Long non-coding RNA DSCAM-AS1 functions as an oncogene in thyroid cancer via regulating miR-211

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Abstract. Long non-coding RNA Down syndrome cell adhesion molecule-antisense 1 (DSCAM-AS1) has been reported to play key roles in the progression and initiation of several cancer types. However, the various functional roles of DSCAM-AS1 in thyroid cancer tumorigenesis remain largely elusive. In the present study, the expression of DSCAM-AS1 was examined in thyroid cancer tissues and cell lines. Cell Counting Kit-8, wound healing, Transwell and clonogenic assays were conducted to detect cell proliferation, migration, invasion and colony formation, respectively. The association of DSCAM-AS1 with microRNA 211 (miR-211) was determined by luciferase reporter assay. It was found that the expression of DSCAM-AS1 was upregulated in thyroid cancer cells and tissues. Furthermore, enhanced DSCAM-AS1 expression was positively associated with lymph node metastasis and tumor-node-metastasis stage. Functional experiments demonstrated that DSCAM-AS1 knockdown inhibited the migration, proliferation and invasion of TPC-1 cells. Mechanistically, DSCAM-AS1 could bind to miR-211. Prevention of miR-211 by a miR-211 inhibitor reversed the effect of DSCAM-AS1 depletion in thyroid cancer tumorigenesis. Briefly, the current findings suggested that knockdown of DSCAM-AS1 suppressed the tumorigenesis of thyroid cancer via regulating miR-211, suggesting that DSCAM-AS1 may be a favorable therapeutic target for thyroid cancer.

Introduction

Thyroid cancer is one of the most prevalent tumors of the endocrine system, and remains a global problem (1). Considerable progression has been made in the therapeutic approaches against this cancer type, such as radio/chemotherapy, surgical resection and target therapy (2). In addition, combination therapy with Lenvatinib and Radioactive Iodine in preclinical models also gained favorable clinical outcomes for patients with advanced thyroid cancer (3). However, the long-time prognosis of patients with thyroid cancer and tumor metastasis is poor due to resistance to radio/chemotherapy and/or post-surgical recurrence (4,5). Therefore, exploring new diagnostic biomarkers or therapeutic objectives is an urgent need to increase the survival rate of patients with thyroid cancer.

One of the main subcategories of non-coding RNA is long non-coding RNA (lncRNA). lncRNAs have no protein-coding capability and are >200 nucleotides in length (6). Previous evidence suggested that lncRNAs were involved in various biological processes, including cancer cell proliferation, cycle, apoptosis, migration, invasion (7,8). A number of lncRNAs were identified to act as oncogenes or tumor suppressors in thyroid cancer (9-11), suggesting that lncRNAs could be implemented as therapy targets and markers of diagnosis for this cancer type.

lncRNAs exert their biological function by sponging microRNAs (miRNAs or miRs) (12). miRNAs were reported to be involved in the progression of various cancer types, including cancer cell proliferation, migration, cell cycle and apoptosis (13,14). miRNAs were shown to serve as diagnostic and therapy targets for thyroid cancer (15,16).

lncRNA Down syndrome cell adhesion molecule-antisense 1 (DSCAM-AS1) was reported to affect cancer cell proliferation, migration and invasion in multiple cancers, such as breast cancer (17), hepatocellular carcinoma (18), ovarian cancer (19), non-small cell lung carcinoma (20), cervical cancer (21) and melanoma (21). However, the clinical significance, functions and strategies associated with DSCAM-AS1 in the context of thyroid cancer have remained mainly ambiguous. The present study investigated the expression of DSCAM-AS1 within samples of patients with thyroid cancer, as well as its clinical significance, and assessed the biological application and potential therapeutic strategy of DSCAM-AS1 in thyroid cancer by conducting a number of *in vivo* and *in vitro* experiments.

Materials and methods

Patient samples. A total of 48 paired adjacent normal tissues and thyroid cancer tissues were harvested from patients (age range, 18-72 years) who underwent surgery at Ningbo Medical

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Centre Lihuili Hospital (Ningbo, China) between January 2019 and January 2020. Clinical stage classification was conducted according to the World Health Organization categorization. None of the patients had received any anticancer treatment prior to the operation. The clinicopathological factors of patients are shown in Table I. A written informed consent was obtained from all patients, and the study was approved (approval no. KY2022SL376-01) by the Ethics Committee of Ningbo Medical Centre Lihuili Hospital (Ningbo, China).

Cell culture and transfection. The human normal thyroid cell line Nthy-ori3-1 and human thyroid cancer TPC-1 cells were obtained from the Conservation Genetics Shanghai Cell Bank. The purchased cells were cultured in RPMI-1640 medium (Invitrogen; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) in the presence of 5% CO_2 at 37˚C.

A total of 3 small hairpin RNAs (shRNAs) suppressing DSCAM-AS1 expression (namely sh-DSCAM-AS1#1, 5'-CCT CCTCCAACTGCCATTTT-3'; sh-DSCAM-AS1#2, 5'-GTT CTGGTCTCATCATGATT-3' and sh-DSCAM-AS1#3, 5'-CAC ATAGGCATGACATACTTT-3'), and corresponding negative control (sh-NC, 5'-TTCTCCGAACGTGTCACGTTT-3') were prepared by Shanghai GenePharma Co., Ltd., and were cloned into pGPU6/Hygro vector. miR-211 mimics (miR-211, 5'-UUCCCUUUGUCAUCCUUCGCCU-3'), miR-211 inhibitor (anti-miR-211, 5'-AGGCGAAGGAUGACAAAGGGAA-3') and their negative controls (miR-NC, 5'-AGAAGCUGUUCCAAG GUGGGCC-3' and anti-miR-NC, 5'-GAACAUCCAGGGUCC CGUUCCU-3' respectively), were obtained from Guangzhou RiboBio Co., Ltd. To perform cell transfection, $5x10^5$ TPC-1 cells were plated in 96-well plates and incubated for 24 h at 37˚C. Transfection of shRNAs (100 nM), inhibitors (100 nM) and mimics (100 nM) into the TPC-1 cells was conducted with Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. After 24 h of transfection at 37˚C, the cells were used for follow-up experiments. G418 (0.5 mg/ml; Sigma-Aldrich; Merck KGaA) was utilized to select stably transfected cells.

Extraction of RNA and reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from cell lines and tissues by using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the protocol of the manufacturer. RT was carried out to synthesize cDNA using the PrimeScript RT Reagent Kit (Takara Biotechnology Co., Ltd.) according to the manufacturer's protocol. For the quantification of DSCAM-AS1 expression, qPCR was performed with SYBR® Premix Ex Taq™ II kit (Takara Biotechnology Co., Ltd.) on a 7500 Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.). For the quantification of miR-211, qPCR was conducted by using TaqMan™ MicroRNA Assays (Applied Biosystems; Thermo Fisher Scientific, Inc.) on a 7500 Real-Time PCR System. The reaction conditions were as follows: 95˚C for 2 min, and 40 cycles of 95˚C for 35 sec and 56˚C for 40 sec. The sequences of the primers were as follows: U6 forward, 5'-AAAGCAAATCATCGGACGACC-3' and reverse, 5'-GTACAACACATTGTTTCCTCGGA-3'; miR-211 forward, 5'-TCGGCAGGTCCCTTTGTCATCC-3' and reverse, 5'-AGGCGAAGGATGACAAAGGGTT-3'; GAPDH forward, 5'-GGAGCGAGATCCCTCCAAAAT-3' and reverse, 5'-GGCTGTTGTCATACTTCTCATGG-3'; and DSCAM-AS1 forward, 5'-ACAGAGATGGACGACGGA TC-3', and reverse, 5'-TTGTGAGCCTGAGAGATCCC-3'. The levels of DSCAM-AS and miR-211 were normalized to those of GAPDH and small nuclear RNA U6, respectively. The $2^{-\Delta\Delta Cq}$ method was utilized to assess gene expression (22).

Cell proliferation assessment. The ability of cell proliferation in thyroid cancer cell lines was assessed via Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Inc.) assay based on our previous study (19).

Colony formation assay. The ability of TPC-1 cells to form colonies was assessed as described in our previous study (19).

Transwell cell invasion assay. A total of $5x10^4$ transfected cells in 200 μl serum-free medium were placed in the upper chamber of a Transwell plate (pore size of the insert, ~8 μm; EMD Millipore) pre-coated with Matrigel (precoating at 37˚C for 2 h; BD Biosciences), while RPMI-1640 containing 10% FBS was added to the lower chamber. After 24 h, the invasive cells located in the lowest part of the surface were fixed with 4% polyoxymethylene at 25˚C for 30 min and subsequently stained by using 0.1% crystal violet (Sigma-Aldrich; Merck KGaA) at 25˚C for 30 min. The invasive cells in 5 randomly selected visual fields were counted under a light microscope (X71; Olympus Corporation).

Wound healing assay. Transfected cells were added to six-well culture plates and cultured in serum-free medium until 100% confluence. Next, a wound area was created by scratching the cell monolayer with a 10-μl pipette tip. Scratch wounds at 0 and 24 h were depicted by utilizing a microscope (X71; Olympus Corporation).

Isolation of cytoplasmic/nuclear RNA. The Cytoplasmic & Nuclear RNA Purification Kit (Norgen Biotek Corp) was utilized to isolate cytoplasmic/nuclear RNA from TPC-1 cells according to the manufacturer's instructions. DSCAM-AS1 expression was assessed by RT-qPCR, as aforementioned. U6 was utilized as the nuclear control, while GAPDH was utilized as the cytoplasmic control.

Luciferase reporter assay. The possible sites of binding among DSCAM-AS1 and miR-211 were estimated using ENCORI (http://starbase.sysu.edu.cn/). The DSCAM-AS1 sequences with the estimated wild-type (Wt) or mutant (Mut) miR-211 binding sites were prepared through chemical synthesis and subsequently cloned into the luciferase reporter vector psi-CHECK-2 (Promega Corporation). The recombinant plasmids were named Wt-DSCAM-AS1 (5'-CUUGUGAUUCUUUCAAAGGGAA-3') and Mut-DSCAM-AS1 (5'-CUUGUGAUUCUUUGUUUCCCU A-3'), respectively. For calculating the luciferase reporter activity, TPC-1 cells were co-transfected with recombinant reporter plasmids and miR-211 mimics or miR-NC with Lipofectamine 3000® (Invitrogen; Thermo Fisher Scientific, Inc.). After 24 h, the luciferase activities were determined with a Dual-Luciferase Reporter Assay (Promega Corporation) according to the manufacturer's instructions, and normalized to *Renilla* luciferase activity.

Table I. Association of DSCAM-AS1 expression with clinicopathologic factors of 48 patients with thyroid cancer.

Clinicopathological factors	Cases, n	DSCAM-AS1 expression		P-value
		High	Low	
Age, years				0.2461
<40	22	9	13	
≥40	26	16	10	
TNM stage				0.0088
I-II	35	14	21	
III-IV	13	11	2	
Tumor size, cm				0.2209
≤1	33	15	18	
>1	15	10	5	
Lymphatic metastasis				0.0002
No	36	14	22	
Yes	12	11	1	

DSCAM-AS1, Down syndrome cell adhesion molecule-antisense 1; TNM, tumor-node-metastasis.

RNA immunoprecipitation (RIP) assay. RIP experiments were implemented to investigate the binding between miR-211 and endogenous DSCAM-AS1 in TPC-1 cells by using the Magna RIP™ RNA-Binding Protein Immunoprecipitation Kit (cat. no. 17-701; Merck KGaA) according to the manufacturer's instructions. In brief, cells were lysed with RIP lysis buffer, and then incubated with RIP buffer containing 50 μl magnetic beads conjugated with human anti-argonaute-2 (Ago2) antibody (EMD Millipore) or negative control IgG (EMD Millipore). Upon protein removal, the RNA precipitates were isolated using TRIzol® reagent according to the manufacturer's protocol, and then subjected to RT-qPCR for detection of DSCAM-AS1 and miR-211 expression.

Statistical analysis. Statistical analysis was performed with SPSS 20.0 (IBM Corp.) and GraphPad Prism 5.0 (GraphPad Software, Inc.). Results are presented as the mean ± standard deviation of ≤3 replicates. An unpaired or paired Student's t-test was used to analyze the comparisons between two groups, while one-way ANOVA followed by the Tukey's post hoc test was utilized for multiple-group comparisons. The association between DSCAM-AS1 and clinical pathology criteria was determined by Pearson's c^2 test. The correlation between miR-211 and DSCAM-AS1 expression was assessed with Spearman's correlation test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Elevated DSCAM-AS1 expression is correlated with weak prognosis of patients with thyroid cancer. To explore the relevance of DSCAM-AS1 in the development of thyroid cancer, the expression of DSCAM-AS1 was detected in 48 pairs of thyroid cancer tissues and adjacent normal tissues. The expression of DSCAM-AS1 was considerably increased in thyroid cancer tissues in comparison with that in adjacent normal tissues (Fig. 1A). The expression of DSCAM-AS1 in human thyroid cancer cell lines was also examined, and its was found that the expression of DSCAM-AS1 in TPC-1 cells was considerably greater than that in normal thyroid cells Nthy-ori3-1 (Fig. 1B).

The association of DSCAM-AS1 with the clinicopathological characteristics of patients with thyroid cancer was investigated. The 48 cases were divided into two categories: i) DSCAM-AS1-low group (n=22) and ii) DSCAM-AS1-high group (n=26) based on the mean value of DSCAM-AS1 expression in thyroid cancer. As illustrated in Table I, high expression of DSCAM-AS1 was positively correlated with lymph node metastasis and advanced clinical stage.

DSCAM-AS1 knockdown inhibits cell proliferation and colony formation of thyroid cancer cells. To explore the role of DSCAM-AS1 in thyroid cancer cells, loss-of function assays were performed in TPC-1 cells by transfection with three shRNAs specific for DSCAM-AS1 (namely sh-DSCAM-AS1#1, sh-DSCAM-AS1#2 and sh-DSCAM-AS1#3). It was found that the three shRNAs against DSCAM-AS1 were all able to decrease DSCAM-AS1 expression in TPC-1 cells (Fig. 2A), and sh-DSCAM-AS1#2 displayed the most effectiveness, and was selected for subsequent experiments (referred to as sh-DSCAM-AS1).

CCK-8 assay revealed that DSCAM-AS1 depletion led to a notable decrease in cell proliferation in TPC-1 cells (Fig. 2B). In addition, colony formation assay revealed that DSCAM-AS1 depletion led to a significant decrease in colony formation in TPC-1 cells (Fig. 2C).

DSCAM-AS1 knockdown inhibits the invasion and migration of thyroid cancer cells. The impact of DSCAM-AS1 depletion on cell invasion and migration in thyroid cancer cells was studied by Transwell and wound healing assays, respectively. It was revealed that the invasion and migration capabilities of TPC-1 cells were significantly reduced when DSCAM-AS1 was downregulated (Fig. 3A and B).

DSCAM-AS1 binds to miR-211 in thyroid cancer cells. To ascertain the mechanism of DSCAM-AS1 in cancer progression, miR-211 was determined to be a potential target of DSCAM-AS1 by using ENCORI software. DSCAM-AS1 has a binding site on miR-211 (Fig. 4A). In addition, DSCAM-AS1 was mainly located in the cytoplasm of TPC-1 cells (Fig. 4B); thus, DSCAM-AS1 may regulate miRNAs as a sponge. To examine this hypothesis, a luciferase assay was conducted. As revealed in Fig. 4C, overexpression of miR-211 in TPC-1 cells by transfection using miR-211 mimics could reduce Wt-DSCAM-AS1 luciferase activity (Wt-DSCAM-AS1). However, it did not affect the mutant type of DSCAM-AS1 (Mut-DSCAM-AS1) (Fig. 4C). To further confirm this result, RIP assay was performed. The results demonstrated that both DSCAM-AS1 and miR-211 expression were increased in Ago2-rich beads in thyroid cancer cells (Fig. 4D).

Moreover, it was found that overexpression of miR-211 significantly suppressed DSCAM-AS1 expression (Fig. 4E), while DSCAM-AS1 depletion enhanced miR-211 expression

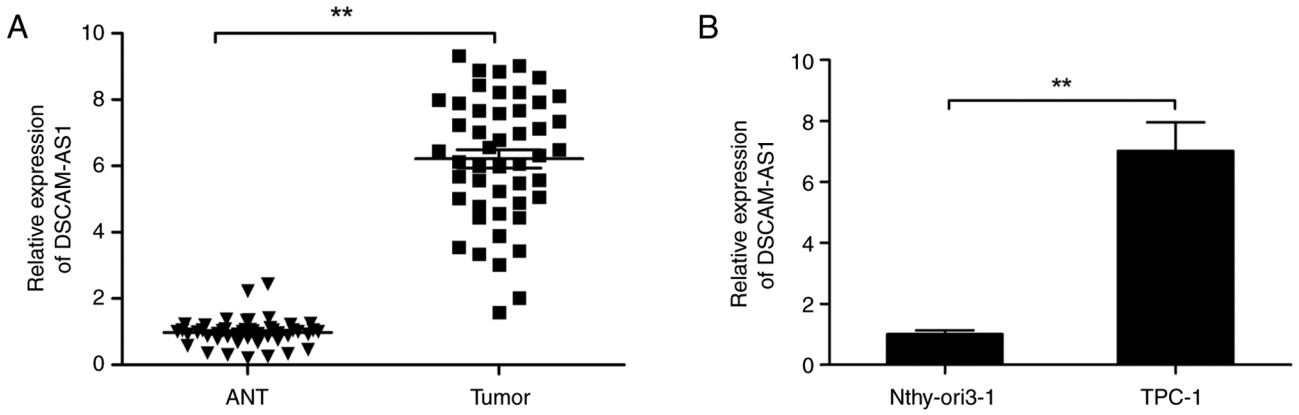

Figure 1. DSCAM-AS1 expression is elevated and associated with poor prognosis of patients with thyroid cancer. (A) DSCAM-AS1 expression was increased in thyroid cancer tissues compared with that in adjacent normal tissues (n=48). (B) DSCAM-AS1 expression was increased in a human thyroid cancer cell line (TPC-1) compared with that in a human normal thyroid cell line (Nthy-ori3-1). The results are shown as the mean ± standard deviation of 3 independent experiments. **P<0.01. DSCAM-AS1, Down syndrome cell adhesion molecule-antisense 1.

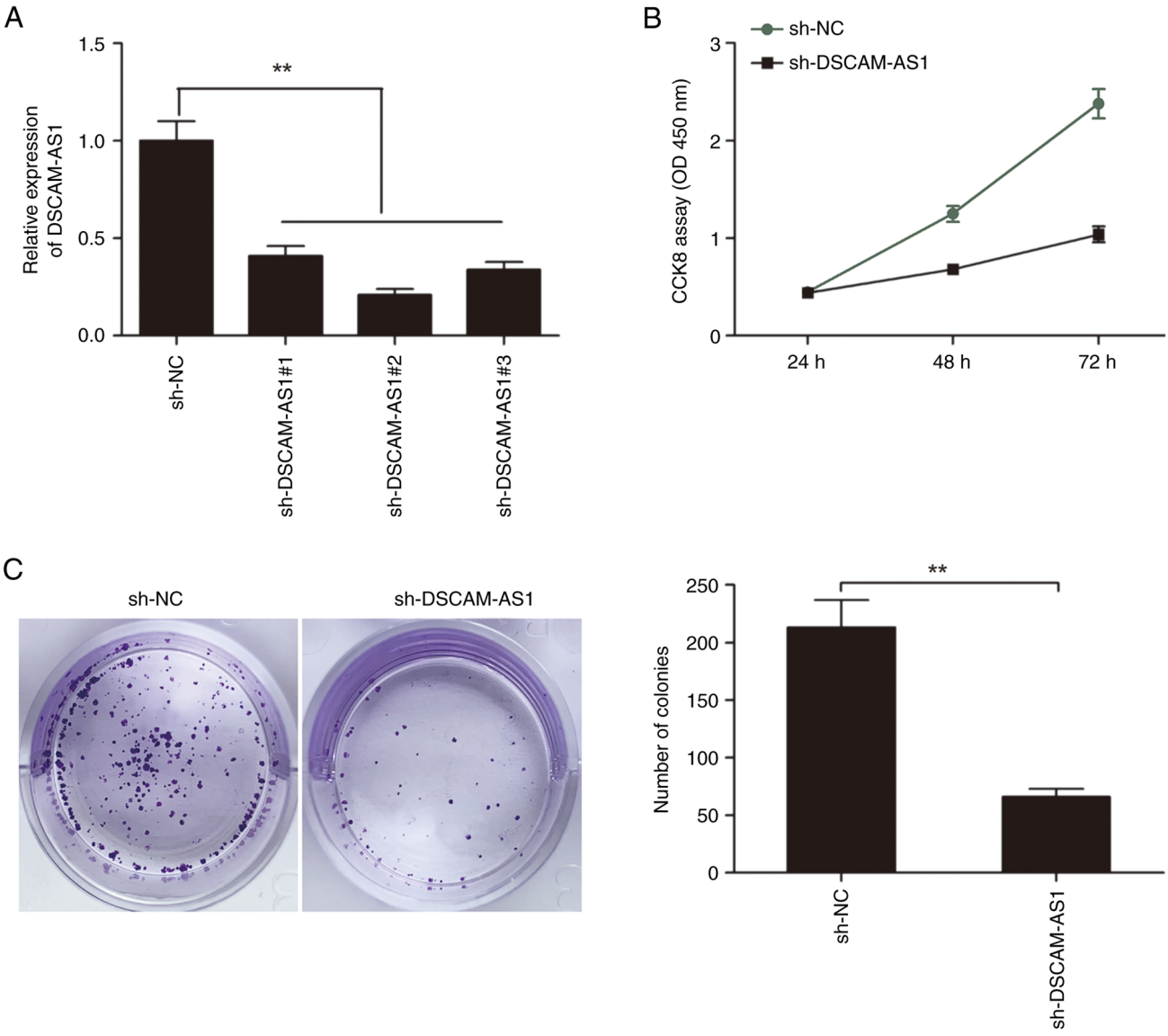

Figure 2. DSCAM-AS1 knockdown inhibits cell proliferation and colony formation in thyroid cancer cells. (A) The transfection efficiency of three shRNAs against DSCAM-AS1 (namely sh-DSCAM-AS1#1, sh-DSCAM-AS1#2 and sh-DSCAM-AS1#3) was confirmed in TPC-1 cells via reverse transcription-quantitative PCR. (B) Knockdown of DSCAM-AS1 inhibited the proliferation ability of TPC-1 cells. (C) Knockdown of DSCAM-AS1 inhibited the colony formation ability of TPC-1 cells. The results are shown as the mean ± standard deviation of 3 independent experiments. **P<0.01. DSCAM-AS1, Down syndrome cell adhesion molecule-antisense 1; shRNA, small hairpin RNA.

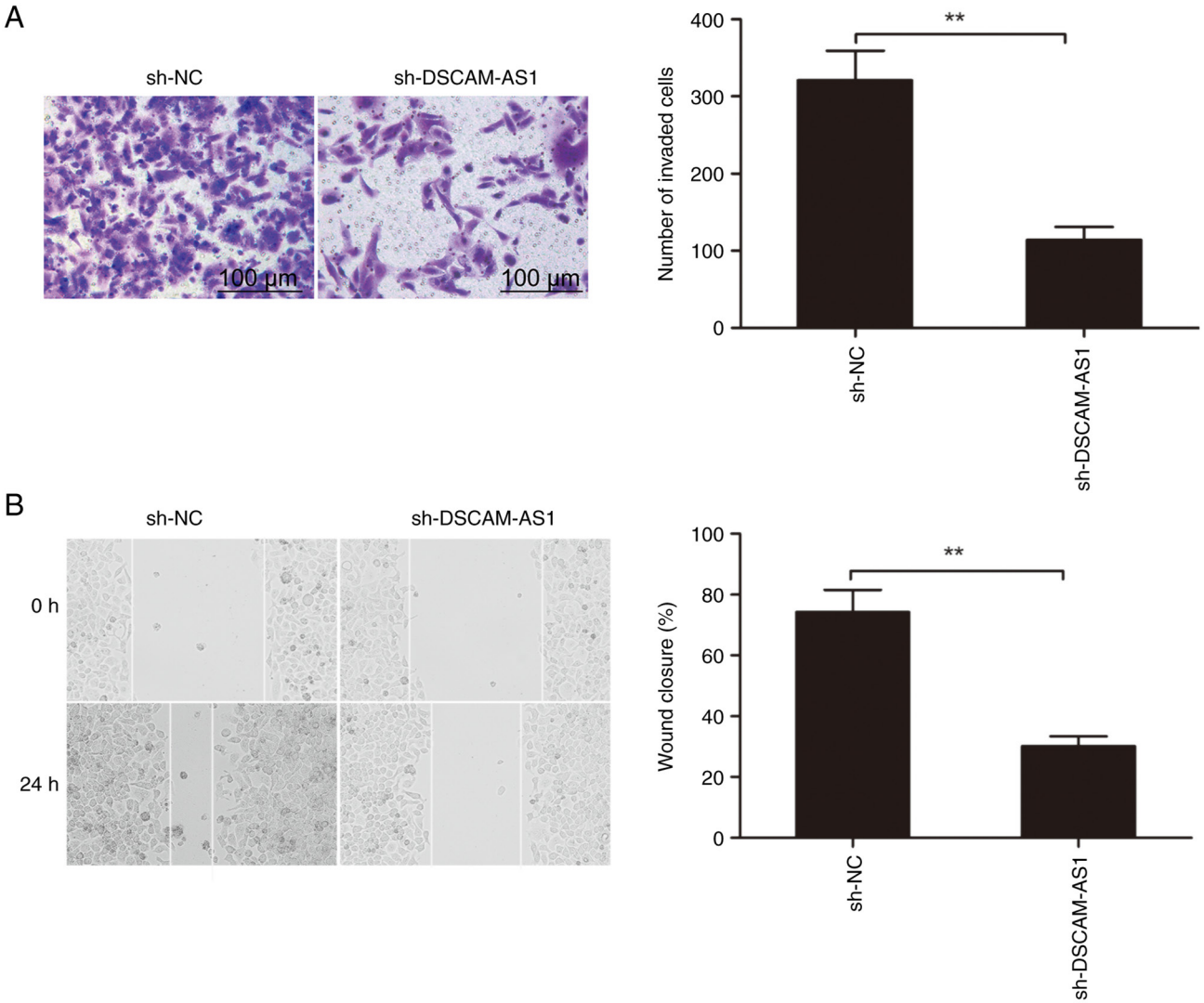

Figure 3. DSCAM-AS1 knockdown inhibits the invasion and migration of thyroid cancer cells. (A and B) Knockdown of DSCAM-AS1 inhibited the (A) invasion and (B) migration of TPC-1 cells. The results are shown as the mean ± standard deviation of 3 independent experiments. **P<0.01. DSCAM-AS1, Down syndrome cell adhesion molecule-antisense 1.

in TPC-1 cells (Fig. 4F). The expression of miR-211 was also examined within tissue samples from 48 clinical patients with thyroid cancer via RT-qPCR. The results demonstrated that miR-211 expression was considerably downregulated (Fig. 4G), and was negatively associated with DSCAM-AS1 in these tissues (ρ=-0.585; Fig. 4H). These data demonstrated that miR-211 was a target of DSCAM-AS1 in thyroid cancer cells.

DSCAM-AS1 knockdown suppresses the development of thyroid cancer via regulation of miR-211. The association between DSCAM-AS1 and miR-211 was investigated. Mimics and inhibitors of miR-211 were transfected into TPC-1 cells, and it was found that transfection of miR-211 mimics significantly increased the expression of miR-211 in TPC-1 cells (Fig. 5A), while transfection of miR-211 inhibitors significantly decreased the expression of miR-211 in TPC-1 cells (Fig. 5A). It was observed that knockdown of DSCAM-AS1 significantly increased the expression of miR-211 in TPC-1 cells, while miR-211 inhibitor rescued the effects of DSCAM-AS1 knockdown (Fig. 5B). Furthermore, it was found that miR-211 downregulation partially reversed the effects of DSCAM-AS1 knockdown on the proliferation, migration and invasion of TPC-1 cells (Fig. 5C-F). Thus, it was suggested that DSCAM-AS1 knockdown suppressed the development of thyroid cancer by regulating miR-211.

Discussion

Numerous lncRNAs have been found to be dysregulated in various cancer types, and to perform an essential role in tumor development, including thyroid cancer (9-11). For instance, Wang *et al* (23) reported that lncRNA maternally expressed 3 inhibited the migration and invasion of thyroid cancer cell by targeting Rac1 protein. Liu *et al* (24) reported that lncRNA-X-inactive specific transcript enhanced the invasion and proliferation of thyroid cancer cells by acting as a competing endogenous RNA to regulate MET-PI3K-AKT signaling via sponging miR-34a. Guo *et al* (25) indicated that lncRNA myocardial infarction associated transcript

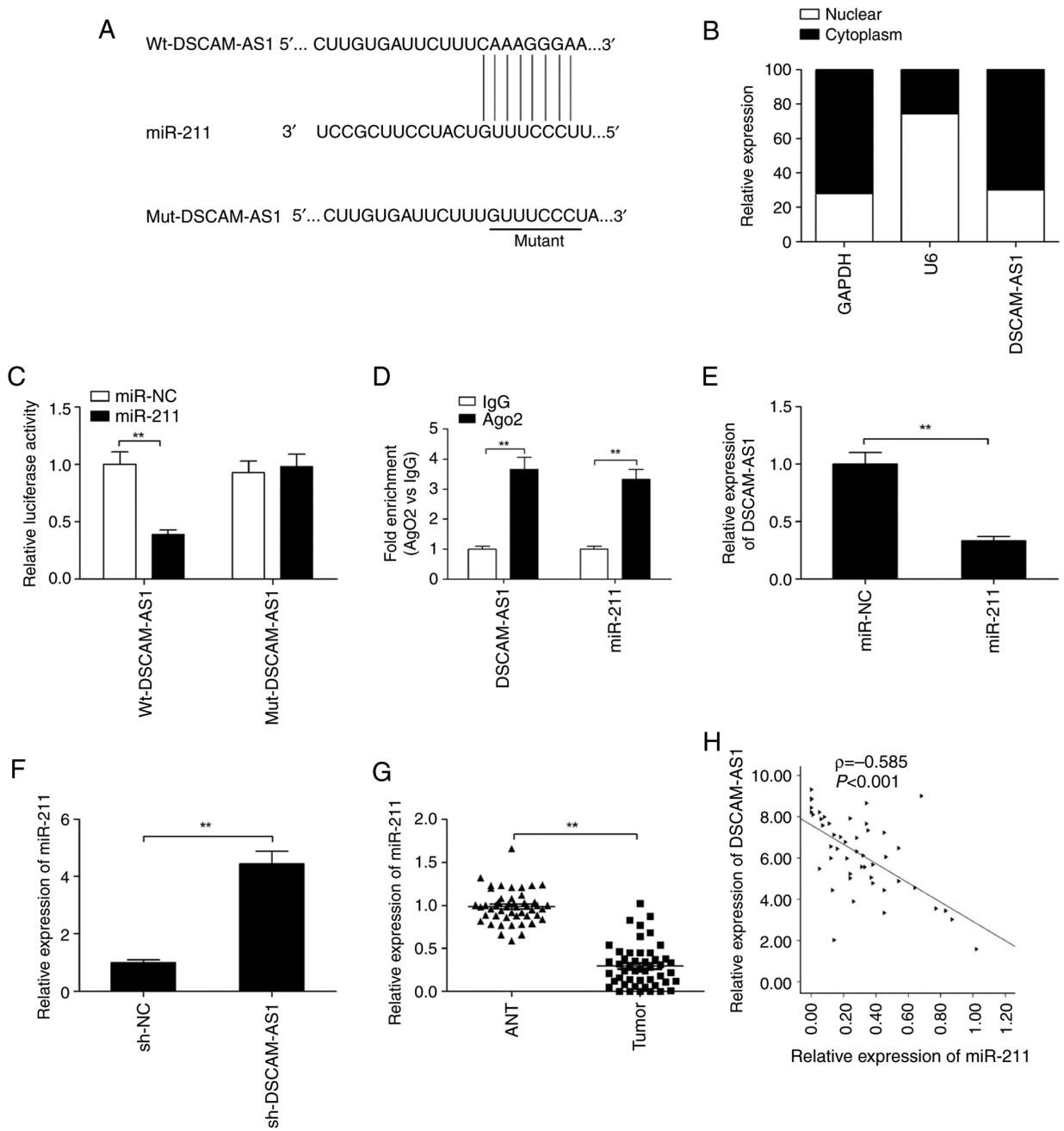

Figure 4. DSCAM-AS1 binds to miR-211 and suppresses its expression in thyroid cancer cells. (A) Complementary binding between miR-211 and Wt/Mut DSCAM-AS1. (B) DSCAM-AS1 was mainly expressed in the cytoplasm of TPC-1 cells. (C) miR-211 mimics significantly reduced the luciferase activity of the Wt-DSCAM-AS1 group. (D) The interaction between miR-211 and DSCAM-AS1 was analyzed by RNA immunoprecipitation assay. (E) The expression of DSCAM-AS1 in TPC-1 cells transfected with miR-211 mimics or miR-NC was examined by RT-qPCR. (F) The expression of miR-211 in TPC-1 cells transfected with sh-NC or sh-DSCAM-AS1 was examined by RT-qPCR. (G) RT-qPCR was used to examine the expression of miR-211 in thyroid cancer and adjacent normal tissues (n=48). (H) The correlation between DSCAM-AS1 and miR-211 in thyroid cancer tissues was analyzed by Spearman's correlation. The results are shown as the mean ± standard deviation of 3 independent experiments. **P<0.01. DSCAM-AS1, Down syndrome cell adhesion molecule-antisense 1; miR, microRNA; Wt, wild-type; Mut, mutant; NC, negative control; RT-qPCR, reverse transcription-quantitative PCR; sh, small hairpin.

enhanced the development of thyroid cancer through modulation of the miR-150-5P/enhancer of zeste homolog 2 axis. The present study investigated a new regulatory network of miR-211 and DSCAM-AS1 involved in the proliferation and invasion of thyroid cancer. The current results demonstrated that DSCAM-AS1 knockdown hindered the development of thyroid cancer via regulation of miR-211, suggesting that DSCAM-AS1 could be a therapeutic target for thyroid cancer.

Recent studies have suggested an oncogenic role for lncRNA DSCAM-AS1 in multiple cancer types (18-21). However, the detailed roles and fundamental mechanism of DSCAM-AS1 in thyroid cancer remain largely unclear. The current study demonstrated that DSCAM-AS1 was highly expressed in thyroid cancer tissues, and was positively correlated with lymph node metastasis and advanced clinical stage. Functional experiments revealed that DSCAM-AS1 depletion considerably diminished the invasion and proliferation

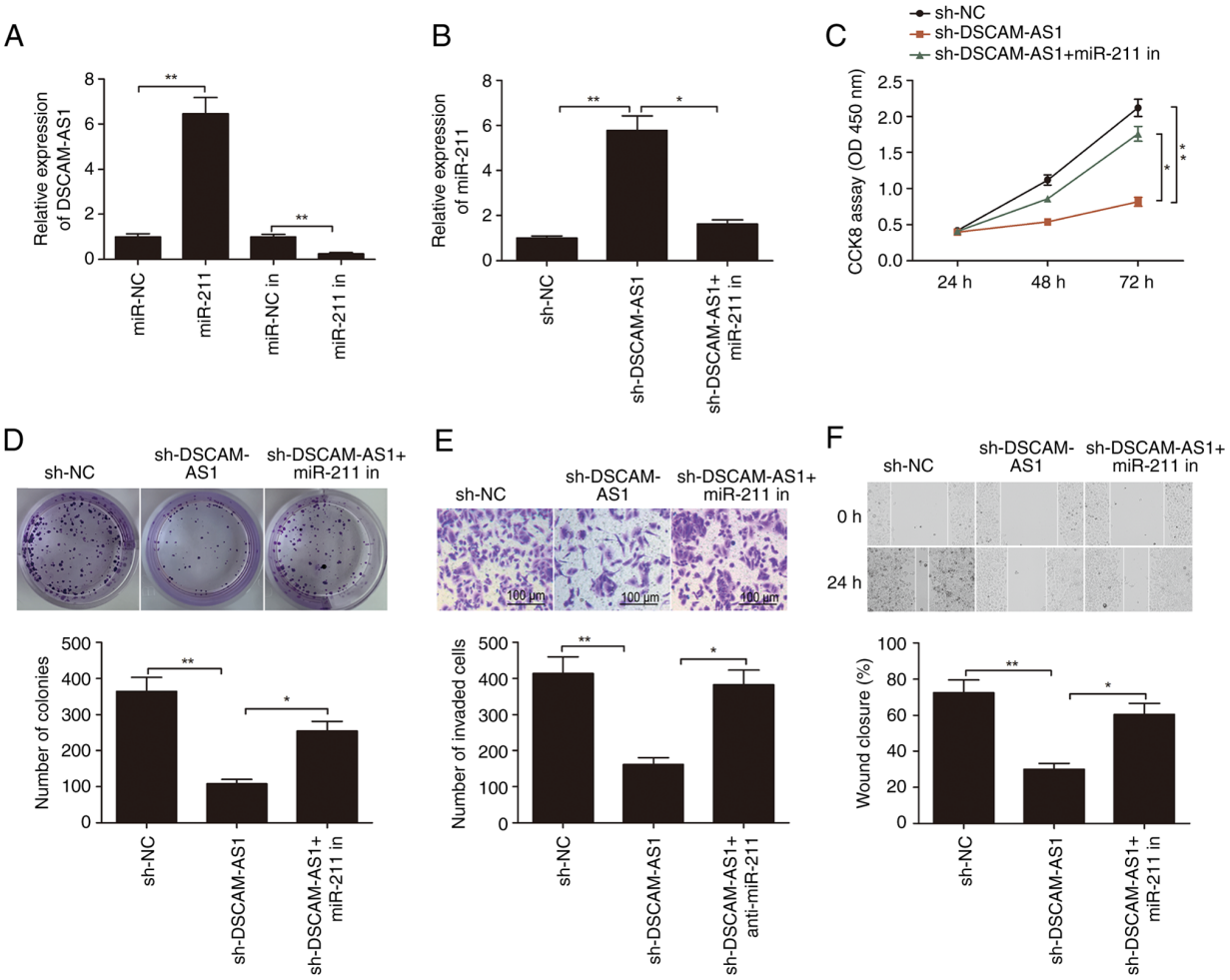

Figure 5. DSCAM-AS1 knockdown suppresses the progression of thyroid cancer via regulating miR-211. (A) The expression levels of miR-211 were determined in TPC-1 cells transfected with miR-211 mimics and inhibitors. (B) The expression of miR-211 was determined in TPC-1 cells transfected with sh-NC, sh-DSCAM-AS1 and sh-DSCAM-AS1 + miR-211 inhibitor (anti-miR-211). (C-F) Cell proliferation, colony formation, invasion and migration abilities were determined in TPC-1 cells transfected with sh-NC, sh-DSCAM-AS1 and sh-DSCAM-AS1 + anti-miR-211. The results are shown as the mean ± standard deviation of 3 independent experiments. *P<0.05 and **P<0.01. DSCAM-AS1, Down syndrome cell adhesion molecule-antisense 1; miR, microRNA; sh, small hairpin; NC, negative control.

abilities of thyroid cancer cells *in vitro*. These results showed that DSCAM-AS1 functioned as an oncogenic lncRNA in thyroid cancer.

Several studies have reported that lncRNAs can affect the tumorigenesis and progression of various cancer types by serving as miRNA sponges to restore the expression of target genes (26,27). DSCAM-AS1 was reported to be able to bind to several miRNAs, such as miR-338-3p (18), miR-136 (21), miR-137 (28) and miR-204-5p (29). Whether DSCAM-AS1 regulates the development of thyroid cancer through a similar mechanism remains unknown. In the present study, bioinformatics suggested that DSCAM-AS1 could interact with miR-211 in thyroid cancer, which was demonstrated by luciferase activity reporter and RIP assays. Furthermore, miR-211 was reported to be downregulated and to play a tumor suppressive role in thyroid cancer (30,31). In the present study, it was also confirmed that the expression of miR-211 was notably reduced in thyroid cancer tissues, which was in consistency with previous studies (30,31). Moreover, there was a negative association between miR-211 and DSCAM-AS1 expression in thyroid cancer tissues. These data indicated that DSCAM-AS1 could interact with miR-211 in thyroid cancer cells.

Several limitations exist in the present study: i) The effects of DSCAM-AS1 on cell cycle and apoptosis were not investigated, which is performed in the related ongoing study; ii) The effect of DSCAM-AS1 on change of related genes expression of cell proliferation, cycle, apoptosis, migration and invasion should be studied, iii) The animal study was not performed, which is performed in the related ongoing study; and iv) The effect of overexpression of DSCAM-AS1 on thyroid cancer cell proliferation, migration and invasion was not investigated, which will be performed in the future study.

Briefly, the present study revealed that DSCAM-AS1 was upregulated in thyroid cancer cells and tissues, and was correlated with lymph node metastasis and clinical stage. Functional assays demonstrated that knockdown of DSCAM-AS1 hindered thyroid cancer cell invasion, proliferation and migration. Mechanistically, DSCAM-AS1 promoted thyroid cancer aggressiveness via regulating miR-211. These results may provide a molecular basis for potential roles of DSCAM-AS1 in the treatment and prognosis of thyroid cancer.

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Availability of data and materials

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

Authors' contributions

TH, YL and JY designed the study, performed the experiments and wrote the manuscript. YZ performed the statistical analysis. All authors have read and approved the final manuscript. TH and YL confirm the authenticity of all the raw data.

Ethics approval and consent to participate

All experimental protocols were approved (approval no. KY2022SL376-01) by the Ethics Committee of Ningbo Medical Centre Lihuili Hospital (Ningbo, China). Written informed consent was provided by all participants.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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