [illegible]SCAM-AS1 level was markedly hig[illegible] ova[illegible]ancer cells (Figure 1B).

[illegible]CAM[illegible] Ov[illegible]pression [illegible]oted [illegible]liferation In Vitro

[illegible]cording [illegible]SCAM-AS1 expression in sev-e[illegible] cells, the authors chose SKOV3 ovarian [illegible]cer [illegible]or overexpression of DSCAM-AS1. [illegible]he DSCAM-AS1 lentivirus (DSCAM-AS1) and [illegible]empty vector (control) were synthesized and t[illegible]duced into SKOV3 cells. Then, the DSCAM-AS1 expression was determined by qRT-PCR (Figure 2A). The authors performed the colony formation assay and found that overexpression of DSCAM-AS1 promoted the growth of ovarian ca[illegible]cell (Figure 2B). [illegible]rthermore, the results o[illegible]CK8 assay showed that the cell proliferation [illegible]varian canc[illegible]ells was promoted after the o[illegible]pression [illegible]SCAM-AS1 (Figure 2C).

DSCA[illegible] verexpression Promoted Cell Invasion In Vitro

[illegible]en, the authors performed wound healing [illegible]d transwell assay, thus finding out that the overexpression of DSCAM-AS1 promoted the migration of ovarian cancer cell (Figure 3A and 3B). Furthermore, the results of the transwell assay showed that cell invasion capacity of ovarian cancer cells was induced after the overexpression of DSCAM-AS1 (Figure 3C).

DSCAM-AS1 Promoted Ovarian Cancer Tumorigenesis Via SOX4

The results of QRT-PCR demonstrated that the SOX4 mRNA expression was upregulated in ovarian cancer cells when transfected with DSCAM-AS1 lentivirus (Figure 4A). Western blot analysis results further verified that the expression level of SOX4 protein was upregulated in ovarian cancer cells when transfected with DSCAM-AS1 lentivirus (Figure 4B). To explore the interaction between DSCAM-AS1 and SOX4, the expression level of SOX4 was detected in ovarian cancer tissues. As a result, SOX4 expression of cervical cancer tissues was markedly higher in ovarian cancer tissues compared with that in the adjacent tissues (Figure 4C). The linear correlation analysis revealed that the SOX4 expression positively correlated to DSCAM-AS1 expression in ovarian cancer tissues (Figure 4D).

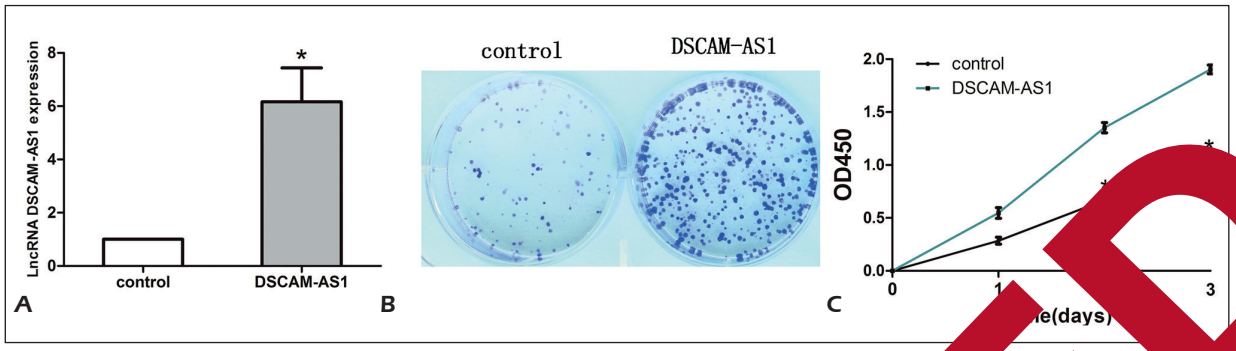

Figure 2. Overexpression of DSCAM-AS1 promoted ovarian cancer cell proliferation. **A**, DSC AS1 exp ion in o cancer cells transduced with control vector (control) or DSCAM-AS1 lentivirus (DSCAM-AS1) v ected b -PCR. GAP was used as an internal control. **B**, Colony formation assay showed that number of colonies i DS tivirus g p was markedly increased compared with empty control group in SKOV3 ovarian cancer cells. **C** s assa d that ov ression of DSCAM-AS1 significantly increased cell proliferation in SKOV3 ovarian cancer cell results rep the a ge of three independent experiments (mean ± standard error of the mean). *$p<0.05$, compared ontrol cells.

Discussion

LncRNAs, which can be utilized as classifiers for personalized therapy, have been reported to be the important factors in ovarian cancer. For instance, lncRNA CCAT2 is overexpressed in ovarian cancer and is reported to be related t e poor prognosis[8]. LncRNA LINC00092 act important driver of metastatic progression e progression of ovarian cancer which is medi by cancer-associated fibroblasts[9]. LncRNA TU up-regulates the proliferation n canc by promoting epithelial-m chy transi- tion[10]. Moreover, after k down ncRNA MNX1-AS1, the cell p ion of ovarian cancer are bite may offer a potential target fo

Previous re [12,13] have s ted that lncRNA DSC -AS s an impo ant role in breast biology a treatment resistance. Ac to the transcr e sequencing data f a cohort of 6,503 cancers and cell lines in t CGA and Michigan Center for Translati Pathology, RNA DSCAM-AS1 exhibits a h cance ecific expression pattern, especia cancer and breast cancer[14]. For example, lncRNA DSCAM-AS1 has as an onic role in ER-positive breast cancer phenop omotes cell reproduction and suppresses cell apoptosis in tamoxifen-resistance breast cancer[12,13]. According to the recent study, it was found out that DSCAM-AS1 was upregulated in both of the tissues and cells of ovarian cancer. Furthermore, after DSCAM-AS1 was overexpressed, the ability of cell growth, migration and invasion was promoted. The data indicated that DSCAM-AS1 functions as an oncogene and enhances the tumorigenesis of ovarian cancer.

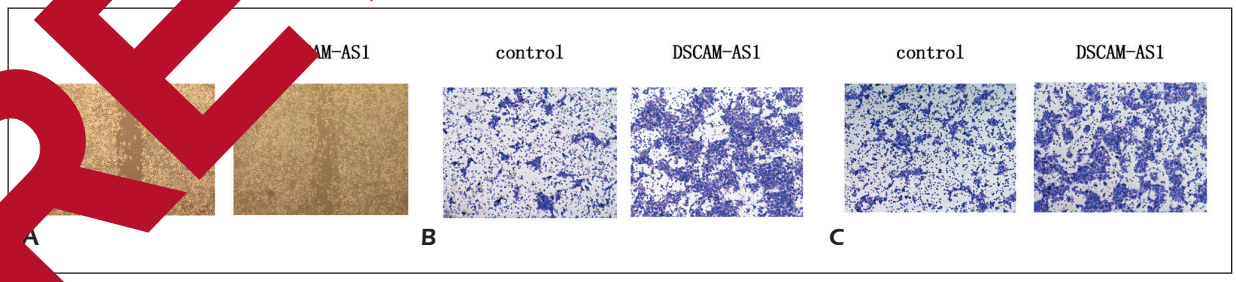

Figure 3. Overexpression of DSCAM-AS1 increased ovarian cancer cell invasion. **A**, Wound healing assay showed that the migrated length of cells in DSCAM-AS1 lentivirus group was remarkably increased compared with empty control group in SKOV3 ovarian cancer cells. **B**, The transwell assay showed that the migrated length of cells in DSCAM-AS1 lentivirus group was significantly increased compared with empty control group in SKOV3 ovarian cancer cells. **C**, The transwell assay showed that overexpression of DSCAM-AS1 markedly increased cell invasion in SKOV3 ovarian cancer cells. The results represent the average of three independent experiments (mean ± standard error of the mean). *$p<0.05$, compared with the control cells.

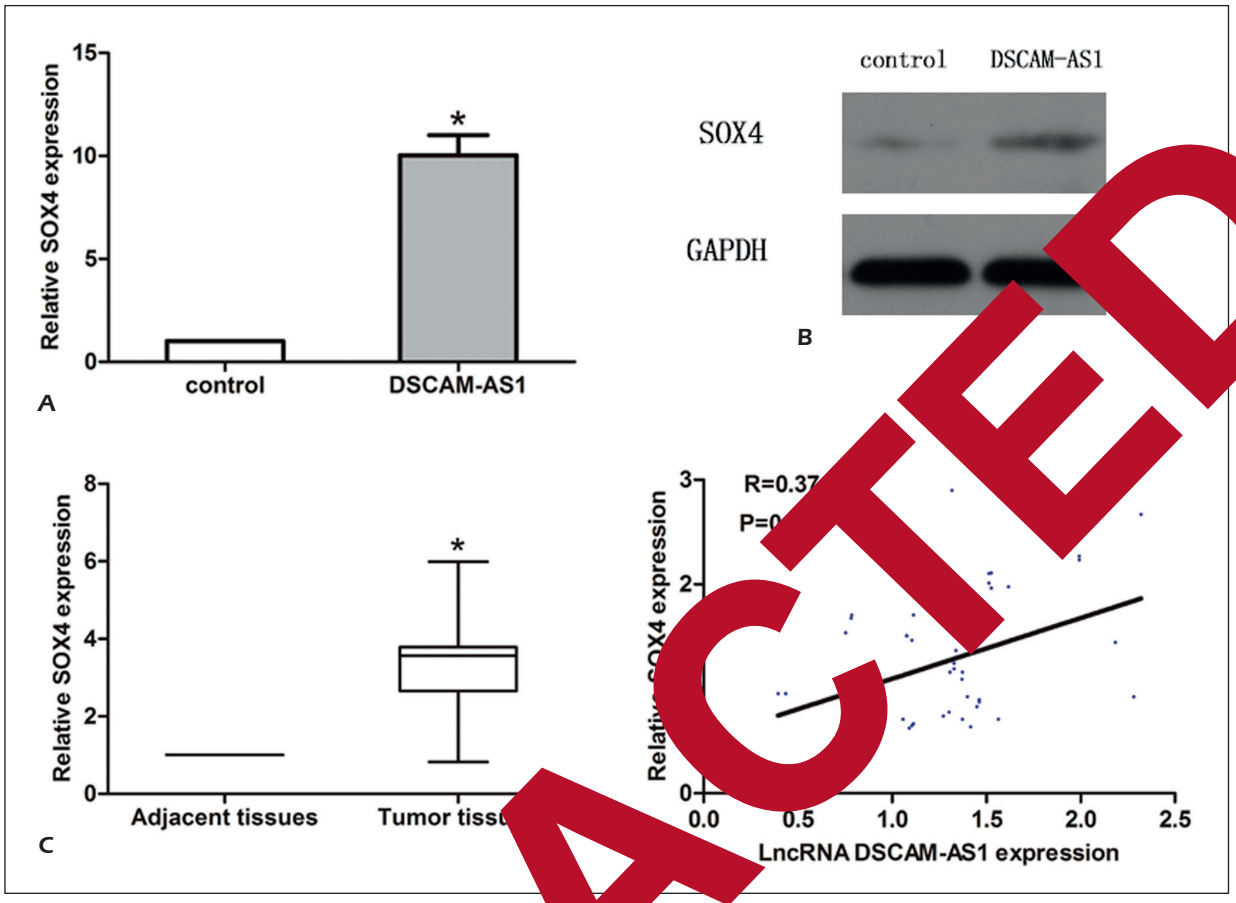

Figure 4. Interaction between SOX4 a AM-AS1 i ian cancer. **A**, The RNA expression level of SOX4 in DSCAM-AS1 cells was significantly increase ith emp trol cells in SKOV3cells. **B**, Protein expression of SOX4 was increased after overexpression of AM-A n SKOV3 **C**, SOX4 was significantly upregulated in ovarian cancer tissues compared with adjacent es. **D**, Th ear correla n between the expression level of SOX4 and DSCAM-AS1 in ovarian cancer tissues. The resu reser three independent experiments. Data are presented as the mean ± standard error of the mea =0.0

As a trans tion r, sex-dete mining region Y-rel high-mob roup box 4 (SOX4) has bee ntified as an on e in various cancers exam by downregulating SOX4 and up ting -132-3p, lncRNA TUG1 facilitates eratio d inhibits apoptosis in an c arcon SOX4 is an important mo- r fact eukemogenesis. Besides, the ov pressio SOX4 is related to poor prog- n cute myeloid leukemia[16]. Furthermore, X4 p otes the ability of cell proliferation nd migration by targeting the epithelial-mesen- mal transition processes in prostate cancer – a p tial therapeutic target[17]. SOX4 also plays an important role in the metastasis of renal cancer by epithelial-mesenchymal transition[18]. In this work, the result of Western blot analysis indicated that SOX4 was upregulated after DSCAM-AS1 was overexpressed *in vitro*. Besides, it was discovered that there had been positive correlation between SOX4 and DSCAM-AS1 expression in tumor tissues. The above results revealed that DSCAM-AS1 may fulfill its function *via* SOX4.

Conclusions

We detected a new biomarker in the development of ovarian cancer. The results also indicate that lncRNA DSCAM-AS1 is vital in the carcinogenesis of ovarian cancer and can be served as a promising mark for ovarian cancer.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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