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Long noncoding RNA DSCAM-AS1 functions as an oncogene in non-small cell lung cancer by targeting BCL11A

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Abstract. – OBJECTIVE: Long noncoding RNAs (lncRNAs) have attracted more attention for their role in tumor progression recently. The aim of this study was to investigate the role of DSCAM-AS1 in the progression of non-small cell lung cancer (NSCLC), and to elucidate its possible underlying mechanism.

PATIENTS AND METHODS: DSCAM-AS1 expression in both NSCLC cells and tissue samples was detected by Real Time quantitative-Polymerase Chain Reaction (RT-qPCR). Moreover, the association between the DSCAM-AS1 expression level and patients' overall survival rate was explored. Furthermore, wound healing assay and transwell assay were conducted. In addition, RT-qPCR and Western blot assay were used to elucidate the underlying mechanism.

RESULTS: DSCAM-AS1 expression level in NSCLC samples was significantly higher than that of the corresponding normal tissues. The expression level of DSCAM-AS1 was associated with an overall survival time of NSCLC patients. Besides, the migration and invasion abilities of NSCLC cells were remarkably promoted after DSCAM-AS1 overexpression in vitro. Moreover, the mRNA and protein expression of BCL11A was significantly upregulated after the overexpression of DSCAM-AS1. Furthermore, the expression of BCL11A was positively correlated with DSCAM-AS1 expression in NSCLC tissues.

CONCLUSIONS: We observed that DSCAM-AS1 could enhance NSCLC cell migration and invasion via upregulating BCL11A. Furthermore, DSCAM-AS1 might be a potential therapeutic target for NSCLC.

Key Words:
Long noncoding RNA, Non-small cell lung cancer (NSCLC), DSCAM-AS1, BCL11A.

Introduction

Lung cancer is one of the most common malignant tumors worldwide, which is also the leading cause of cancer-related mortality. There are two major subtypes of lung cancer, including 15% of small cell lung cancer (SCLC) and 85% of non-small cell lung cancer (NSCLC)[1]. The prognosis of NSCLC patients is relatively poor; meanwhile, the five-year survival rate of these patients is approximately 15%[2]. However, limited targeted therapies can be used for NSCLC. Therefore, it is urgent to elucidate the underlying mechanism and find new treatment strategies.

Numerous researches have indicated that non-coding RNAs (ncRNAs) participate in various biological behaviors during tumor progression. Known as a subgroup of the ncRNA family, long noncoding RNAs (lncRNAs) are non-coding RNA molecules with over than 200 bps in length. They cannot be transcribed into proteins. Moreover, it has been found that lncRNAs are related to various kinds of cellular functions, including carcinogenesis and metastasis. For instance, lncRNA EWSAT1 can facilitate the proliferation and formation of Ewing sarcoma[3]. LncRNA HOTAIR plays an important role in the carcinogenesis of endometrial cancer[4]. Moreover, HOTAIR promotes the development and metastasis of gastric cancer by suppressing PCBP1[5]. In addition, lncRNA GHET1 has been demonstrated to promote the growth and formation of bladder cancer[6].

Previous researches have suggested that lncRNA DSCAM-AS1 plays a vital role in breast cancer biology and therapeutic resistance. For example, DSCAM-AS1 acts as an oncogene in tamoxifen-resistance breast cancer by promoting cell proliferation and suppressing cell apoptosis[7]. Moreover, DSCAM-AS1 serves as an oncogene in ER-positive breast cancer phenotypes[8]. However, the exact role of DSCAM-AS1 in NSCLC has not been elucidated. Our study found that the DSCAM-AS1 expression was significantly higher in NSCLC tissues. DSCAM-AS1 significantly promoted NSCLC cell migration and invasion *in*

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vitro. Moreover, we further explored the underlying mechanism of DSCAM-AS1 function in the NSCLC development.

Patients and Methods

Cell Lines and Clinical Samples

56 NSCLC patients who received surgery at Yantaishan Hospital were enrolled in this study. Meanwhile, human tissues were collected. Before the operation, written informed consent was obtained from each subject. No radiotherapy or chemotherapy was performed before surgery. The tissues collected from the surgery were immediately stored at -80°C. All tissues were confirmed by an experienced pathologist. This study was approved by the Ethics Committee of Yantaishan Hospital.

NSCLC cell lines (SPCA1, A549, PC-9, H1975) and normal human bronchial epithelial cell (16HBE) were obtained from the Shanghai Model Cell Bank (Shanghai, China). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) and penicillin. Besides, cells were maintained in a 5% CO_2 and 37°C incubator.

Cell Transfection

After synthesis, the lentiviral virus targeting DSCAM-AS1 was cloned into a pLenti-EF1a-EGFP-F2A-Puro vector (Biosettia Inc., San Diego, CA, USA). 293T cells were used for packaging DSCAM-AS1 lentiviruses (DSCAM-AS1) and empty vector (control). Subsequently, constructed plasmids were transfected into NSCLC cells. 48 h later, the DSCAM-AS1 expression level in cells was detected using Real Time-quantitative Polymerase Chain Reaction (RT-qPCR).

RNA Extraction and RT-qPCR

Total RNA was extracted according to the instructions of TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Subsequently, extracted total RNA was reverse transcribed into complementary deoxyribose nucleic acid (cDNA) through the reverse Transcription Kit (TaKaRa Biotechnology Co., Ltd., Dalian, China). The thermal cycle was as follows: 30 sec at 95°C, 5 sec for 40 cycles at 95°C, 35 sec at 60°C. The primer sequences used in this study were as follows: DSCAM-AS1, F: 5'-CCAGGAACCAATCCTTACTC-3', R: 5'-CCCTAGGGATGTGACCGAAGGA-3'; BCL11A, F: 5'-ATAAGTGTAAACATCCTCGACTG-3', R: 5'-CTCCCGTGTCGTGGAGTCG-3'; β-actin: F: 5'-CCTGGCACCCAGCACAAT-3', R: 5'-GCTGATCCACATCTGCTGGAA-3'.

Western Blot Analysis

Reagent radioimmunoprecipitation assay (RIPA) (Beyotime, Shanghai, China) was utilized to extract total protein from cells. The concentration of extracted protein was detected by the BCA protein assay kit (TaKaRa, Dalian, China). The protein samples were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membranes were then incubated with primary and corresponding secondary antibodies. Rabbit anti-GAPDH and rabbit anti-BCL11A, as well as goat anti-rabbit secondary antibody were provided by Cell Signaling Technology (CST; Danvers, MA, USA). The chemiluminescent film was applied for protein expression detection with Image J software.

Wound Healing Assay

Cells were seeded into 6-well plates and cultured in DMEM medium overnight. After scratching with a plastic tip, the cells were cultured in serum-free DMEM. Wound closure was observed at different time points. Each assay was independently repeated in triplicate.

Matrigel Assay

5×10^4 cells in 200 μL serum-free DMEM were added to the upper chamber of an 8 μm pore size insert (Millipore, Billerica, MA, USA) coated with 50 μg Matrigel (BD Biosciences, Franklin Lakes, NJ, USA). Meanwhile, the lower chamber was added with DMEM and FBS. 48 h later, after being wiped by cotton swab, the top surface of the chamber was immersed in pre-cooled methanol for 10 min. Then the cells were stained with crystal violet for 30 min. Three fields were randomly selected for each sample, and the number of invading cells was counted.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 (SPSS, Chicago, IL, USA) was used for all statistical analyses. Data were expressed as mean ± SD. Chi-square test, student *t*-test and Kaplan-Meier method were selected when appropriate. $p<0.05$ was considered statistically significant.

Results

DSCAM-AS1 Expression Level in NSCLC Tissues and Cells

First, RT-qPCR was conducted for the DSCAM-AS1 expression detection in 56 tumor tissue samples and 4 NSCLC cell lines. As a result, DSCAM-AS1 was significantly upregulated in tumor tissue samples (Figure 1A). Meanwhile, the expression of DSCAM-AS1 in NSCLC cells was remarkably higher than that of the normal human bronchial epithelial cells (16HBE) (Figure 1B).

High Expression of DSCAM-AS1 was Correlated with Poor Overall Survival of NSCLC Patients

The overall survival of NSCLC patients after surgery was analyzed through the Kaplan-Meier method. According to the median expression, 56 NSCLC patients were divided into two groups, including the high-DSCAM-AS1 group and low-DSCAM-AS1 group. The results of the Kaplan-Meier analysis showed that a higher DSCAM-AS1 level indicated worse overall survival of NSCLC patients (Figure 1C).

Overexpression of DSCAM-AS1 Promoted Migration and Invasion of NSCLC Cells

In our work, NSCLC cell line SPCA1 was selected for DSCAM-AS1 overexpression *in vitro*. Then RT-qPCR was utilized to detect the expression of DSCAM-AS1 (Figure 2A). The results of wound healing assay revealed that after DSCAM-AS1 overexpression, the migration ability of NSCLC cells was significantly enhanced (Figure 2B). Moreover, transwell assay demonstrated that after DSCAM-AS1 was overexpressed in NSCLC cells, the number of migrated and invaded cells was remarkably increased (Figure 3A, 3B).

Interaction Between BCL11A and DSCAM-AS1 in NSCLC

RT-qPCR results showed that compared with the empty vector (control) group, the expression level of BCL11A in NSCLC cells of DSCAM-AS1 lentiviruses (DSCAM-AS1) group was significantly higher (Figure 4A). Western blot assay found that, after the DSCAM-AS1 overexpression, the protein expression of BCL11A was upregulated (Figure 4B). We further found that BCL11A expression in NSCLC tissues was significantly higher when compared with that of the adjacent tissues (Figure 4C). The correlation analysis demonstrated that the BCL11A expression was positively correlated with the DSCAM-AS1 expression in NSCLC tissues (Figure 4D).

Discussion

Previous studies have demonstrated that lncRNAs are a kind of important regulators in lung cancer development and progression. For example, lncRNA PRNCR1 promotes the progression of NSCLC by upregulating HEY2 through the PRNCR1-miR-488-HEY2 network[9]. FGF1 upregulation resulted from lncRNA RAB1A-2 overexpression promotes lung cancer development, eventually leading to poor prognosis[10]. LncRNA AGER plays an inhibitory role in the development of lung cancer by targeting AGER[11]. Our study showed that DSCAM-AS1 was significantly upre-

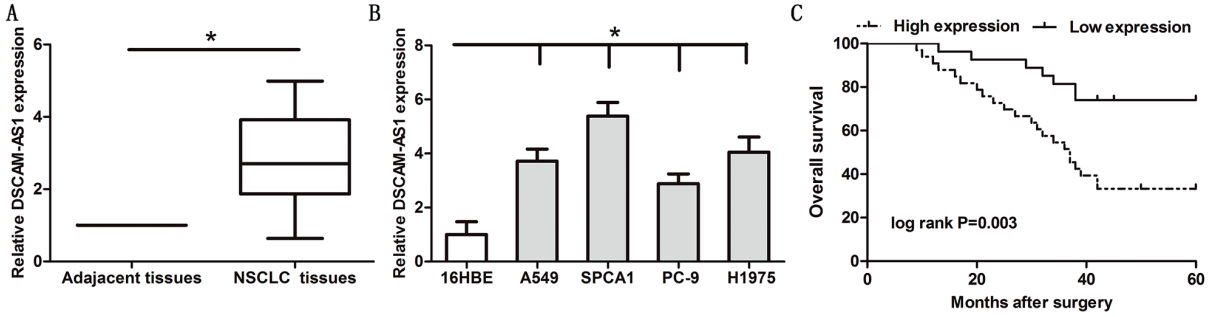

Figure 1. Expression level of DSCAM-AS1 in NSCLC tissues and cell lines. ***A,*** DSCAM-AS1 expression in NSCLC tissues was significantly up-regulated when compared with adjacent tissues. ***B,*** Expression levels of DSCAM-AS1 relative to β-actin in human NSCLC cell lines and 16HBE (normal human bronchial epithelial cell line) were determined by RT-qPCR. ***C,*** The higher expression of DSCAM-AS1 was associated with worse overall survival of NSCLC patients. Data were presented as mean ± standard error of mean. **p*<0.05.

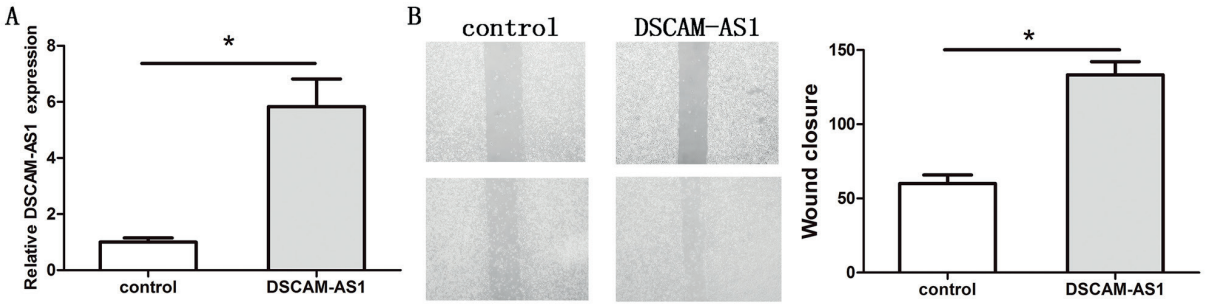

Figure 2. Overexpression of DSCAM-AS1 promoted the migration ability of NSCLC cells. ***A,*** DSCAM-AS1 expression in NSCLC cells transfected with DSCAM-AS1 lentiviruses (DSCAM-AS1) and empty vector (control) was detected by RT-qPCR. β-actin was used as an internal control. ***B,*** Wound healing assay showed that the overexpression of DSCAM-AS1 significantly increased the migration of SPCA1 NSCLC cells. The results represented the average of three independent experiments (mean ± standard error of mean). *p<0.05, as compared with control cells. *p<0.05.

gulated in NSCLC samples and cell lines. Besides, a significant correlation was observed between the prognosis of NSCLC patients and the DSCAM-AS1 expression. Furthermore, after DSCAM-AS1 overexpression, NSCLC cell migration and invasion abilities were markedly enhanced. The above results indicated that DSCAM-AS1 promoted tumorigenesis of NSCLC and might act as an oncogene.

B cell leukemia 11A (*BCL11A*) gene is essential for pre-B-cell development, lymphocyte maturation and goblin switching. It has also been identified as a proto-oncogene in hematopoietic cell malignancies and breast cancer[12-14]. Researchers[15] have found that *BCL11A* is upregulated in lung squamous cell carcinoma by acting with SOX2. By directly targeting BCL11A, miR-146a acts as a potential tumor suppressor gene in human neuro-

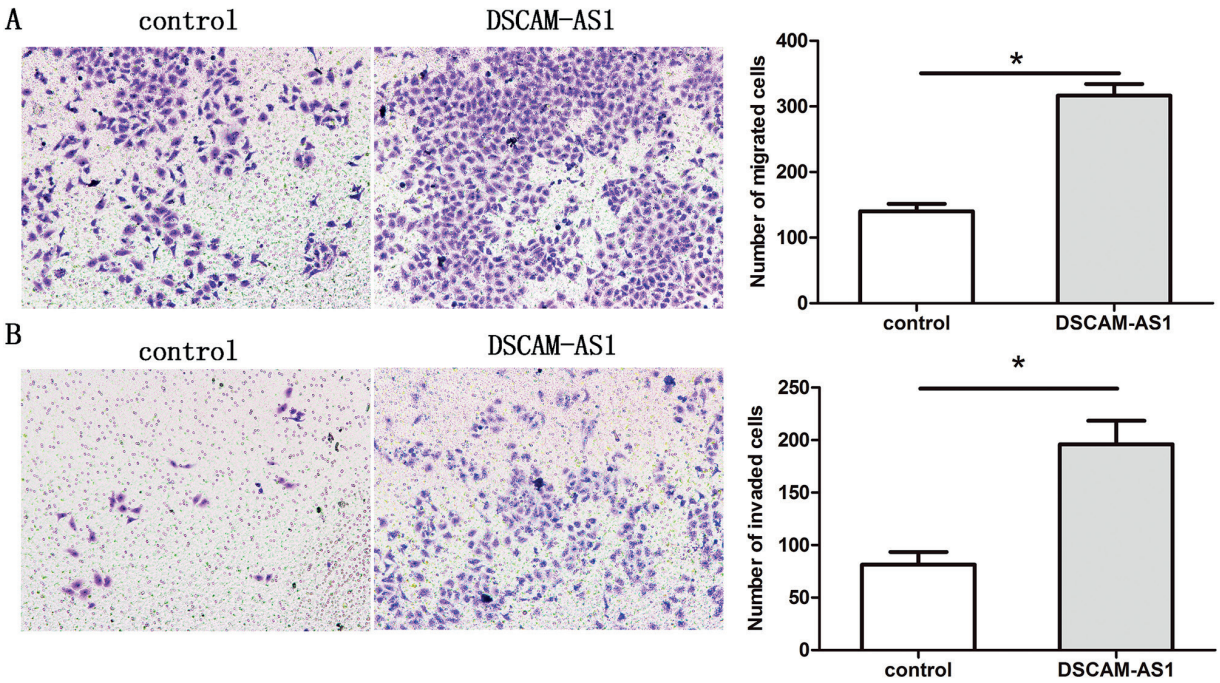

Figure 3. Overexpression of DSCAM-AS1 promoted NSCLC cell migration and invasion. ***A,*** transwell assay showed that the number of migrated cells was significantly increased after DSCAM-AS1 overexpression in SPCA1 NSCLC cells. ***B,*** transwell assay showed that the number of invaded cells was significantly increased after overexpression of DSCAM-AS1 in SPCA1 NSCLC cells. The results represented the average of three independent experiments (mean ± standard error of mean). *p<0.05, as compared with control cells. *p<0.05.

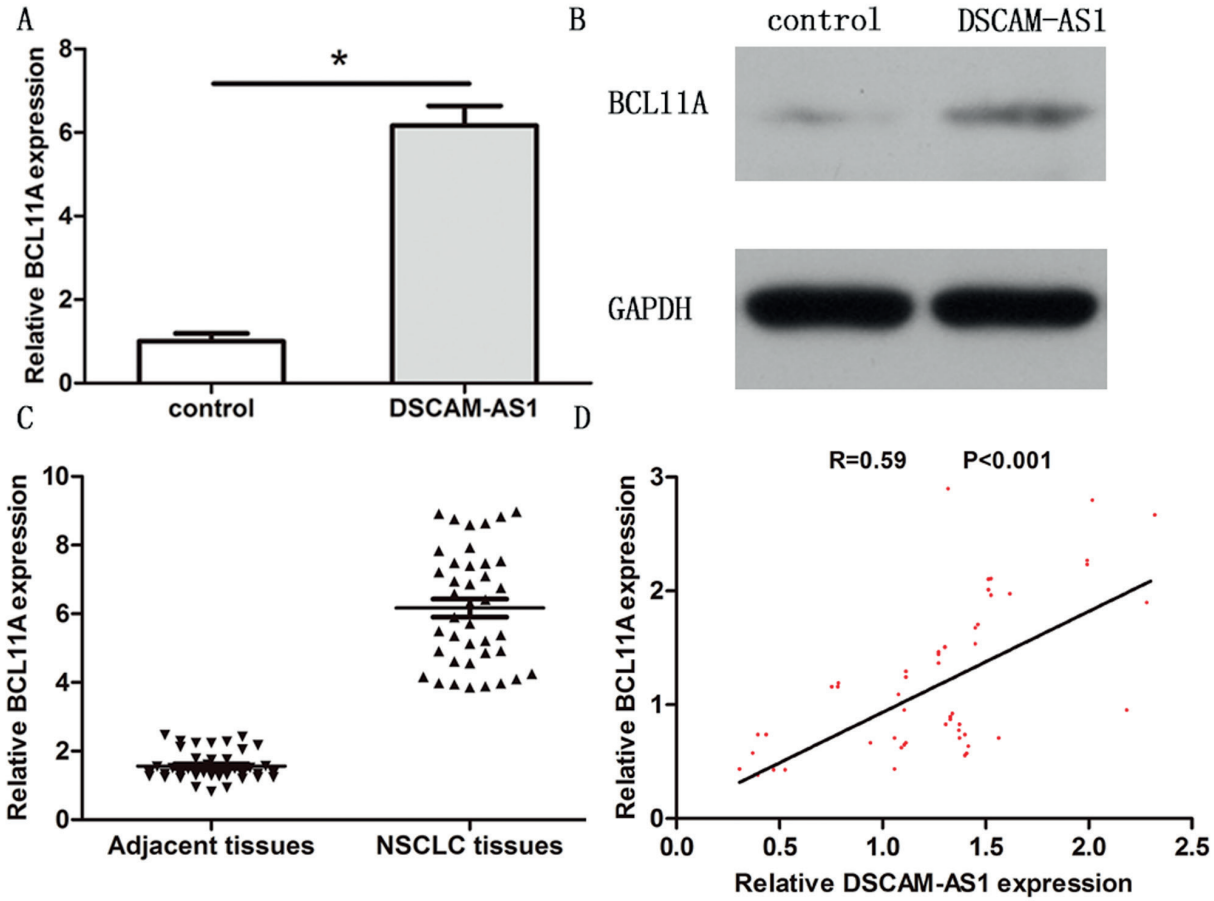

Figure 4. Interaction between DSCAM-AS1 and BCL11A. ***A,*** RT-qPCR results showed that BCL11A expression was significantly increased in the DSCAM-AS1 lentiviruses (DSCAM-AS1) group compared with the empty vector (control) group. ***B,*** Western blot assay revealed that the protein expression BCL11A in the DSCAM-AS1 lentiviruses (DSCAM-AS1) group was remarkably higher than the empty vector (control) group. ***C,*** BCL11A was significantly up-regulated in NSCLC tissues compared with adjacent tissues. ***D,*** Linear correlation between the expression levels of BCL11A and DSCAM-AS1 in NSCLC tissues. The results represented the average of three independent experiments. Data were presented as mean ± standard error of mean. *$p<0.05$.

blastoma[16]. Meanwhile, *BCL11A* has been shown to play an oncogenic role in B-cell lymphoma and triple negative breast cancer[17]. In the present study, BCL11A expression was significantly upregulated after overexpression of DSCAM-AS1. Moreover, the BCL11A expression in NSCLC tissues was positively associated with the DSCAM-AS1 expression. All the above results suggested that DSCAM-AS1 might promote tumorigenesis of NSCLC *via* targeting BCL11A.

Conclusions

We showed that DSCAM-AS1 was remarkably upregulated in NSCLC, which was negatively related to overall survival of NSCLC patients. Besides, DSCAM-AS1 could significantly enhance the NSCLC cell migration and invasion by targeting BCL11A. These findings indicated that DSCAM-AS1 might contribute to the treatment of NSCLC as a candidate target.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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