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Long noncoding RNA DSCAM-AS1 is associated with poor clinical prognosis and contributes to melanoma development by sponging miR-136

Y.-L. HUANG[1], Q. XU[2], X. WANG[1]

[1]Department of Dermatology, Shanghai Sixth People's Hospital East Affiliated to Shanghai University of Medicine and Health Sciences, Shanghai, China
[2]Department of Cosmetology, Hubei Provincial Hospital of Traditional Chinese Medicine, Wuhan, China

Yan-Li Huang and Qing Xu have equal contribution

Abstract. – OBJECTIVE: Accumulating evidence suggests that long non-coding RNAs (lncRNAs) are playing critical roles in tumorigenesis. The present study aimed to investigate the expression pattern and effects of lncRNA DSCAM-AS1 (DSCAM-AS1) that was a newly discovered lncRNA in melanoma.

PATIENTS AND METHODS: Real-time quantitative PCR (polymerase chain reaction) was performed to determine the expression of DSCAM-AS1 in melanoma tissues and cell lines. Kaplan-Meier and Cox regression analyses were utilized to assess the association between the DSCAM-AS1 and overall survival of patients in melanoma patients. The CCK-8 assay, colony formation assay, flow cytometry assays, transwell and wound scratch assays were performed to determine the biological function of DSCAM-AS1 in tumor cells behaviors. Then, DSCAM-AS1-specific miRNA was further confirmed using the dual-luciferase reporter assay and Western blotting.

RESULTS: In this research, we showed that the expression of DSCAM-AS1 was significantly upregulated in melanoma samples and cell lines. Clinical investigation indicated that higher expression of DSCAM-AS1 was associated with ulceration and advanced stage and led to significantly poorer survival time. High DSCAM-AS1 expression in melanoma was confirmed to be an independent predictor of poor survival of patients using univariate and multivariate analysis. Functional investigations revealed that knockdown of DSCAM-AS1 inhibited the ability of cell proliferation, colony formation, migration, invasion, whereas promoted cell apoptosis. Furthermore, mechanistic investigations indicated that DSCAM-AS1 could interact with miR-136 and negatively influence the expression of miR-136.

CONCLUSIONS: Our findings showed that DSCAM-AS1 is a novel tumor-related molecule involved in melanoma progression as well as a potential prognostic biomarker and therapeutic target.

Key Words
LncRNA DSCAM-AS1, Prognosis, Melanoma, Metastasis, miR-136.

Introduction

Melanoma is an aggressive skin cancer often arising from the skin that accounts for more than 70% of skin cancer-related deaths[1]. The incidence of melanoma has continued to increase in recent years, and it metastasizes quickly and is lethal in most cases of advanced disease[2,3]. Although melanoma just accounts for approximately 5% of all skin cancers, it leads to the highest number of mortalities. Although major advances have been made in the diagnosis and therapy of uveal melanoma, the median survival of patients with metastatic melanoma, following treatment with systematic treatment, is only 6-9 months[4,5]. Thus, further understanding the underlying mechanisms of metastatic melanoma is urgently required. Long noncoding RNAs (lncRNAs) are a kind of noncoding RNAs with the length > 200 nucleotides and have no or weak protein-coding abilities[6]. Thousands of lncRNAs are encoded in mammalian genomes, many of which have vital functions in a wide range of cellular processes, such as X chromosome inactivation, imprinting, genes epigenetic control and transcription modulation[7,8]. Increasing studies indicate that lncRNAs play important roles in various biological processes involving in the pathogenesis and progression of diverse cancer[9,10]. Substantial basic and clinical evidence suggested that lncRNAs display tumor suppressor or oncogenic roles in tumorigenesis. For instance, lncRNA LINC01186 was reported to inhibit migration and invasion

Corresponding Author: Xiong Wang, MD; e-mail: wahaa1191@yeah.net

in lung cancer[11]. LncRNA ILF3-AS1 was found to promote cell proliferation and metastasis via modulating miR-200b in melanoma[12]. Although many lncRNAs have been functionally identified, a large number of lncRNAs remain to be elucidated. LncRNA DSCAM-AS1 (DSCAM-AS1) is one of the few intensively studied tumor-related lncRNAs whose length is 1.4 kb and locates on chromosome 21q22.3[13]. Previously, the up-regulation of DSCAM-AS1 and its oncogenic roles have been reported in several tumors, such as breast cancer[14] and non-small cell lung cancer[15]. However, DSCAM-AS1 has not been characterized in human melanoma samples to date. This study aimed to explore the expression pattern of DSCAM-AS1 and its clinical significance and potential biological effects on tumor behaviors.

Patients and Methods

Human Tissue Samples

A total of 104 melanoma specimens and corresponding non-cancerous tissue samples were obtained from Shanghai Sixth People's Hospital East Affiliated to Shanghai University of Medicine and Health Sciences from January 2008 to May 2012. No patients received anti-cancer therapy before the operation. The tissues were immediately preserved at -80°C after surgerical resection. Written informed consent was obtained from all participants, and this study was approved by the Ethics Committee of Shanghai Sixth People's Hospital East Affiliated to Shanghai University of Medicine and Health Sciences. The patients' clinical information was shown in Table II.

Cell Lines and Cell Transfection

Five human melanoma cells lines (1205Lu, CHL-1, A-375, UACC903, and SK-MEL-2) and one normal human epidermal melanocyte (HEMa-LP) were all purchased from BeNa Culture Collection (Chaoyang, Beijing, China). Cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics. The cells were cultured in an atmosphere of 5% CO_2 at 37°C.

Small interfering RNAs (siRNAs) were synthesized by GenePharma Inc. (Suzhou, Jiangsu, China) to target DSCAM-AS1 (siRNA#1, siRNA#2, and siRNA#3). The miRNA mimics (miR-136 mimics and control mimics) were purchased from RiboBio Corporation (Guangzhou, Guangdong, China). Ectopic expression of DSCAM-AS1 was achieved through transfecting pcDNA3.1-DSCAM-AS1 plasmids and the pcDNA3.1-DSCAM-AS1 plasmids were constructed by HanBio Company (Pudong, Shanghai, China). Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA) was applied to transfect the siRNAs, miRNA mimics or plasmids into cells in accordance with the relevant protocols.

Real-Time Quantitative PCR Analysis

Total RNA in this study was extracted using TRIzol reagent (Sigma-Aldrich, St. Louis, MO, USA) and isolated by standard phenol-chloroform extraction protocols. Then, 2 μg total RNAs were reverse transcribed to cDNA using iScript cDNA Synthesis kit (Bio-Rad, Hercules, CA, USA). Next, the qRT-PCR analysis for DSCAM-AS1 determination was conducted by a SYBR Green Master Mix kit (WuxinBio, Hangzhou, Zhejiang, China) on a TL988-II Real-Time PCR system (Tianlong, Suzhou, Jiangsu, China). For miR-136 detection, a TransScript Green miRNA Two-Step qRT-PCR SuperMix kit (Transgen, Haidian, Beijing, China) was utilized. DSCAM-AS1 and miR-136 expression levels were normalized to that of GAPDH and U6, respectively, and calculated using the $2^{-\Delta\Delta Ct}$ method. Relevant primers used in this study were summarized in Table I.

Cell Counting Kit-8 (CCK-8) Assays

Cell proliferation was determined by a CCK-8 assay kit (KeTe, Yancheng, Jiangsu, China). The cells after transfection with corresponding siRNAs were collected, seeded into 96-well plates (2×10^3 cells/well), and incubated for indicated times (24, 48, 72, and 96 h). After adding 10 μl CCK-8 reagents into each well, the absorbance (OD450 nm) of the plates was determined using a microplate reader.

Cell Colony Formation Assays

After transfection with indicated siRNAs, the cells were plated into 6-well plates and allowed to grow for about 2-3 weeks until the colonies were visible. Next, the colonies were photographed using a microscope after they were fixed with polyoxymethylene (4%) and stained with 0.1% crystal violet.

Cell Apoptosis Analysis

We used an annexin V-FITC/PI apoptosis assay kit (GeminBio, Minhang, Shanghai, China) to determine the cell apoptosis of the siRNAs-treat-

Table I. Primer sets used in the present study.

Name	Sequences (5′-3′)
DSCAM-AS1-F	GTGACACAGCAAGACTCCCT
DSCAM-AS1-R	GATCCGTCGTCCATCTCTGT
miR-136-F	GCGCTGGAGTGTGACAATGGTG
miR-136-R	GTGCAGGGTCCGAGGT
GAPDH-F	GGGTGTGAACCATGAGAAGT
GAPDH-R	TGAGTCCTTCCACGATACCAA

ed cells. In short, cells after treatment were harvested and washed with PBS. Subsequently, cells were added into binding buffer supplemented with annexin V-FITC (5 μl) and PI (5 μl). The mixture was incubated at room temperature for 20-30 min keeping away from light. After the cells were washed using PBS, the cells were subjected to flow cytometry analysis.

Caspase 3/9 Activity Detection Assays

The caspase 3 and caspase 9-activity assay kit (Beyotime, Haimen, Jiangsu, China) was applied to detect the activity of caspase 3 and caspase 9, respectively. In short, the treated cells were lysed by the lysis buffer and the supernatants were collected after the cell lysates were centrifuged with high speed. Then, the cell lysates were added with Ac-DEVD-pNA (final concentration: 2 mM) and the absorbance at 405 nm was determined using a microplate reader.

Wound Healing Assays

The cells were transfected with corresponding siRNAs and collected using trypsinization. After the treated cells were placed into 24-well plates and cultured until 95% confluent, a pipette tip (200 μl) was utilized to scratching across the cell monolayer, generating a wound. Then, the wound closures were photographed by a microscope at 0 h and 48 h. These results were expressed as the percentage of wound closure (migration) relative to control conditions.

Transwell Invasion Assays

In brief, the upper sides of the transwell inserts (pore size: 8.0 μm; BD Biosciences, Franklin Lakes, NJ, USA) was added with 200 μl treated cells-suspension (without serum). The lower chamber was added with 7500 μl culture medium (with 15% FBS). After culturing for 24 h, the cells on the lower surface of the insert membrane were fixed with polyoxymethylene (4%) and stained with 0.1% crystal violet. Finally, the stained cells were photographed using a microscope.

Table II. Summary ORs of non-vertebral fracture for each direct comparison.

Variables	Case (N)	DSCAM-AS1 expression		*p*-value
		High	Low	
Age				0.330
≤ 55	54	24	30	
> 55	50	27	23	
Gender				0.720
Male	63	30	33	
Female	41	21	20	
Thickness (mm)				0.075
≤ 2.0	66	28	38	
> 2.0	38	23	15	
Ulceration				0.006
Absent	55	20	35	
Present	39	31	18	
Histologic type				0.439
SSM	49	26	23	
LMM	55	25	30	
Site				0.323
Sun exposed	54	29	25	
Sun protected	50	22	28	
Stage				0.002
I/II	59	21	38	
III/IV	45	30	15	

RNA Pull-Down Assays

Biotin-labeled DSCAM-AS1 (Biotin-DSCAM-AS1) and matched biotin labeled control RNA (Biotin-control) were purchased from Zoon Biotechnology company (Nanjing, Jiangsu, China). Then, the Biotin-DSCAM-AS1 and Biotin-control were separately incubated with A 375 cell lysates. The beads from Invitrogen Dynabeads M-280 Streptavidin kit (Pudong, Shanghai, China) were then added into each binding reaction solution, and finally, the eluted RNAs were detected by qRT-PCR analysis.

Dual-Luciferase Reporter Assays

The predicted wild type binding sequence of DSCAM-AS1 (DSCAM-AS1 wt) or predicted mutant binding sequence of DSCAM-AS1 (DSCAM-AS1 mut) was constructed into pGL3 luciferase reporter vector by ShuangLing Biotechnology Company (Nanjing, Jiangsu, China). The A375 and SK-MEL-2 cells were maintained overnight until the confluence reached about 70%. Subsequently, miR-136 mimics were co-transfected with DSCAM-AS1 wt or DSCAM-AS1 mut into A375 or SK-MEL-2 cells. The cells were collected for luciferase evaluation using the dual-luciferase reporter assay kit (BoSun, Haidian, Beijing, China) forty-eight hours post-transfection.

Statistical Analysis

SPSS 19.0 statistical software (IBM Corp., IBM SPSS Statistics for Windows, Armonk, NY, USA) was utilized to conduct statistical analysis. Differences between two groups were analyzed by the Student's *t*-test. The multi-group comparison was performed using one-way analysis of variance. The paired comparison was performed by SNK approach. The associations between DSCAM-AS1 expression and clinical-pathological parameters were evaluated by chi-square tests. The survival rate was calculated using Kaplan-Meier analysis and log-rank test. Cox regression analysis was performed to identify the factor with significant influence on overall survival. A p-value < 0.05 was defined as statistically significant.

Results

The Expression of DSCAM-AS1 is Up-Regulated in Melanoma Tissues and Cell Lines

Firstly, to examine whether DSCAM-AS1 is differentially expressed in melanoma, we determined the expression levels of DSCAM-AS1 in 104 paired melanoma and pair-matched adjacent skin tissues by qRT-PCR. As shown in Figure 1A, we found that the expression of DSCAM-AS1 in melanoma tissues was dramatically higher than that in pair-matched adjacent skin tissues (p<0.01). We also showed that patients with advanced clinical stages showed a higher level of DSCAM-AS1 (Figure 1B). Furthermore, elevated expressions of DSCAM-AS1 were also observed in melanoma cell lines (1205Lu, CHL-1, A-375, UACC903, and SK-MEL-2) compare to one normal human epidermal melanocyte (HEMa-LP) (Figure 1C). Overall, our findings suggested the possible associations between DSCAM-AS1 expression and progression of melanoma.

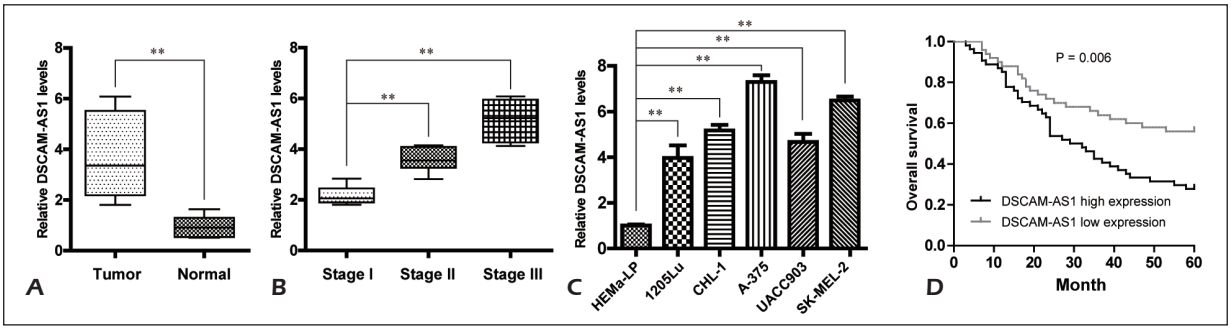

Figure 1. DSCAM-AS1 was highly expressed in melanoma and associated with poor prognosis. **A**, The expression of DSCAM-AS1 in normal skin tissues and malignant melanoma tissues was measured using quantitative RT-PCR. **B**, The expression level of DSCAM-AS1 was compared between patients with different clinical stages. **C**, The relative expression level of miR-145 was determined by real-time qPCR in 1205Lu, CHL-1, A-375, UACC903 cells and SK-MEL-2 and one normal human epidermal melanocyte (HEMa-LP). **D**, The overall survival of melanoma patients with high or low level of DSCAM-AS1 was analyzed with Kaplan-Meier method. *p<0.05, **p<0.01.

Overexpression of DSCAM-AS1 is Associated with Poor Prognosis in Patients with Melanoma

The correlations of DSCAM-AS1 expression with various clinicopathological parameters of osteosarcoma tissues are summarized in Table II. Based on DSCAM-AS1 expression level, we divided all of the melanoma patients into two groups (High and Low). As shown in Table II, we found that increased expression of DSCAM-AS1 positively correlated with ulceration (p = 0.006) and stage (p=0.002). However, there was no association between DSCAM-AS1 expression and other clinical factors, such as gender, age, thickness, histologic type, and site (All p>0.05). Furthermore, Kaplan-Meier analysis revealed that high level of DSCAM-AS1 was associated with poor overall survival in melanoma patients (Figure 1D, p=0.006). Then, we used Cox proportional-hazards regression to determine the correlations between DSCAM-AS1 expression and prognosis further. According to the results of univariate analysis, high expression of DSCAM-AS1, ulceration and stage were significantly associated with the overall survival (all p<0.01, Table III). More importantly, the results of multivariate assays further confirmed that high DSCAM-AS1 expression (HR=3.016, 95% CI: 1.126-4.219, p=0.009) could be an independent prognostic factor of overall survival for patients with melanoma, in addition to ulceration and stage (Table III). Taken together, these results indicated that the elevated expression of DSCAM-AS1 indicated the poor prognosis of melanoma patients.

Silencing of DSCAM-AS1 Inhibited the Proliferation of Melanoma Cells and Promoted Cell Apoptosis

To investigate whether DSCAM-AS1 had an impact on the cell proliferation and apoptosis of melanoma cells, loss-of-function studies via transfecting DSCAM-AS1 siRNAs (siRNA#1, siRNA#2, and siRNA#3) were conducted in A375 and SK-MEL-2 cells. The results from qRT-PCR assays revealed that DSCAM-AS1 siRNAs could significantly reduce the expression of DSCAM-AS1 in A375 and SK-MEL-2 cells; and siRNA#1 and siRNA#2 had the highest knockdown efficiency of DSCAM-AS1 (Figure 2A and B). Then, CCK-8 assays illustrated that DSCAM-AS1 knockdown remarkably repressed the proliferative rates of A375 and SK-MEL-2 cells (Figure 2C and D). In addition, after silencing of DSCAM-AS1, the cell colony numbers of A375 and SK-MEL-2 cells were notably reduced when compared with the siControl-transfected cells (Figure 2E). Furthermore, we next carried out flow cytometry analysis to evaluate the effects of DSCAM-AS1 on cell apoptosis. DSCAM-AS1 depletion was found to markedly accelerate the cell apoptosis of A375 and SK-MEL-2 cells (Figure 2F). Besides, molecular mechanism study demonstrated that repressing the levels of DSCAM-AS1 markedly elevated the activities of caspase 3 and caspase 9 in both A375 and SK-MEL-2 cells (Figure 2G). Taken together, these data indicated that DSCAM-AS1 promoted cell proliferation of melanoma and inhibited apoptosis.

DSCAM-AS1 Modulated the Metastatic Potentials of Melanoma Cells

To explore whether DSCAM-AS1 contributed to the metastatic potentials of melanoma cells, we next determined the migration and invasion capacities of melanoma cells using wound healing and transwell invasion assays, respectively. As results from wound healing assays presented, DSCAM-AS1 knockdown notably suppressed the migration of A375 and SK-MEL-2 cells compared with the siControl-transfected cells (Figure

Table III. Univariate and multivariate analysis of overall survival in melanoma patients.

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p*-value	HR	95 % CI	*p*-value
Age	1.581	0.733-2.152	0.452	–	–	–
Gender	1.362	0.844-2.437	0.233	–	–	–
Thickness	2.132	0.778-2.782	0.089	–	–	–
Ulceration	3.442	1.426-4.783	0.004	2.895	1.226-4.216	0.009
Histologic type	1.642	0.775-2.341	0.144		–	–
Site	1.443	0.462-1.998	0.443	–	–	–
Stage	3.886	1.645-5.273	0.001	3.277	1.289-4.562	0.004
DSCAM-AS1 expression	3.543	1.437-4.779	0.005	3.016	1.126-4.219	0.009

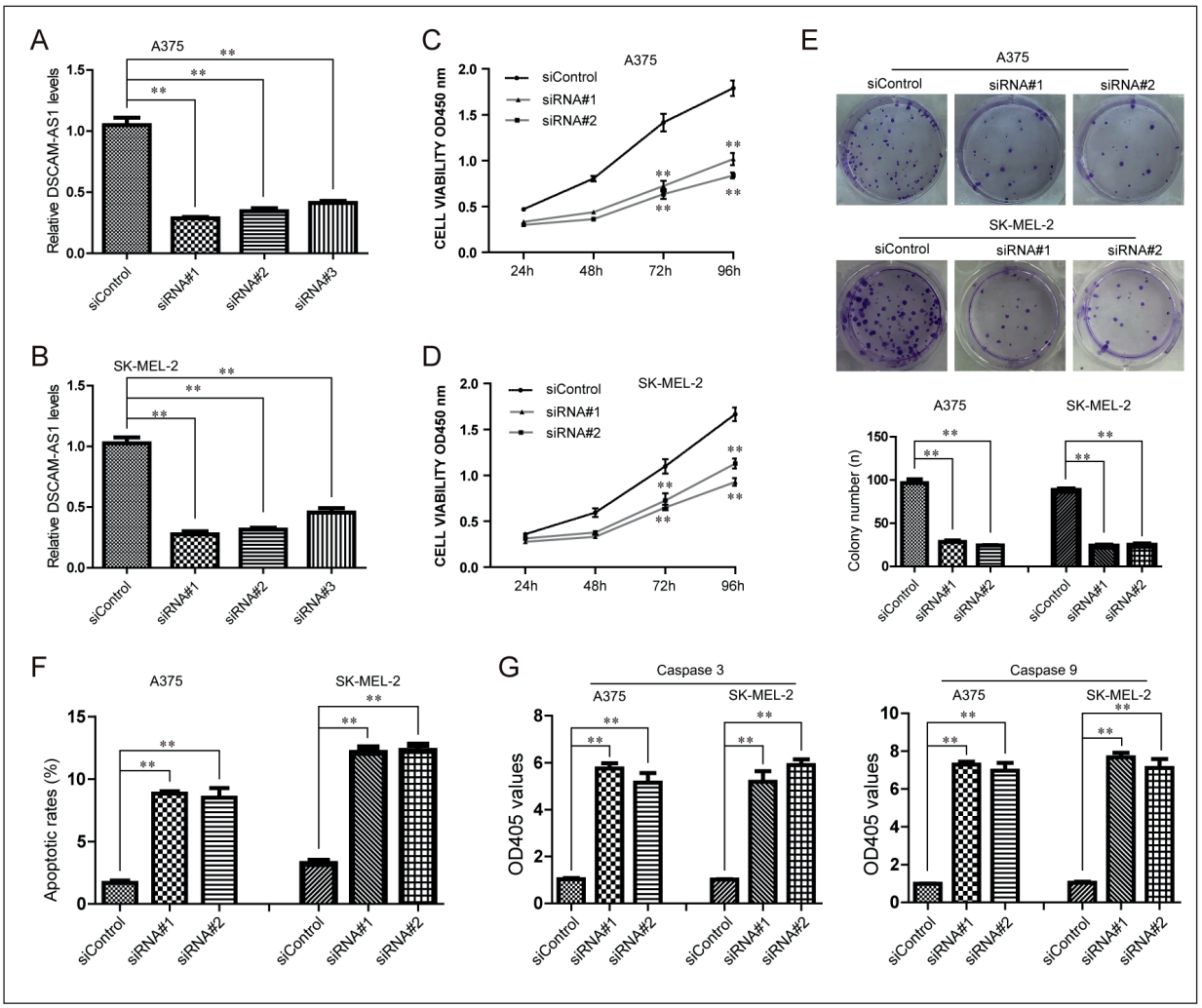

Figure 2. The effects of DSCAM-AS1 on melanoma cell proliferation and apoptosis. (**A** and **B**) DSCAM-AS1 expression in A375 and SK-MEL-2 cells after transfection with DSCAM-AS1 siRNAs (siRNA#1, siRNA#2 and siRNA#3). (**C** and **D**) The effects of DSCAM-AS1 knockdown on the proliferation of A375 and SK-MEL-2 cells were evaluated by CCK-8 assays. **E**, The cell colony number of DSCAM-AS1 siRNAs-transfected A375 and SK-MEL-2 cells was lower compared with siControl-transfected cells. **F**, The effects of DSCAM-AS1 knockdown on A375 and SK-MEL-2 cells apoptosis were detected by flow cytometry. **G**, Caspase 3/9 activity assays determined the caspase 3 and caspase 9 activity of A375 and SK-MEL-2 cells after transfection with DSCAM-AS1 siRNAs. *p<0.05, **p<0.01.

3A). Moreover, the invasive abilities of A375 and SK-MEL-2 cells were markedly weakened by DSCAM-AS1 silence in comparison with siControl treatment (Figure 3B). Therefore, these data shed light on that DSCAM-AS1 was able to modulate the metastatic potentials of melanoma cells.

DSCAM-AS1 Directly Interacted with miR-136 in Melanoma Cells

The above data confirmed that DSCAM-AS1 was capable to modulate the proliferation, apoptosis, migration, and invasion of melanoma cells, and served as an oncogenic role in the development of melanoma. Therefore, we next aimed to discover the molecular mechanisms behind that. First, the subcellular location of DSCAM-AS1 was clarified and the results indicated that DSCAM-AS1 was mainly in the cytoplasm (Figure 4A). Considering numerous studies had demonstrated that lncRNA in the cytoplasm might function as miRNA sponges to exert its roles, we thereby postulated that DSCAM-AS1 might act as miRNA sponge in the oncogenesis of melanoma. Using the online bioinformatics tool "starbase" (http://starbase.sysu.edu.cn/), we found that miR-136, which had been certified

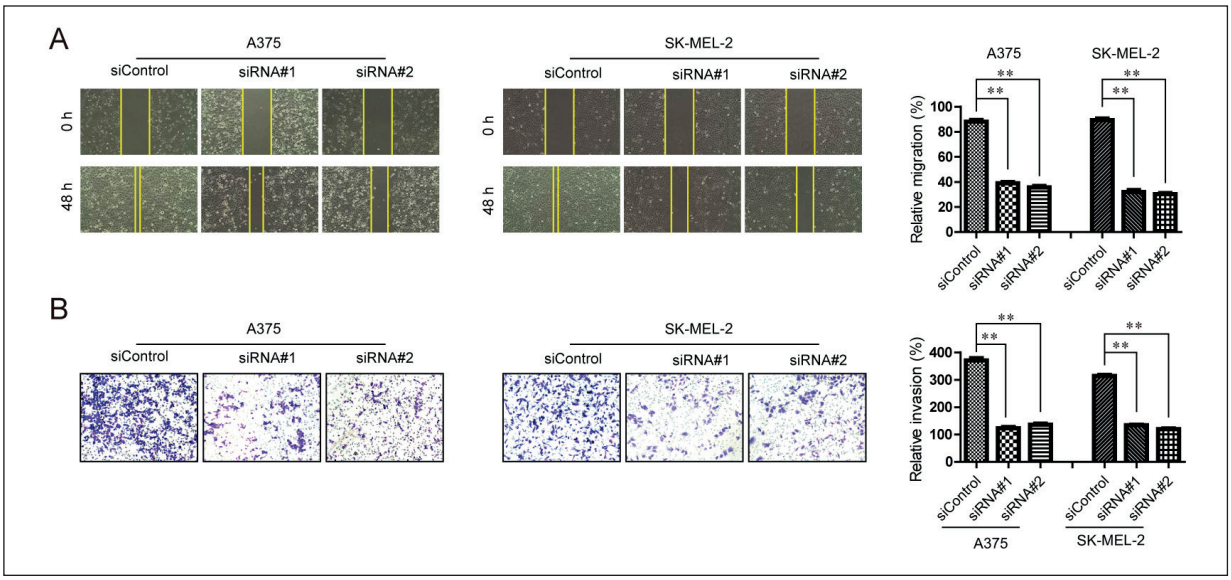

Figure 3. The migration and invasion of A375 and SK-MEL-2 cells were affected by DSCAM-AS1. **A**, Knockdown of DSCAM-AS1 reduced the migration of A375 and SK-MEL-2 cells. **B**, Silence of DSCAM-AS1 impaired the invasion of A375 and SK-MEL-2 cells. *p<0.05, **p<0.01.

as a tumor suppressor in diverse cancer types, was a potential target of DSCAM-AS1 (Figure 4B). To validate that, we next carried out dual-luciferase reporter assays. The data suggested that co-transfection of miR-136 mimics with DSCAM-AS1 wild type (DSCAM-AS1 wt) but not DSCAM-AS1 mutant (DSCAM-AS1 mut) reporter plasmids significantly reduced the relative luciferase activities in A375 and SK-MEL-2 cells (Figure 4C). Moreover, the direct interaction be-

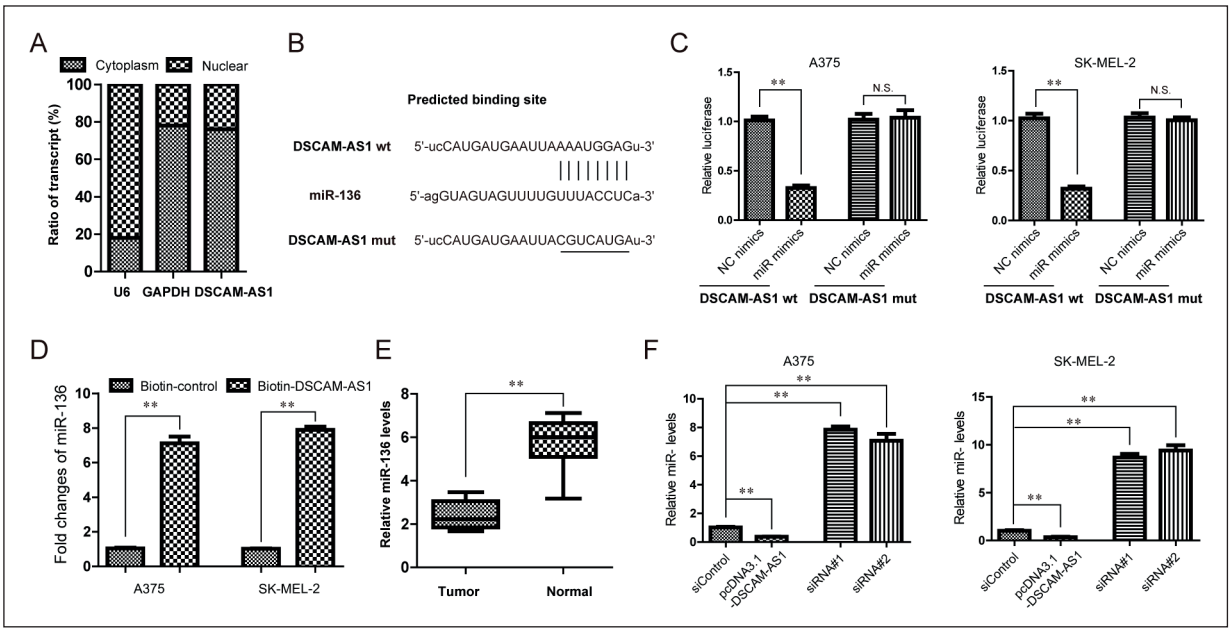

Figure 4. MiR-136 was directly interacted with DSCAM-AS1. **A**, The subcellular location of DSCAM-AS1. **B**, The predicted binding site between miR-136 and DSCAM-AS1. **C**, The relative luciferase activities of A375 and SK-MEL-2 cells were determined by dual-luciferase activity assays. **D**, RNA pull-down assays showed that DSCAM-AS1 could precipitate miR-136 in A375 and SK-MEL-2 cells. **E**, The relative expression of miR-136 in melanoma tissue samples. **F**, The relative expression of miR-136 in A375 and SK-MEL-2 cells after transfection with pcDNA3.1-DSCAM-AS1 plasmids or DSCAM-AS1 siRNAs. *p<0.05, **p<0.01.

tween DSCAM-AS1 and miR-136 in A375 and SK-MEL-2 cells was further verified by RNA pull-down assays (Figure 4D). In addition, the expressing levels of miR-136 in melanoma tissue samples and paired normal tissue specimens were measured by qRT-PCR assays, suggesting the lower expression of miR-136 in melanoma tissue specimens (Figure 4E). The qRT-PCR analysis also revealed that through enhancing the expression of DSCAM-AS1 markedly depressed the miR-136 levels in A375 and SK-MEL-2 cells, knockdown of DSCAM-AS1 notably promoted the expression of miR-136 (Figure 4F). Overall, these results detected that miR-136 was a direct target of DSCAM-AS1 in melanoma cells.

Discussion

Metastatic melanoma is associated with poor clinical significance and highly refractory to chemotherapy and radiotherapy[16,17]. Identification of new cancer biomarkers is very important for the management of clinical treatments. Recently, more and more lncRNAs were reported to have the potential to act as novel diagnostic and prognostic biomarker due to its frequent dysregulation and important regulator effects in tumor progression[18-20]. In this study, we identified a novel melanoma-related lncRNA DSCAM-AS1. We firstly showed that DSCAM-AS1 expression was significantly upregulated in both melanoma tissues and cell lines. In addition, we found that increasing expression of DSCAM-AS was positively associated with ulceration and advanced stage. Clinical assays indicated that melanoma patients with high DSCAM-AS1 had a shorter overall survival compared with those with low DSCAM-AS1 expression. Finally, multivariate Cox regression analysis showed that DSCAM-AS1 was an independent prognostic marker. To the best of our knowledge, this is the first study to determine the clinical significance of tissues DSCAM-AS1 based clinical case investigation. Previously, the function of DSCAM-AS1 had been shown in two tumors, including lung cancer and breast cancer. For instance, Liao et al[15] reported that DSCAM-AS1 expression was up-regulated in non-small cell lung cancer and predicated shorter overall survival of patients. Functionally, knockdown of DSCAM-AS1 suppressed the metastasis abilities of tumor cells by targeting BCL11A. Sun et al[21] showed that DSCAM-AS1 was highly expressed in breast cancer tissues and distinctly associated with poorer clinical outcome of patients with breast cancer. Mechanistic researches revealed that promoted cells proliferation. In addition, several other studies also confirmed the up-regulation of DSCAM-AS1 and its oncogenic roles in breast cancer. However, the potential effects of DSCAM-AS1 remain unknown. In this investigation, we also reported that knockdown of DSCAM-AS1 suppressed melanoma cells proliferation and promoted apoptosis, suggesting that DSCAM-AS1 served as an oncogene in progress of melanoma. In addition, to explore the mechanism by which DSCAM-AS1 promoted apoptosis, we performed RT-PCR to determine the influence of DSCAM-AS1 on the expression of Caspase 3 and Caspase 9, which were important apoptosis-related proteins. Our results revealed the inhibition effects of DSCAM-AS1 on the expression levels of the above two proteins. On the other hand, we also provided evidence that knockdown of DSCAM-AS1 suppressed the migration and invasion of melanoma cells. Overall, our present findings, together with previous observation, indicated that DSCAM-AS1 acted as a tumor promoter in melanoma and may be a new therapeutic target for melanoma patients. MicroRNAs (miRNAs) have been identified as essential regulators of tumor progression via acting as a tumor promoter or anti-oncogenes[22,23]. Besides, novel evidence indicated that some functional miRNAs and lncRNAs can also involve in the lncRNA-miRNA regulatory network to modulate the development and progression of tumors[24,25]. For instance, Chen et al[26] reported that double-negative feedback loop between lncRNA FOXC2-AS1 and miR-1253 promotes the proliferation and metastasis in prostate cancer cells. Zhou et al[27] showed that lncRNA HOXD-AS1 was highly expressed in glioma and its down-regulation enhances cisplatin sensitivity of glioma cells by sponging miR-204. The regulator effects of miRNA and lncRNA were also reported in melanoma[28,29]. To explore the potential mechanism of DSCAM-AS1 involved in the progression of melanoma, we used the bioinformatics predictive tools and luciferase reporter assay to explore the possible targets of DSCAM-AS1. We found that miR-136 targeted DSCAM-AS1, constituting the RNA-induced silencing complex (RISC). Previously, miR-136 has been confirmed to act as a tumor suppressor in various tumors, including melanoma[30-32]. Thus, our results indicated that DSCAM-AS1 displayed its tumor-promotive roles via modulating miR-136. However, further cells experiments were needed to confirm our results and explore the deeply molecular mechanism.

Conclusions

We found that DSCAM-AS1 is upregulated in melanoma and predicts poor survival of melanoma patients. In addition, DSCAM-AS1 may exert its tumor promoter role via modulating miR-136 in melanoma. Therefore, DSCAM-AS1 may be used as a new target for prognosis and treatment of melanoma.

Conflict of Interests

The authors declare no conflicts of interest.

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