

LncRNA DS Cell Adhesion Molecule Antisense RNA 1 Facilitates Oral Squamous Cell Carcinoma Progression through The microRNA-138-5p/ Enhancer of Zeste 2 Polycomb Repressive Complex 2 Subunit Axis

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Abstract

Objective: A lot of lncRNAs are implicated in oral squamous cell carcinoma (OSCC) progression. The study aimed at investigating lncRNA DS cell adhesion molecule antisense RNA 1 (DSCAM-AS1)'s functional role and molecular mechanism in OSCC.

Materials and Methods: In this experimental study, a total of 46 pairs of OSCC samples and para-cancerous tissues were collected during surgery. In OSCC tissues and cell lines, quantitative real time polymerase chain reaction (qRT-PCR) was performed for detecting DSCAM-AS1 and microRNA-138-5p (miR-138-5p) expression levels. Western blot was conducted to examine the enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2) expression level. Then, DSCAM-AS1 was knocked down with siRNA in OSCC cells and MTT and EdU assays were conducted to evaluate OSCC cell proliferation. Transwell assay was utilized for detecting OSCC cell migration and invasion capacities. Besides, the relationships among DSCAM-AS1, miR-138-5p, and EZH2 were explored through RNA immunoprecipitation, dual-luciferase reporter assay, qRT-PCR, and Western blot.

Results: DSCAM-AS1 expression was remarkably increased in OSCC tissues and cell lines, and DSCAM-AS1 knockdown could significantly restrain OSCC cell proliferation, migration, and invasion. MiR-138-5p was identified as a target of DSCAM-AS1, and its inhibitor could reverse the suppressive effects of DSCAM-AS1 knockdown on OSCC progression. EZH2 was verified as a target of miR-138-5p, and EZH2 knockdown could counteract the promotional impact of miR-138-5p inhibitor on OSCC progression. Additionally, DSCAM-AS1, as a ceRNA, could regulate EZH2 expression via miR-138-5p.

Conclusion: DSCAM-AS1 can play a tumor-promoting role in OSCC via miR-138-5p/EZH2 axis.

Keywords: DSCAM-AS1, Oral Squamous Cell Carcinoma, Proliferation

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Introduction

Oral squamous cell carcinoma (OSCC) is a common malignancy occurring in the head and neck, accounting for more than 90% of cases of oral malignant diseases (1). There are more than 350,000 new cases each year and about 180,000 death cases worldwide (2). Although great progress has been made in surgery, chemotherapy, and radiotherapy, OSCC patients' five-year survival rate is merely 40-50% due to metastasis and chemotherapy/radiotherapy resistance (3). In such a context, a full understanding of the molecular mechanism underlying OSCC progression is urgently needed to uncover new therapeutic targets.

LncRNAs can take part in modulating various biological processes, such as cell proliferation, migration, and apoptosis (4). Previous research have shown that many lncRNAs, as tumor suppressors or promoters, partake in OSCC tumorigenesis and development, such as urothelial cancer associated 1 (5), homeobox A11 antisense RNA

(6), and TINCR ubiquitin domain containing (7). DS cell adhesion molecule antisense RNA 1 (DSCAM-AS1) expression is reported to be up-regulated in several tumors, for example, breast cancer (8), hepatocellular carcinoma (9), etc. However, its expression characteristics and functional role in OSCC are unclear.

MicroRNAs (miRs or miRNAs) target the 3'-UTR of mRNA to regulate gene expressions (10). The aberrant expression of miRNAs is linked with the pathogenesis of OSCC (11). Reportedly, miR-138-5p is underexpressed in OSCC, and it inhibits OSCC cell proliferation and invasion via targeting ISG15 ubiquitin like modifier (12); additionally, enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2) is reported as a target gene of miR-138-5p (13).

A large amount of research have demonstrated that the ceRNA network is involved in regulating tumor occurrence and development (14). Interestingly, bioinformatics analysis suggested that the sequence of DSCAM-AS1

contained the potential binding site for miR-138-5p. Given that EZH2 is a target gene of miR-138-5p, we supposed that DSCAM-AS1 could probably be a ceRNA to regulate miR-138-5p and EZH2 expressions.

Materials and Methods

Cell culture and transfection

Normal human oral epithelial cell line (NHOK) was purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China); OSCC cell lines (HSC-3, SCC-15, SCC-4, and CAL-27) were bought from the American Type Culture Collection (ATCC, Manassas, VA, USA). The above cell lines were cultured in RPMI-1640 medium (Sigma-Aldrich, St. Louis, MO, USA) with 100 U/ml penicillin, 100 µg/ml streptomycin, and 10% fetal bovine serum (FBS, Sigma-Aldrich, St. Louis, MO, USA) at 37°C in 5% CO₂.

Three small interference RNAs (siRNAs) against DSCAM-AS1 (si-DSCAM-AS1), scrambled siRNA control (si-NC), miR-138-5p mimics (miR-138-5p mi), negative control mimics (miR-NC), miR-138-5p inhibitor (miR-138-5p in), and EZH2 siRNA (si-EZH) were bought from the GenePharma (Shanghai, China). Lipofectamine™ 3000 (Invitrogen, Carlsbad, CA, USA) was adopted for transfecting them into cells.

Clinical sample collection

From March 2013 to January 2019, para-cancerous tissues and tumor tissues of 46 patients diagnosed with OSCC in the Affiliated Hospital of Chengde Medical College were collected during surgery, and two pathologists completed the pathological diagnosis. The tissues were immediately maintained in liquid nitrogen after the collection until RNA extraction. The subjects had not received any anti-tumor therapies such as radiotherapy and chemotherapy before the surgery.

Ethical approval

This study was reviewed, discussed, and endorsed by the Ethics Committee of the Affiliated Hospital of Chengde Medical College (Approval No. 201301006), and all of the subjects offered the signed informed consent before this study was performed.

Quantitative real time polymerase chain reaction

Total RNA of cell lines and tissues was extracted by TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and cDNA Reverse Transcription Kit (TaKaRa, Ltd., Dalian, China) was employed for reversely transcribing RNA into cDNA. Subsequently, the SYBR Premix Ex Taq™ kit (TaKaRa, Otsu, Shiga, Japan) was utilized for amplification, and eventually, the relative RNA expression was obtained through the 2^{-ΔΔCt} method. *U6* and *GAPDH* served as the internal references. Below are the quantitative real time polymerase chain reaction (qRT-PCR) primer sequences:

DSCAM-AS1-

F: 5'-GATCGGGAAAGCCAACCA-3'
R: 5'-TGGAGGAGGGACAGAGAAGG-3'

miR-138-5p-

F: 5'-AGCTGGTGTGTGAATCAGGCCG-3'
R: 5'-TGGTGTCGTGGAGTCG-3'

EZH2-

F: 5'-CCCTGACCTCTGTCTTACTTGTGGA-3'
R: 5'-ACGTCAG ATGGTGCCAGCAATA-3'

GAPDH-

F: 5'-TGCACCACCAACTGCTTAGC-3'
R: 5'-GGCATGGAC TGTGGTCATGAG-3'

U6-

F: 5'-CTCGCTTCGGCAGCACACA-3'
R: 5'-AACGCTTCACGAATTTGC GT-3'

MTT assay

The measurement of transfected cell proliferation was conducted with a MTT kit (Roche, Basel, Switzerland). After the cells were attached to the bottom of the wells and grew stably, 20 µL of MTT solution (5 mg/ml) was added to each well, and then the cells were incubated for 4 hours. Subsequently, 150 µL of dimethyl sulfoxide was added to each well, and the plate was shaken for 10 minutes to dissolve the formazan crystals. After that, a microplate reader (Bio-Rad, Hercules, CA, USA) was utilized to detect the absorbance of the cells at 490 nm. With the same method, the absorbance values were detected on the 24 hours, 48 hours, and 72 hours, respectively.

EdU assay

EdU Kit (RiboBio, Guangzhou, China) was used for EdU assay to detect the proliferation of the transfected cells. OSCC cells were transferred into 96-well plates, and then EdU staining solution was added to each well before the cells were incubated. 2 hours later, cells were fixed for 30 minutes with 4% paraformaldehyde and then incubated with glycine (ThermoFisher Scientific, Waltham, MA, USA) for 10 minutes. Next, Apollo Dye Solution was adopted for staining the cells, and DAPI solution (Beyotime, Shanghai, China) was used to stain the nuclei of the cells. Ultimately, a fluorescence microscope (Nikon, Tokyo, Japan) was employed to observe and count the EdU-positive cells.

Western blot

RIPA buffer (Sigma-Aldrich, Darmstadt, Germany) was used to lyse the transfected cells. Then SDS-PAGE was used to separate the protein samples in each group. Next, the proteins were transferred to PVDF membranes (GE Healthcare Life Sciences, Little Chalfont, UK). After blocking with 5% skimmed milk at 37°C for 2 hours, the PVDF membranes were incubated overnight with

primary antibodies (anti-EZH2 antibody: cat. no. 4905, 1:1000, Cell Signaling Technology, USA; anti-GAPDH: ab181602, 1:5000, Abcam, UK) at 4°C and subsequently incubated with the secondary antibody (Proteintech, Rosemont, IL, USA) for 1 hour at room temperature. The protein detection was performed by the enhanced chemiluminescence (ECL) substrate kit (Amersham Biosciences, Little Chalfont, UK).

Transwell assay

The migration and invasion of transfected cells were assessed by the Transwell chambers (8 µm pore size; Corning, Corning, NY, USA). For the invasion assay, the membranes were pre-coated with Matrigel (BD, Bedford, MA, USA), and this procedure was not performed in the migration assay. OSCC cells (2×10^4 /well) were transferred into the upper compartments with serum-free medium and RPMI-1640 medium was added to the bottom compartments (with 10% FBS). Following the incubation at 37°C for 24 hours, cells that passed through the filter to the bottom surface of the membrane were stained with crystal violet solution and then counted by a microscope (Nikon, Tokyo, Japan).

Dual-luciferase reporter assay

DSCAM-AS1 or *EZH2* 3'-UTR sequence with wild type (WT) or mutant (MUT) binding sites for *miR-138-5p* was respectively inserted into pmirGLO luciferase reporter plasmids (Promega, Madison, WI, USA). Then, Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) was employed to co-transfect the above-mentioned reporter vectors and *miR-138-5p* mimics or miR-NC into 293T cells. 36 hours after the transfection, the luciferase activity was detected by the dual-luciferase reporter assay system (Promega, Madison, WI, USA).

RIP assay

The Magna RIP Kit (Millipore, Billerica, MA, USA) was used for the RIP assay. In brief, RIP lysis buffer was utilized for lysing the transfected cells, which were incubated with magnetic beads (Millipore, Billerica, MA, USA) coated with Ago2 antibody (Anti-Ago2) or IgG antibody (Anti-IgG). Then the immunoprecipitated RNA was isolated with the TRIzol method and reversely transcribed into cDNA. Subsequently, the enrichment of *DSCAM-AS1* and *miR-138-5p* in the immunoprecipitate was analyzed by qRT-PCR.

Statistical analysis

The experiments were performed in triplicate, and the data were statistically analyzed by SPSS 19.0 software (SPSS Inc., Chicago, IL, USA) and expressed as mean \pm standard deviation. The student's *t*-test was conducted for the difference analysis between two groups, and the Chi-square test was conducted for examining the relationship between *DSCAM-AS1* expression and clinicopathological characteristics. $P < 0.05$ signified statistical significance.

Results

DSCAM-AS1 and *EZH2* expressions were increased in OSCC cell lines and tissues

qRT-PCR was used for detecting the expression of *DSCAM-AS1* in 46 OSCC patients' tumor tissues and para-cancerous tissues. It was revealed that *DSCAM-AS1* expression was remarkably increased in OSCC tissues compared to that in the paracancerous tissues (Fig.1A). Similarly, it was also uncovered that *DSCAM-AS1* expression was markedly enhanced in OSCC cell lines, including CAL-27, HSC-3, SCC-15, and SCC-4, in comparison with those in the cell line NHOK (Fig.1B, Fig.S1, See Supplementary Online Information at www.celljournal.org). Similarly, *EZH2* mRNA expression level was increased in OSCC tissues (Fig.1C) that was reflected at the protein level confirmed by the western blot analysis (Fig.1D). Then the 46 OSCC samples were averagely divided into high expression group and low expression group ($n=23$ in each group). We analyzed the association between *DSCAM-AS1* expression and OSCC patients' pathological features and found that high *DSCAM-AS1* expression was significantly correlated with lymph node metastasis and advanced clinical stage (Table 1). Therefore, *DSCAM-AS1* is likely to be a vital regulator in the OSCC progression.

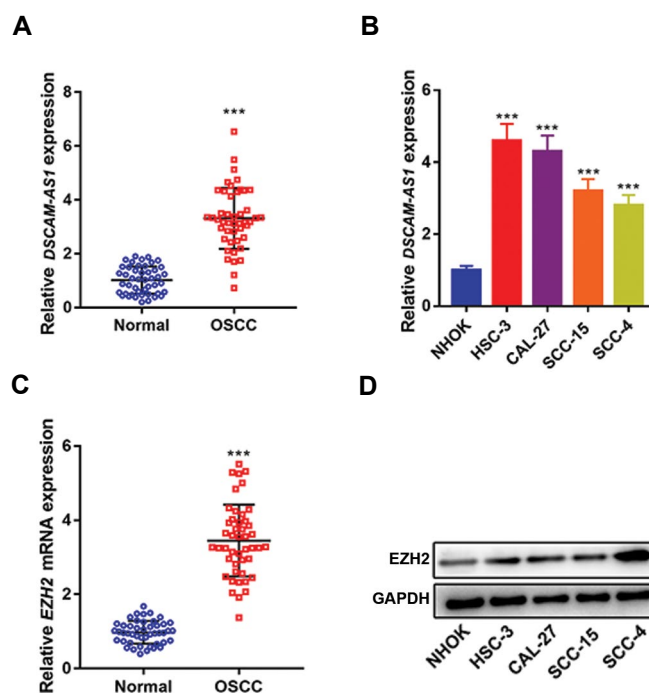


Fig.1: *DSCAM-AS1* and *EZH2* expressions were up-regulated in OSCC tissues and cell lines. **A.** qRT-PCR showed that *DSCAM-AS1* expression was upregulated in OSCC tissues. **B.** qRT-PCR showed that *DSCAM-AS1* expression was upregulated in OSCC cell lines. **C.** qRT-PCR showed that *EZH2* mRNA expression was upregulated in OSCC tissues. **D.** Western blot showed that *EZH2* expression was upregulated in OSCC cell lines. The experiments were performed in triplicate. ***, $P < 0.001$, OSCC; Oral squamous cell carcinoma, and qRT-PCR; Quantitative real time polymerase chain reaction.

Table 1: Correlation between *DSCAM-AS1* expression and pathological characteristics

Parameter	The number of patients	DSCAM-AS1 expression		P value
		High (n=23)	Low (n=23)	
Age (Y)				
≥ 60	28	11	17	0.069
< 60	18	12	6	
Gender				
Male	24	11	13	0.554
Female	22	12	10	
Tumor size (cm)				
≥ 5	34	16	18	0.501
< 5	12	7	5	
Clinical stage				
III-IV	22	17	5	0.0003
I-II	24	6	18	
Lymphatic metastasis				
Negative	25	16	9	0.038
Positive	21	7	14	

DSCAM-AS1; DS cell adhesion molecule antisense RNA 1.

Knocking down *DSCAM-AS1* could inhibit OSCC cell proliferation, migration, and invasion

To investigate *DSCAM-AS1*'s biological functions in OSCC progression, three siRNAs (si-*DSCAM-AS1*#1, #2, and #3) were used to knock down *DSCAM-AS1* expression in CAL-27 and HSC-3 cell lines. As shown in Figure 2, transfection of these *DSCAM-AS1* siRNAs notably decreased *DSCAM-AS1* expression, and *DSCAM-AS1* expression in the si-*DSCAM-AS1*#1 group was the lowest, thus si-*DSCAM-AS1*#1 was used for the subsequent experiments (Fig.2A). MTT assay showed that knocking down *DSCAM-AS1* markedly inhibited the proliferation of HSC-3 and CAL-27 cells (Fig.2B, C). EdU staining was used to detect proliferating cells, and DAPI staining the cell nuclei of alive cells (15). EdU assay showed that the percentage of EDU-positive cells was markedly decreased in the si-*DSCAM-AS1*#1 group. In addition, the Transwell assay manifested that silencing of *DSCAM-AS1* could dramatically inhibit HSC-3 and CAL-27 cell migration and invasion (Fig.2E, F).

MiR-138-5p was a target of *DSCAM-AS1*

To expound the potential mechanism by which *DSCAM-AS1* participated in OSCC progression, the StarBase v2.0 database (<http://starbase.sysu.edu.cn/>) was used to retrieve possible target miRNAs of *DSCAM-AS1*, and it was unmasked that *DSCAM-AS1* contained a binding site for *miR-138-5p* (Fig.3A). Subsequently, we used the luciferase reporter assay for examining the binding relationship between them and found that *miR-*

138-5p mimics could significantly reduce WT reporter's luciferase activity but would not affect MUT reporter's luciferase activity (Fig.3B). In addition, the RIP assay also confirmed that *DSCAM-AS1* was able to interact with *miR-138-5p* directly (Fig.3C, D). qRT-PCR indicated that *miR-138-5p* expression was markedly down-regulated in OSCC tissues and cell lines (Fig.3E, F). Notably, *DSCAM-AS1* knockdown in HSC-3 and CAL-27 cell lines induced a significant increase of *miR-138-5p* expression (Fig.3G). Therefore, it was concluded that *DSCAM-AS1* could sponge *miR-138-5p* and repress its expression.

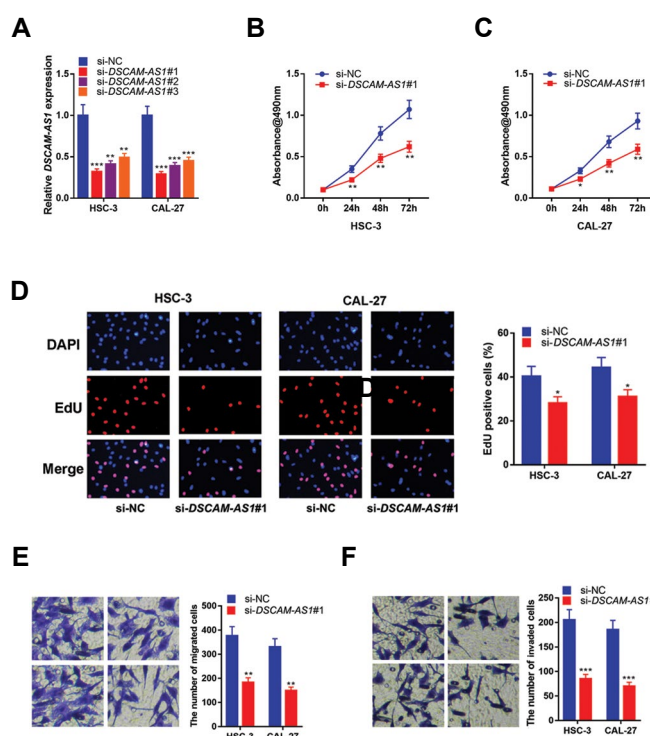


Fig.2: Knockdown of *DSCAM-AS1* inhibited OSCC cell proliferation, migration, and invasion. **A.** After transfection of *DSCAM-AS1* siRNA, *DSCAM-AS1* was downregulated in HSC-3 and CAL-27 cells, which was verified by qRT-PCR. **B.** **C.** MTT assay showed that OSCC cell proliferation was lower in the si-*DSCAM-AS1*#1 group. **D.** EdU assay showed that detect OSCC cell proliferation level was lower in the si-*DSCAM-AS1*#1 group. **E.** **F.** Transwell assay showed that OSCC cell migration and invasion abilities were decreased in the si-*DSCAM-AS1*#1 group. The experiments were performed in triplicate. *, P<0.05, **, P<0.01, ***, P<0.001, OSCC; Oral squamous cell carcinoma, qRT-PCR; Quantitative real time polymerase chain reaction, siRNA; Small interfering RNA, MTT; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, and EdU; 5-ethynyl-2'-deoxyuridine.

DSCAM-AS1 played a role in OSCC cells through *miR-138-5p*

Next, *miR-138-5p* inhibitors were transfected into HSC-3 and CAL-27 cells, and MTT, EdU, and Transwell assays were performed. The results elucidated that *miR-138-5p* inhibition markedly facilitated OSCC cell proliferation, migration, and invasion capabilities (Fig.4A-E). To confirm that *DSCAM-AS1* was involved in OSCC progression through repressing *miR-138-5p* expression, rescue experiments were performed. The

results suggested that *miR-138-5p* inhibitors were able to significantly abolish *DSCAM-AS1* knockdown-induced inhibitory impact on OSCC cell proliferation, migration,

and invasion (Fig.4F, J). These findings suggested that *DSCAM-AS1* could promote OSCC progression via suppressing *miR-138-5p* expression.

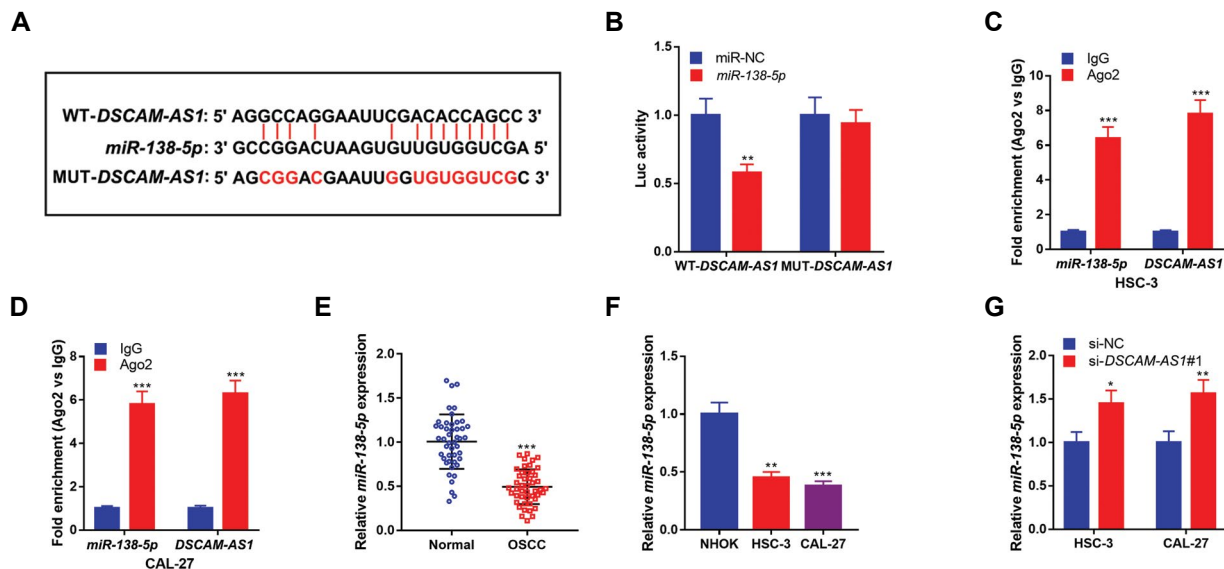


Fig.3: *MIR-138-5p* was a target of *DSCAM-AS1*. **A**, StarBase database predicted that *DSCAM-AS1* contained the complementary binding site for *miR-138-5p*. **B**, Luciferase reporter assay confirmed that the *DSCAM-AS1* and *miR-138-5p* could bind directly to each other. **C, D**, RIP assay verified the direct interaction between *miR-138-5p* and *DSCAM-AS1*. **E, F**, qRT-PCR showed that the expression of *miR-138-5p* was downregulated in the OSCC tissues and cell lines. **G**, qRT-PCR showed that *miR-138-5p* expression was upregulated in OSCC cell lines transfected with *DSCAM-AS1* siRNA. The experiments were performed in triplicate. *, P<0.05, **, P<0.01, ***, P<0.001, RIP; RNA immunoprecipitation, qRT-PCR; Quantitative real time polymerase chain reaction, OSCC; Oral squamous cell carcinoma, and siRNA; Small interfering RNA.

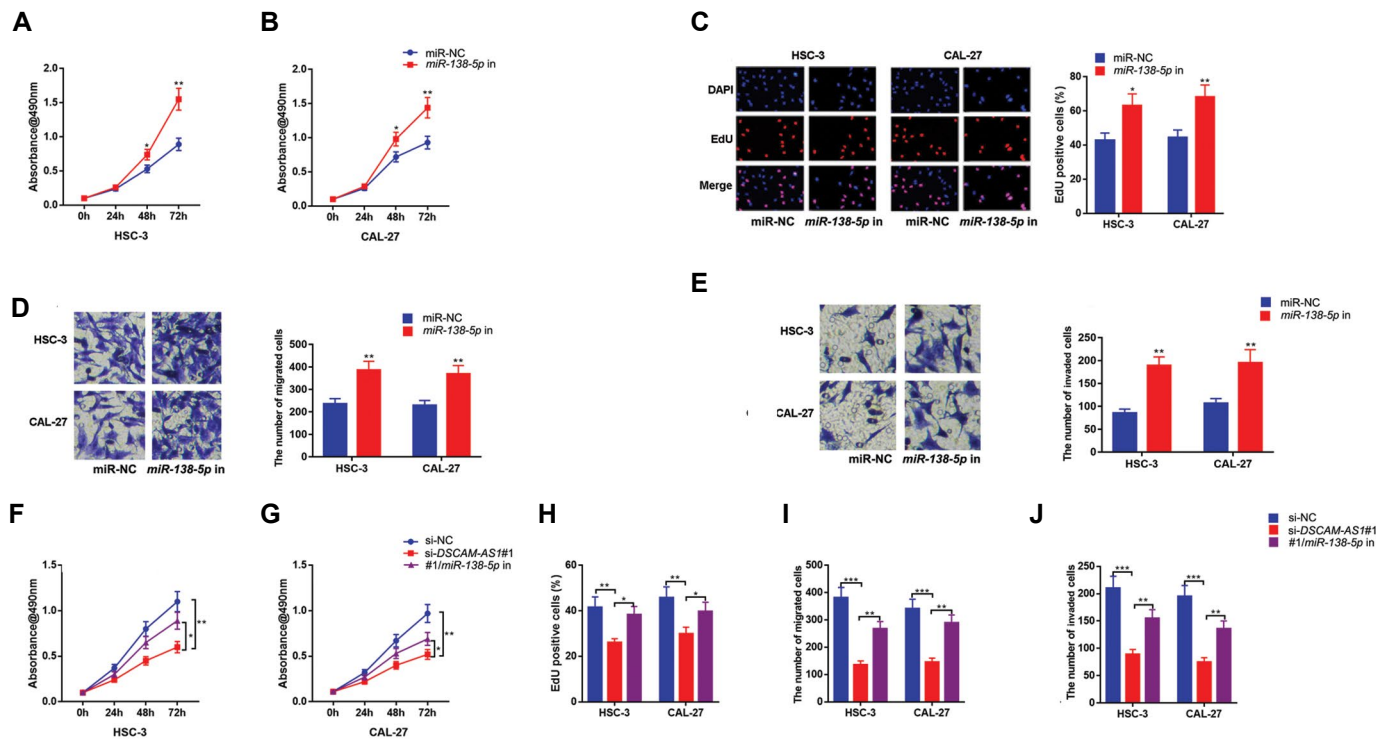


Fig.4: *DSCAM-AS1* played a role in OSCC cells via regulating *miR-138-5p* expression. **A-C**, MTT assay and EdU assay showed that OSCC cell proliferation was increased in OSCC cells transfected with *miR-138-5p* inhibitor. **D, E**, Transwell assay showed that cell migration and invasion abilities were increased in OSCC cells transfected with *miR-138-5p* inhibitor. **F-H**, MTT and EdU assay showed that OSCC cell proliferation was increased in the OSCC cells co-transfected with *DSCAM-AS1* siRNA and *miR-138-5p* inhibitors compared with OSCC cells transfected with *DSCAM-AS1* siRNA. **I, J**, Transwell assay showed that OSCC cell migration and invasion abilities were increased in the OSCC cells co-transfected with *DSCAM-AS1* siRNA and *miR-138-5p* inhibitors compared with OSCC cells transfected with *DSCAM-AS1* siRNA. The experiments were performed in triplicate. *, P<0.05, **, P<0.01, ***, P<0.001, OSCC; Oral squamous cell carcinoma, MTT; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, EdU; 5-ethynyl-2'-deoxyuridine, and siRNA; Small interfering RNA.

MiR-138-5p could directly target EZH2 to inhibit OSCC progression

Next, we use 5 online databases (microT, TargetScan, PicTar, miRmap, and miRanda) to search the potential targets of *miR-138-5p*, and a Venn diagram depicted that there were 85 genes predicted by all of the bioinformatics tools, and *EZH2* was among them (Fig.5A). Subsequently, dual-luciferase reporter assay showed that *miR-138-5p* mimics notably reduced the luciferase activity of the WT *EZH2* reporter vector but had no impact on the MUT *EZH2* reporter (Fig.5B). In addition, the RIP assay showed that *miR-138-5p* and *EZH2* mRNA were directly interacted (Fig.5C, D). Moreover, rescue experiments revealed that *EZH2* siRNA could partly counteract the *miR-138-5p* inhibitor-induced promoting impact on OSCC cell proliferation, migration, and invasion (Fig.5E-I). The

aforementioned evidence confirmed that *miR-138-5p* could regulate OSCC progression by inhibiting *EZH2*.

DSCAM-AS1 regulated EZH2 expression via miR-138-5p

Eventually, to substantiate the regulatory effects of *DSCAM-AS1* on *miR-138-5p* and *EZH2* expressions in OSCC cells, we transfected *DSCAM-AS1* siRNA or *DSCAM-AS1* siRNA+*miR-138-5p* inhibitors into HSC-3 and CAL-27 cell lines, respectively. Our data demonstrated that knocking down *DSCAM-AS1* observably inhibited *EZH2* expression, and the co-transfection of *miR-138-5p* inhibitors partially abolished the inhibiting impact of *DSCAM-AS1* knockdown on the *EZH2* expression (Fig.5J, K). Hence, it was concluded that *DSCAM-AS1* could regulate *EZH2* expression through repressing *miR-138-5p* expression.

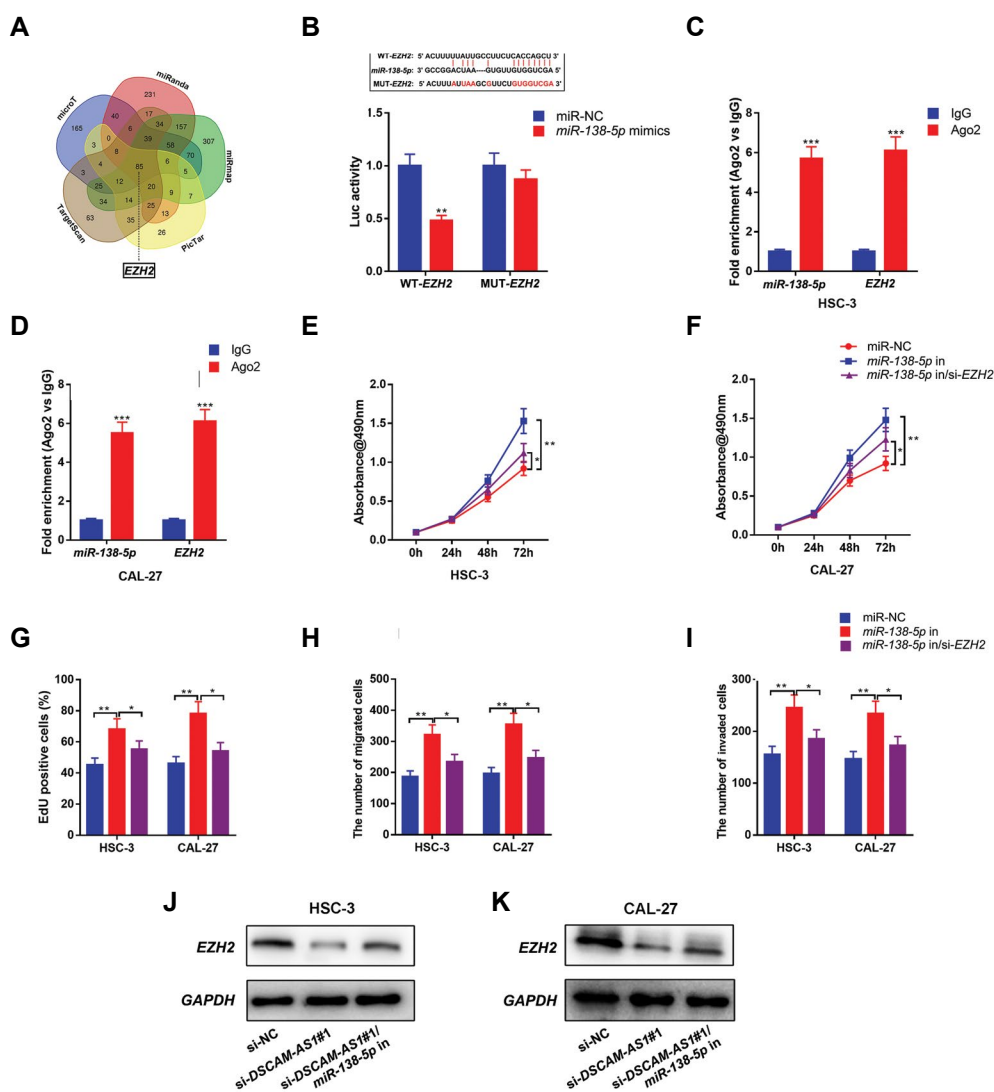


Fig.5: *MIR-138-5p* could directly target *EZH2* to inhibit OSCC progression. **A.** The targets of *miR-138-5p* were predicted by five online databases. **B.** StarBase predicted that the 3'UTR of *EZH2* contained a binding site for *miR-138-5p*, and dual-luciferase reporter assay confirmed that *miR-138-5p* and the 3'UTR of *EZH2* could directly bind to each other. **C, D.** RIP assay verified the relationship between *miR-138-5p* and *DSCAM-AS1*. **E-G.** MTT and EdU assay showed that OSCC cell proliferation was decreased in OSCC cells co-transfected with *miR-138-5p* inhibitors and *EZH2* siRNA compared with OSCC cells transfected with *miR-138-5p* inhibitors. **H, I.** Transwell assay showed that OSCC cell proliferation was decreased in OSCC cells co-transfected with *miR-138-5p* inhibitors and *EZH2* siRNA compared with OSCC cells transfected with *miR-138-5p* inhibitors. **J, K.** Western blot showed that the expression of *EZH2* in OSCC cell lines was increased in the OSCC cells co-transfected with *DSCAM-AS1* siRNA and *miR-138-5p* inhibitors compared with OSCC cells transfected with *DSCAM-AS1* siRNA. The experiments were performed in triplicate. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$, OSCC; Oral squamous cell carcinoma, siRNA; Small interfering RNA, RIP; RNA immunoprecipitation, MTT; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, and EdU; 5-ethynyl-2'-deoxyuridine.

Discussion

LncRNAs feature prominently in cancer biology. Accumulating studies confirmed that DSCAM-AS1 contributes to promoting the carcinogenesis and disease progression of multiple cancers. For example, DSCAM-AS1 can enhance ribonucleotide reductase regulatory subunit M2 expression via inhibiting miR-204-5p expression, thus promoting breast cancer cell proliferation (8). DSCAM-AS1 expression is remarkably elevated in hepatocellular carcinoma cell lines and tissues, and DSCAM-AS1 promotes hepatocellular carcinoma cell proliferation and migration through targeting miR-338-3p (9). In this study, it was found that DSCAM-AS1 expression was significantly up-regulated in OSCC tumor tissues and cell lines; high DSCAM-AS1 expression was linked to unfavorable pathological characteristics of OSCC patients; *In vitro* experiments confirmed that knocking down DSCAM-AS1 significantly inhibited OSCC cell proliferation, migration, and invasion. Such findings exhibited that DSCAM-AS1 could probably play a role as a cancer-promoting factor in OSCC.

LncRNAs can act as ceRNAs by sponging miRNAs to affect the expression of mRNAs and thus play a role in OSCC (16). For example, lncRNA nuclear paraspeckle assembly transcript 1 can absorb miR-365 as a molecular sponge to regulate regulator of G protein signaling 20, thus promoting OSCC cell proliferation and invasion (17). LncRNA relaxin 1, as a ceRNA of miR-138, regulates EZH2 and promotes OSCC cell proliferation and invasion (13). HOXA11-AS adsorbs miR-98-5p to up-regulate the expression of Y-box binding protein 2, thereby facilitating OSCC development (18). To delve deeper into the underlying mechanism of DSCAM-AS1 in OSCC, we used the StarBase database to predict the miRNAs that were potentially regulated by DSCAM-AS1, and then miR-138-5p was selected for further research. As a tumor suppressor, miR-138-5p participates in the development of multiple tumors. For instance, miR-138-5p expression is significantly reduced in colorectal cancer cell lines and tissues, and miR-138-5p can inhibit cell proliferation by inhibiting programmed cell death 1 ligand 1 (19). In OSCC, it's been shown that miR-138 expression is notably down-regulated in cancer tissues and cell lines, and miR-138 is able to suppress cell proliferation, migration, and invasion via modulating ISG15 (12). Another study reported that lncRNA H19 imprinted maternally expressed transcript suppresses the expression of EZH2 by inhibiting miR-138 expression to play a cancer-promoting role in OSCC (13). In this work, we demonstrated that DSCAM-AS1 and miR-138-5p were able to bind directly to each other, miR-138-5p expression was markedly reduced in OSCC cell lines and tissues, and miR-138-5p could repress the malignant biological behaviors of OSCC cells. Furthermore, DSCAM-AS1 knockdown in OSCC cells elevated miR-138-5p expression, and miR-138-5p inhibitors partly abolished the inhibitory effect

of DSCAM-AS1 knockdown on the malignancy of OSCC cells. Therefore, our findings unearthed that miR-138-5p was likely to suppress OSCC expression, miR-138-5p was a downstream target of DSCAM-AS1, and DSCAM-AS1 played a cancer-promoting role in OSCC by inhibiting miR-138-5p expression.

Given that lncRNA acts as a ceRNA to eliminate the inhibitory effect of miRNA on target genes, the target genes of miRNA are important parts of the ceRNA network. In the present work, EZH2 was proven to be a target of miR-138-5p, which is consistent with the previous report (13). As the core part of the polycomb repressive complex 2, EZH2 plays a cancer-promoting role in many tumors. A previous study verified that EZH2 can inhibit cyclin dependent kinase inhibitor 1A expression to promote the proliferation of gastric cancer cells (20); in the head and neck squamous cell carcinoma, EZH2 participates in tumor progression by regulating EMT (21). In the current study, we uncovered that EZH2 expression was markedly enhanced in OSCC cell lines and tissues. Besides, knocking down EZH2 could partially counteract the promotional impact of miR-138-5p inhibition on OSCC progression. These findings indicated that miR-138-5p repressed OSCC development via suppressing EZH2 expression. Further experiments showed that EZH2 expression was modulated by DSCAM-AS1/miR-138-5p axis. The above-mentioned evidence confirmed that the DSCAM-AS1/miR-138-5p/EZH2 axis participated in regulating OSCC progression.

Conclusion

We identified a novel oncogenic lncRNA, DSCAM-AS1, in OSCC. It was demonstrated that DSCAM-AS1 increases OSCC cell proliferation, migration, and invasion through modulating miR-138-5p/EZH2 axis. DSCAM-AS1 may be a potential biomarker and target for OSCC diagnosis and therapies.

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Authors' Contributions

Y.Z., F.H.; Participated in study design and drafting the manuscript. Y.Z., X.W., P.W.; Contributed extensively in interpretation of the data and the conclusion. Y.Z., X.W., X.Z., S.H.; Performed data collection and experiments. All authors performed editing read and approved the final manuscript.

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