WITHDRAWN: LncRNA DSCAM-AS1 Promotes Proliferation, Migration and Invasion of Colorectal Cancer Cells via Modulating miR-144-5p/CDKL1

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The full text of this preprint has been withdrawn by the authors while they make corrections to the work. Therefore, the authors do not wish this work to be cited as a reference. Questions should be directed to the corresponding author.

Abstract

Background LncRNA DSCAM-AS1 is oncogenic in several cancers. However, DSCAM-AS1 expression and function in colorectal cancer (CRC) remain far from being fully elucidated.

Methods Paired CRC tissues/adjacent tissues were collected, and the expression levels of DSCAM-AS1, miR-144-5p and CDKL1 were examined by qRT-PCR; DSCAM-AS1 shRNA was transfected into HCT-116 and SW480 cell lines to establish cell models. The proliferation was detected through CCK-8 assay and plate colony formation assay. Transwell assay was used to evaluate the migration and invasion. QRT-PCR and western blot were adopted to analyze changes in miR-144-5p and CDKL1; luciferase reporter gene assay was performed to determine the regulatory relationship between miR-144-5p and DSCAM-AS1, miR-144-5p and CDKL1.

Results DSCAM-AS1 was notably up-regulated in CRC samples, positively correlated with CDKL1 expression, while negatively correlated with miR-144-5p. After the transfection of DSCAM-AS1 shRNAs into cancer cells, the proliferative and metastatic ability of cancer cells were impeded. DSCAM-AS1 could reduce the expression level of miR-144-5p by binding with it. Additionally, CDKL1 was also validated as a target gene of miR-144-5p, and DSCAM-AS1 was proved to indirectly regulate CDKL1 expression.

Conclusion DSCAM-AS1 was aberrantly up-regulated in CRC, and it can modulate the cells proliferative and metastatic ability. It has the ability to be the “ceRNA” to regulate CDKL1 expression via sponging miR-144-5p.

1. Introduction

Colorectal cancer (CRC) is a frequently diagnosed cancer worldwide. In 2012, the global incidence of CRC was about 17.2/100000, with a high mortality rate of 8.4/100000 [1, 2]. CRC ranks the fourth in the incidence of malignant tumors in males and the third in females in China [3]. Even though surgery combined with adjuvant chemotherapy could be of benefit, the prognosis of CRC still remains unsatisfactory with only about 50% survival rate in 5 years [4]. Due to this fact, it’s imperative to explore the biological mechanism of CRC to make the contribute to exploration of novel therapy targets and a reduction in CRC mortality.

Long non-coding RNAs (lncRNAs) emerge as functional regulatory elements in mediating gene expression. Accumulating lncRNAs were proved to be linked to diverse death-related diseases [5, 6]. For instance, in CRC, lncRNA CPS1-IT1 was associated with tumorigenesis, progression and prognosis [7]; The highly expressed lncRNA BANCR in CRC tissues has a positive correlation with lymph node metastasis, indicating a low survival rate [8]. LncRNA DSCAM-AS1 has also been confirmed to be closely linked to the tumorigenesis and progression of breast cancer, non-small cell lung cancer and other cancers in several previous studies [9, 10]. Unfortunately, the role of it in CRC and its downstream molecular mechanism are poorly understood.

It has been confirmed that exceeding 30% coding genes in human body were regulated by miRNAs, by which miRNAs were widely involved in the cell development and differentiation, energy metabolism, cell cycle and other biological process [11]. miRNA is also a participant in CRC progression [12]. In details, the expressions of miR-148a and miR-625-3p were proven to be down-regulated, which was significantly linked to epithelial-interstitial transformation in CRC. For this reason, miR-148a and miR-625-3p can be used as biomarkers for the prognosis of CRC [13]. miR-144-5p has been validated to be abnormally expressed and affect the progress of diverse diseases, such as bladder cancer, non-small cell lung cancer and so on [14, 15]. But the its role in CRC needs to be explored in detail.

Cell division cycle gene 2 (CDC2) is a key player in mitosis and Cyclinin-dependent kinase-like protein 1 (CDKL1) is a member of CDC2-related serine/threonine protein kinase family. CDKL1 has been revealed to regulate cell cycle, whose abnormal expression has a close connection with the disorder of cell cycle regulation in malignant tumors [16]. Some scholars have pointed out that CDKL1 expression was markedly up-regulated in neuroblastoma, which can induce cell cycle G0/G1 phase arrest and apoptosis, thereby impeding the migration and invasion of tumor cells [17]. With regard to the function of CDKL1 in CRC, Qin C et al reckoned that CDKL1 protein expression was notably up-regulated in CRC and the knockdown of CDKL1 could inhibit cell growth, migration and invasion [18]. However, there was a lack of research on the upstream molecular mechanism of CDKL1.

We identified in present study DSCAM-AS1 as a novel player in modulating CRC progression. The investigation on expressions and functions of DSCAM-AS1, miR-144-5p and CDKL1 in CRC would help clarify the mechanism of CRC progression and provide potential therapy targets.

2. Materials And Methods

2.1. Tissue samples

We collected tumor tissues and paracancerous normal tissues from 40 patients who had histologically confirmed CRC and underwent radical colectomy in Jiangsu Province Hospital from January 2019 to January 2018. The control samples were procured from the adjacent normal tissue in the same patient, in which no cancer cells were found by pathological examination after operation. The tissue samples were surgically removed and immediately preserved with liquid nitrogen at -196 ℃. The written informed consent was obtained from participants enrolled. The protocol was approved by the review board of Nanjing Medical University.

2.2. Cell culture

Human CRC cell lines HCT-116, SW480, SW620, DLD-1, HT29 and normal colon epithelial cell line CCD841 were purchased from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). All cell lines were cultured in RPMI-1640 medium (Gibco, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS; Carlsbad, CA, USA) and 100U/ml penicillin/streptomycin (Carlsbad, CA, USA) at 37 ℃ and 5% CO_2. The solution was replaced every 3 days, and the cells were passaged when covered with the bottom of the culture bottle. The cells in logarithmic growth phase were used for the following assays.

2.3. Cell transfection

The cells were washed clean with PBS for 3 times and trypsinized by trypsin for 2min. Then, after treatment, the cells were transferred to 15ml aseptic centrifugal tube, centrifuged and counted. Cells were then and inoculated in 6-well plate with 4×10^5 cells per well, with the fusion rate of about 70%. The transfection reagent was diluted with serum-free medium to 3 μL/L and incubated at 37 ℃ for 20 min. DSCAM-AS1 siRNA, DSCAM-AS1 plasmid, miR-144-5p mimics, miR-144-5p inhibitor and control groups were diluted to 50 μmol/L and incubated at room temperature for 5min. Ultimately, they were mixed with the same volume of the transfection reagent and incubated in the incubator at 37 ℃. 12 hours later, the serum-free medium was replaced with the complete medium. After 48 hours of culture, RNA was extracted to verify the efficiency of transfection. DSCAM-AS1 plasmid and miR-144-5p mimics were integrated by GenePharma (Shanghai, China), empty plasmid was used as control, and DSCAM-AS1

lentivirus and miR-144-5p inhibitor were purchased from RiboBio Company (Guangzhou, China). The stably transfected cells were selected by Geneticin (Sigma-Aldrich, St Louis, MO, USA).

2.4. RNA isolation and qRT-PCR

Total RNA was isolated and purified from tissues and cell lines with the use of TRIzol reagent (Invitrogen, Carlsbad, USA). The purity and concentration of RNA were measured by NanoDrop ND-2000 spectrophotometer (NanoDrop Wilmington DE). A total RNA of 5 μg was reversed to cDNA by M-MLV (Thermo Fisher Scientific, Inc., Rockford, IL, USA). SYBR Green PCR Master mix (Thermo Fisher Scientific) was adopted to perform qRT-PCR on a ABI 7300 machine (Applied Biosystems, Foster City, CA, USA). The qRT-PCR conditions were as follows: pre-denaturation at 95 ℃ for 10 min, 95 ℃ for 15 s and 60 ℃ for 15 s for 45 cycles. The fluorescence signal was obtained at 60 ℃. GAPDH and U6 were used as internal references to detect the expressions of DSCAM-AS1 and miR-144-5p, respectively. The relative expressions of DSCAM-AS1, miR-144-5p and CDKL1 were calculated by $2^{-\Delta\Delta Ct}$ method. The primers sequences were listed in **Table 1**.

Table 1
qRT-PCR Primer Sequences

Name	Primer sequences(5'-3')
DSCAM-AS1	Forward:GTGACACAGCAAGACTCCCT
	Reverse:GATCCGTCGTCCATCTCTGT
miR-144-5p	Forward:CGGGCGATATCATCATATACTG
	Reverse:GTGCAGGGTCCGAGGT
CDKL1	Forward:CGAATGCTCAAGCAACTCAAGC
	Reverse:GCCAAGTTATGCTCTTCACGAG
GAPDH	Forward:TGACCCCTTCATTGACCTCA
	Reverse:GGACTCCACGACATACTCAG
U6	Forward:CTCGCTTCGGCAGCACA
	Reverse:AACGCTTCACGAATTTGCGT

2.5. *CCK-8 assay*

The logarithmic phase cells were trypsinized with trypsin, and the cell suspension was prepared by 10%FBS and cultured in the culture medium. HCT-116 and SW480 cells (3000 cells per well) were incubated in 96 well plates for 1, 2, 3 and 4 days, respectively. Then 10 μl Enhanced Cell Counting Kit-8 (Beyotime, Beijing, China) was added to incubate at 37 ℃ for 1 hour. After continuous culture at 37 ℃ for 2 hours, the culture was terminated. The absorbance value (OD) of each well was measured at 450nm, and the relative OD ratio was used to represent the viability of the cells.

2.6. Colony formation assay

Cells in each group were inoculated in 12 well plates with 1000 cells per well. The culture medium was removed after two weeks, and the colonies were washed with PBS for 3 times. Afterwards, cells were fixed with methanol for 20 minutes, stained with methylene blue for 40 min, and subsequently washed with deionized water twice and dried. The clone number with over 50 cells was calculated under microscope, and the clone formation rate was calculated.

2.7. Transwell assay

5×10^4 transfected cells resuspended in serum-free medium in each group were placed in the upper chamber of transwell chambers, and medium containing 10% FBS was added into the lower chamber. After cultured at 37 ℃ for 24 hours, the cells failing to migrate or invade were removed from the membrane. Then the cells was fixed with 4% paraformaldehyde for 10 minutes and stained with 0.5% crystal violet for 30 minutes. After rinsing with the tap water, the cells was counted under the inverted microscope. Matrigel was required in the invasion experiment, but not in the migration assay.

2.8. Flow cytometry

The cells were seeded in 96 well plates with 1×10^4 cells per well. After 24 hours of culture, the cells were washed twice with PBS, fixed with 70% ethanol and stored overnight at 4 ℃. After washing once with PBS, the cell density was adjusted to 1×10^6 cells/mL. Propidium iodide staining solution was subsequently added to adjust the final concentration to 0.05mg/mL, and the cells were dyed at 4 ℃ for 30min. Then the cells were analyzed by flow cytometry.

2.9. Dual luciferase reporter assay

The target fragments of wild type and mutant type DSCAM-AS1 were integrated into pGL3 vector (Promega, Madison, WI, USA) to construct pGL3-lncDSCAM-AS1-wild type and pGL3-lncDSCAM-AS1-mutant reporter vector. DSCAM-AS1-wt or DSCAM-AS1-mut was co-transfected with miR-144-5p mimics or negative control into HCT-116 and SW480 cells. 48 hours after transfection, the luciferase activity was determined. Luciferase activity was measured using the Dual-luciferase reporter assay system (Promega, Madison, WI, USA).

2.10. Western blot

CRC cells were lysed with RIPA buffer (Beyotime, China) containing protease inhibitor PMSF. SDS-PAGE was conducted to separate the proteins and then the proteins were transferred to the nitrocellulose (NC) membrane. Then the membranes were blocked with 5% silk milk. Next the membranes were incubated with the primary antibodies and the secondary antibody. Ultimately, color rendering was performed using hypersensitive ECL (Hubei Biossci Biotechnology Co, Ltd.). The primary antibodies includes CDKL1 antibody (1:1000, Cell Signaling Technology) and anti-GAPDH antibody (1:2000, Santa Cruz).

2.11. *Mice test*

BALB/C nude mice aged 6 weeks were enrolled. The animal-related experiments were approved by the Institutional Animal Care and Use Committee. We randomly divided the mice into two groups with 10 mice in each group. HCT-116 cells (2×10^7/ml) with DSCAM-AS1 overexpression or the control cells were resuspended in PBS. The cell suspension was then injected subcutaneously into the bilateral posterior back of nude mice. The longest and shortest diameters of the tumors were measured every 3 days using a caliper. The formula was performed to calculate the tumor volume: volume = ($0.5\times\text{length}\times\text{width}^2$). At the 18 days after the injection, the mice were sacrificed and the tumors were removed and weighted. In the lung metastasis study, 1×10^7 cells in every group were injected into caudal vein of 10 mice respectively. Two weeks later, mice were killed and lung colonization was quantified through pathological examination.

2.12. Statistical analysis

All statistical analysis was performed using SPSS18.0. Experimental results were presented as Mean ± SD. Comparisons among different groups were conducted using Student's *t*-test. Chi-square test was adopted to analyse the correlation between the expressions of DSCAM-AS1 as well as miR-144-5p and pathological features. Differences with $p<0.05$ were considered to be statistically significant.

3. Results

3.1. High expression of DSCAM-AS1 and low expression of miR-144-5p were observed in CRC tissues and cells

The qRT-PCR was performed to detect the expressions of DSCAM-AS1 and miR-144-5p in 40 pairs of CRC cancer tissues and adjacent tissues. Highly expressed DSCAM-AS1 in CRC tissues was observed as compared with normal tissues (**Fig. 1A**). Similarly in CRC cells, DSCAM-AS1 expression was also up-regulated (**Fig. 1B**). Conversely, miR-144-5p was significantly down-regulated in CRC tissues and cells (**Fig. 1C** and **1D**). Additionally, we also observed that the high expression of DSCAM-AS1 and low expression of miR-144-5p were associated with the lymphatic invasion (**Table 2**).

Table 2
Correlation between expressions of DSCAM-AS1 and miR-144-5p with clinical feature

Clinicopathologic factors	Patients number	DSCAM-AS1 expression		X^2	*P*	miR-144-5p expression		X^2	*P*
	(n=40)	Lower (n=18)	Higher (n=22)			Lower (n=19)	Higher (n=21)		
Gender				0.803	0.370			1.283	0.257
Male	26	15	11			10	16		
Female	14	6	8			8	6		
Age									
≤60	28	15	13	0.043	0.836	12	16	0.173	0.677
>60	12	6	6			6	6		
Tumor position				0.973	0.324			0.004	0.949
Colon	22	10	12			10	12		
Rectum	18	11	7			8	10		
Tumor size(cm)				0.382	0.536			0.123	0.726
<5	19	9	10			8	11		
≥5	21	12	9			10	11		
Histological grade				0.173	0.677			0.4173	0.519
I-II	36	6	30			21	15		
III-IV	4	1	3			3	1		
Lymphatic invasion									
Yes	24			13.949	<0.001***			6.077	0.014*
No	16	14	2			11	5		

*, ** and *** represent $p<0.05$, $p<0.01$ and $p<0.001$, respectively.

3.2. High expression of DSCAM-AS1 can promote the proliferation, migration and invasion of CRC cells

Since DSCAM-AS1 was expressed highly in HCT-116 cell line and lower in SW480 cell line, we selected HCT-116 cell line to conduct DSCAM-AS1 knock-down and SW480 cell line to conduct DSCAM-AS1 overexpression (**Fig. 2A**). The results of CCK8 and colony formation assay indicated that the DSCAM-AS1 overexpression could promote the proliferation of SW480 cells, while DSCAM-AS1 knockdown could significantly restrain the proliferation of HCT-116

cells (**Fig. 2B**-**2D**). Through transwell assay, we observed that DSCAM-AS1 knock-down can significantly repress the invasion and migration of HCT-116 cells, while DSCAM-AS1 overexpression can accelerate that of SW480 cells (**Fig. 2E** and **2F**). The results of flow cytometry analysis demonstrated that DSCAM-AS1 knockdown could significantly enhance the apoptosis of HCT-116 cells, while DSCAM-AS1 overexpression suppressed the apoptosis of SW480 cells (**Fig. 2G**). Taken all results mentioned above together, we can draw a conclusion that DSCAM-AS1 could promote the growth and metastasis of CRC cells.

3.3. DSCAM-AS1 can directly act on miR-144-5p

We carried out bioinformatics analysis through StarBase (http://www.starbase.com) and found that DSCAM-AS1 contained a potential target site of miR-144-5p (**Fig. 3A**). Afterwards, qRT-PCR results revealed that the expressions of DSCAM-AS1 and miR-144-5p in CRC tissues were significantly negatively correlated (**Fig. 3B**). In order to further verify that DSCAM-AS1 can directly bind to miR-144-5p, we adopted dual luciferase reporter assay. The results informed us that miR-144-5p mimics reduced the luciferase activity of DSCAM-AS1-wt, but had no significant effect on DSCAM-AS1-mut (**Fig. 3C**). Moreover, we found that overexpression of DSCAM-AS1 in SW480 cells could down-regulate miR-144-5p expression, while miR-144-5p expression was up-regulated after knocking down DSCAM-AS1 in HCT-116 cells (**Fig. 3D** and **3E**). However, knockdown or overexpression of miR-144-5p in HCT-116 and SW480 cells has no effect on DSCAM-AS1 expression (**Fig. 3F**). These results implied that miR-144-4p was a downstream target of DSCAM-AS1 and could be negatively regulated by it.

3.4. Low-expressed miR-144-5p can facilitate CRC progression

In order to determine how miR-144-5p affect CRC progression, we constructed overexpression and inhibition of miR-144-5p models in HCT-116 and SW480 cells, respectively (**Fig. 4A**). It was indicated that overexpressed miR-144-5p can significantly restrain the proliferation of HCT-116 cells, while knockdown of miR-144-5p can speed up the proliferation of SW480 cells (**Fig. 4B** and **4C**). And the results of colony formation assay showed the same trend (**Fig. 4D**). By transwell experiment, we observed that the significant inhibition of invasion and migration of HCT-116 cells were induced by overexpressed miR-144-5p, while inhibition of miR-144-5p can promote SW480 cells (**Fig. 4E** and **4F**). Flow cytometry results demonstrated that overexpression of miR-144-5p significantly promoted apoptosis of HCT-116 cells, whereas knockdown of miR-144-5p significantly inhibited apoptosis of SW480 cells ($P<0.05$, **Fig. 4G**). The above results signified that miR-144-5p had the obvious inhibitory effect on the growth and metastasis of CRC cells.

3.5. CDKL1 can directly bind miR-144-5p

For the exploration of the downstream molecular mechanism of miR-144-5p, we conducted bioinformatics analysis through targetscan (http://www.targetscan.org) and found that miR-144-5p and CDKL1 have two binding sites (**Fig. 5A**). We further verified this conjecture by dual luciferase activity assay. The results confirmed that miR-144-5p reduced the luciferase activity of wild type CDKL1, but exerted no effect on mutant CDKL1 (**Fig. 5B** and **5C**). The above results showed that CDKL1 can bind miR-144-5p in a targeted manner. Therefore, we studied the expression and function of CDKL1 in CRC. qRT-PCR indicated that CDKL1 expression was significantly up-regulated in CRC (**Fig. 5D**), and miR-144-5p expression was significantly negatively correlated with CDKL1 expression (**Fig. 5E**). In addition, we set up overexpression and inhibition of miR-144-5p in HCT-116 and SW480 cells. qRT-PCR and western blot

showed that compared with the control group, overexpression of miR-144-5p could significantly reduce CDKL1 expression and inhibition of miR-144-5p can increase CDKL1 in HCT-116 and SW480 cells (**Fig. 5F**).

3.6. DSCAM-AS1 promotes the proliferation, migration and invasion of CRC cells via miR-144-5p and CDKL1

In order to determine whether DSCAM-AS1 regulates proliferation and metastasis of CRC cells through miR-144-5p/CDKL1 axis, we transfected miR-144-5p mimics into SW480 cells with overexpressed DSCAM-AS1 (**Fig. 6A** and **6B**). CCK8 and colony formation assay proved that transfection of miR-144-5p mimics reduced the proliferation ability of SW480 cells, while overexpression of DSCAM-AS1 inhibited the effect of miR-144-5p (**Fig. 6C** and **6D**). Transwell experiment confirmed that transfection of miR-144-5p mimics offsetted the enhancement of cell migration and invasion caused by overexpression of DSCAM-AS1 (**Fig. 6E-6G**). Flow cytometry demonstrated that compared with SW480 cells with overexpressed DSCAM-AS1, the transfection of miR-144-5p mimics improved apoptosis ($P<0.05$, **Fig. 6H**). Additionally, qRT-PCR and western blot were performed to detect CDKL1 expression after overexpression of DSCAM-AS1. The results informed us that CDKL1 expression was significantly increased after overexpression of DSCAM-AS1, while the opposite result occurred when DSCAM-AS1 was knocked down (**Fig. 6I** and **6G**). Collectively, these results implied that the oncognic function of DSCAM-AS1 in CRC was partly mediated by miR-144-5p and CDKL1

3.7. DSCAM-AS1/miR-144-5p/CDKL1 axis promotes the growth and metastasis of CRC tumor in vivo

The results form tumorigenicity experiment in nude mice showed that the tumor volume and weight in the DSCAM-AS1 overexpression group were significantly higher than those in the control group (**Fig. 7A** and **7B**), and the lung metastasis in the DSCAM-AS1 overexpression group was significantly more obvious than that in the control group (**Fig. 7C**). In addition, the qRT-PCR results for the tumor tissue of mice demonstrated that miR-144-5p expression in the DSCAM-AS1 overexpression group was significantly lower than that of the control group (**Fig. 7D**), whereas DSCAM-AS1 overexpression group had higher CDKL1 expression than that of the control group (**Fig. 7E**). These *in vivo* data further validated the role of DSCAM-AS1/miR-144-5p/CDKL1 axis in CRC progression.

4. Discussion

In this study, we found that DSCAM-AS1 and CDKL1 were significantly up-regulated in CRC. Furthermore, we demonstrated that they could promote the proliferation and metastasis of CRC cells, while miR-144-5p has the opposite effect. Apart from that, our study verified that DSCAM-AS1 can target miR-144-5p to increase CDKL1 expression, thus promoting the tumorigenesis of CRC. This research revealed for the first time the role of DSCAM-AS1/miR-144-5p/CDKL1 axis in CRC, providing potential biomarkers and therapy targets for diagnosis and treatment of CRC.

LncRNA widely exists in the nucleus and cytoplasm, which modulate gene expression at epigenetics, transcription and post-transcription levels through gene silencing, histone modification, transcription activation and interference, thus regulating the tumorigenesis and progression of cancer [19]. LncRNA regulates genes in CRC via multiple mechanisms, including epigenetic modification and interaction with miRNA and proteins [20-22]. The role of

DSCAM-AS1 has also been investigated in several studies. It is confirmed that DSCAM-AS1 expression was up-regulated in breast cancer [23]; DSCAM-AS1 can target miR-137 and EPS8 to facilitate the proliferation of breast cancer cells while arrest their apoptosis, and DSCAM-AS1 can enhance the drug resistance of tamoxifen [24]. The above researches uncovered that DSCAM-AS1 had the function as an oncogene. Our study firstly validated that DSCAM-AS1 was up-regulated in CRC, and modulated the malignant phenotypes of cancer cells, suggesting that DSCAM-AS1 can be a potential therapeutic target.

For the in-depth exploration of the downstream molecular mechanism of DSCAM-AS1, we conducted bioinformatics analysis with the help of StarBase. DSCAM-AS1 and miR-144-5p were found to have a binding site. The upstream mechanism of miR-144-5p in CRC has not been reported much in the past, but miR-144, as a miRNA with extensive research, has been proved to be significantly down-regulated in CRC tissues and cells [25]. The overexpression of miR-144 in CRC patients' feces can be used as a screening diagnostic marker for CRC [26]. There are also studies on the molecular mechanism of miR-144 in CRC. It is proved that downregulation of miR-144 can activate mTOR signaling pathway, and its expression is related to poor prognosis of patients [25]. It was also found that lncRNA ZNFX1-AS1 was significantly up-regulated in CRC and had a negative regulatory relationship with miR-144 and DSCAM-AS1/miR-144/EZH2 axis was involved in CRC regulation [27]. The targeted binding relationship between DSCAM-AS1 and miR-144-5p as well as miR-144-5p as a tumor suppressor gene in CRC were verified in the present research.

A large body of evidences have supported the idea that CDKL1 was tightly linked to multiple tumors. For instance, the knockdown of CDKL1 can impede the migration and invasion of breast cancer cell [28]. It has also been illustrated by scholars that CDKL1-siRNA can potentiate the progression of gastric cancer [29]. Previous studies have verified that CDKL1 can promote the progression of CRC [18]. Intriguingly, with the use of TargetScan, we found that there were two binding sites between miR-144-5p and CDKL1. Hence, we reckoned that, in CRC, DSCAM-AS1 can serve as a competitive endogenous RNA of miR-144-5p to up-regulate CDKL1 expression, thus promoting the progression and metastasis of CRC tumors. In the present study, we validated the target relationship between miR-144-5p and CDKL1. In detail, both the down-regulation of miR-144-5p and the up-regulation of DSCAM-AS1 can induce the elevated expression of CDKL1, thereby triggering the proliferation and invasion of CRC cells.

5. Conclusions

In conclusion, our study represented a paradigm that DSCAM-AS1 can serve as a ceRNA of miR-144-5p to modulate CDKL1 expression, by which it can be involved in biological behaviors of CRC cells. Furthermore, our study results herein indicated an innovative web to depict lncRNA-miRNA-mRNA ceRNA in CRC. This novel mechanism would help clarify the tumorigensis and progression of CRC.

Declarations

Ethics approval and consent to participate

This study obtained the approval of the Ethics Committee of Shanghai Pulmonary Hospital. The research has been carried out in accordance with the World Medical Association Declaration of Helsinki. All of the patients were consent to participate in this study.

Consent for publication

Yes.

Availability of data and materials

The datas used to support the findings of this study and related materials are available from the corresponding author upon request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Jiaping Pei conducted the experiments and data analysis and drafted the manuscript. Xiaozhao Deng conceived and designed the study and revised the manuscript. All authors read and approved the final manuscript.

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Figures

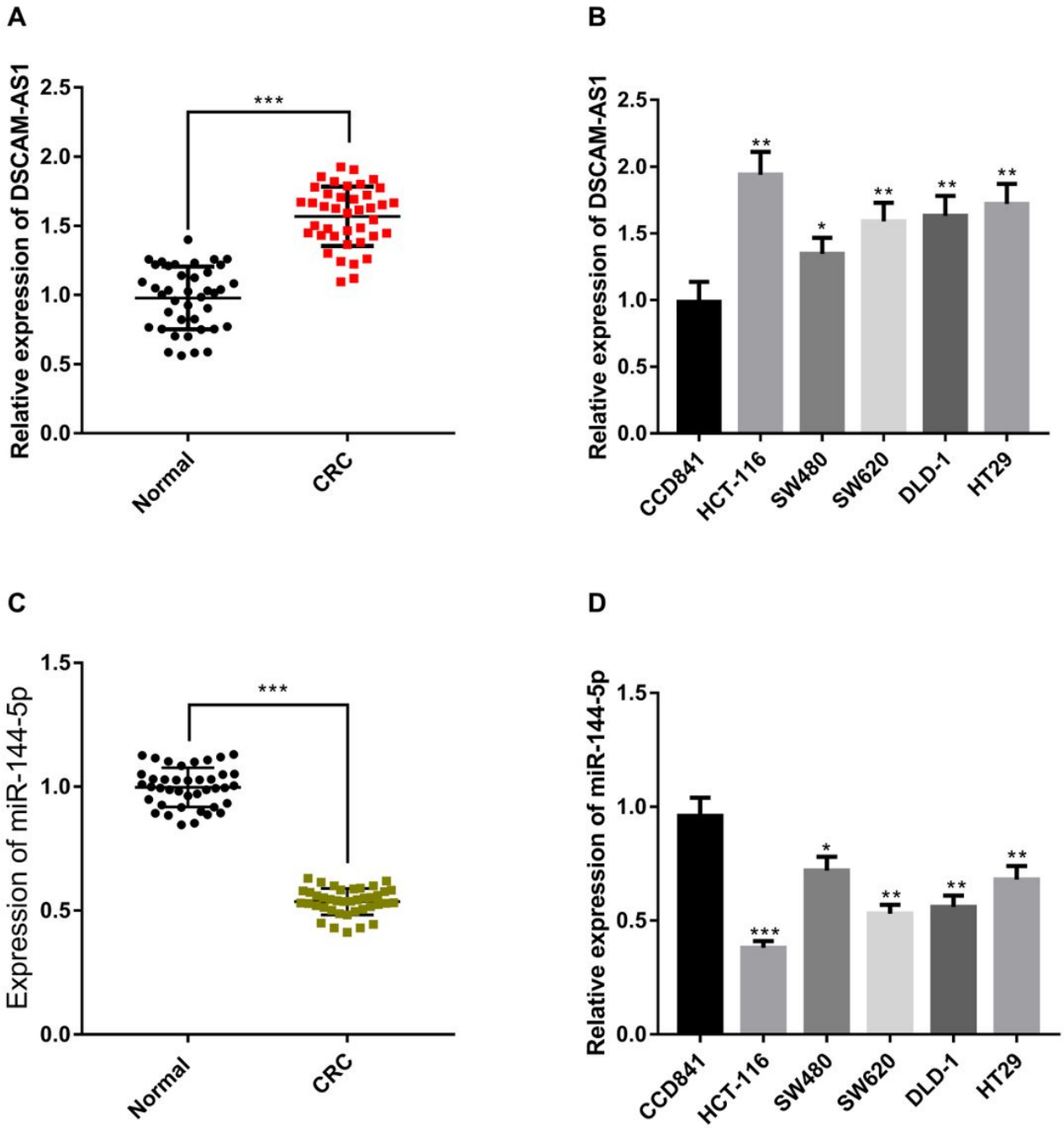

Figure 1

DSCAM-AS1 was highly expressed in CRC and miR-144-5p was lowly expressed in CRC (A) DSCAM-AS1 expressions in CRC tissues and normal tissues adjacent to cancer in 40 patients were analyzed by qRT-PCR. (B) qRT-PCR was performed to detect DSCAM-AS1 expression in CRC cells and normal cells. (C) miR-144-5p expressions in CRC tissues and normal tissues adjacent to cancer in 40 patients were analyzed by qRT-PCR. (D) qRT-PCR was done to

detect miR-144-5p expressions in CRC cells and normal cells. (Compared with Normal or NC group, *P<0.05; **P<0.01; ***P<0.001; NS P>0.05)

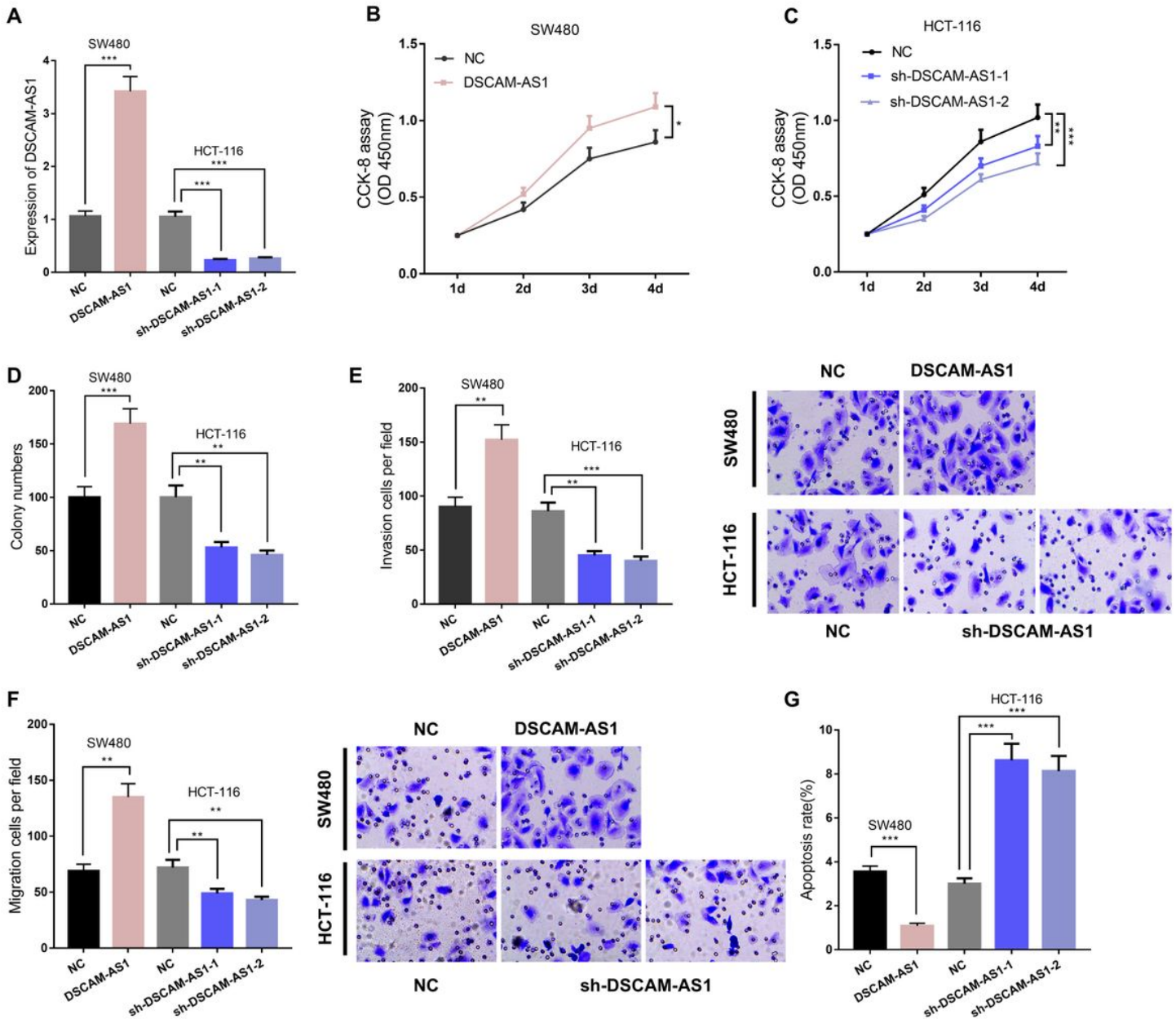

Figure 2

Overexpressed DSCAM-AS1 can promote the proliferation, migration and invasion of CRC cells (A) The cell line constructed by DSCAM-AS1 shRNA and DSCAM-AS1 plasmid was detected by qRT-PCR. (B-D) Cell proliferation was detected by CCK-8 and colony formation assay after DSCAM-AS1 overexpression in SW480 cells and DSCAM-AS1 knockdown in HCT-116 cells. (E-F) The invasion and migration of HCT-116 cells with DSCAM-AS1 knocked down and SW480 cells with DSCAM-AS1 overexpression were monitored via transwell assay. (G) Flow cytometry was performed to detect cell apoptosis after DSCAM-AS1 knocked down in HCT-116 cell line and DSCAM-AS1 overexpression in SW480 cell line. (Compared with Normal or NC group, *P<0.05; **P<0.01; ***P<0.001; NS P>0.05)

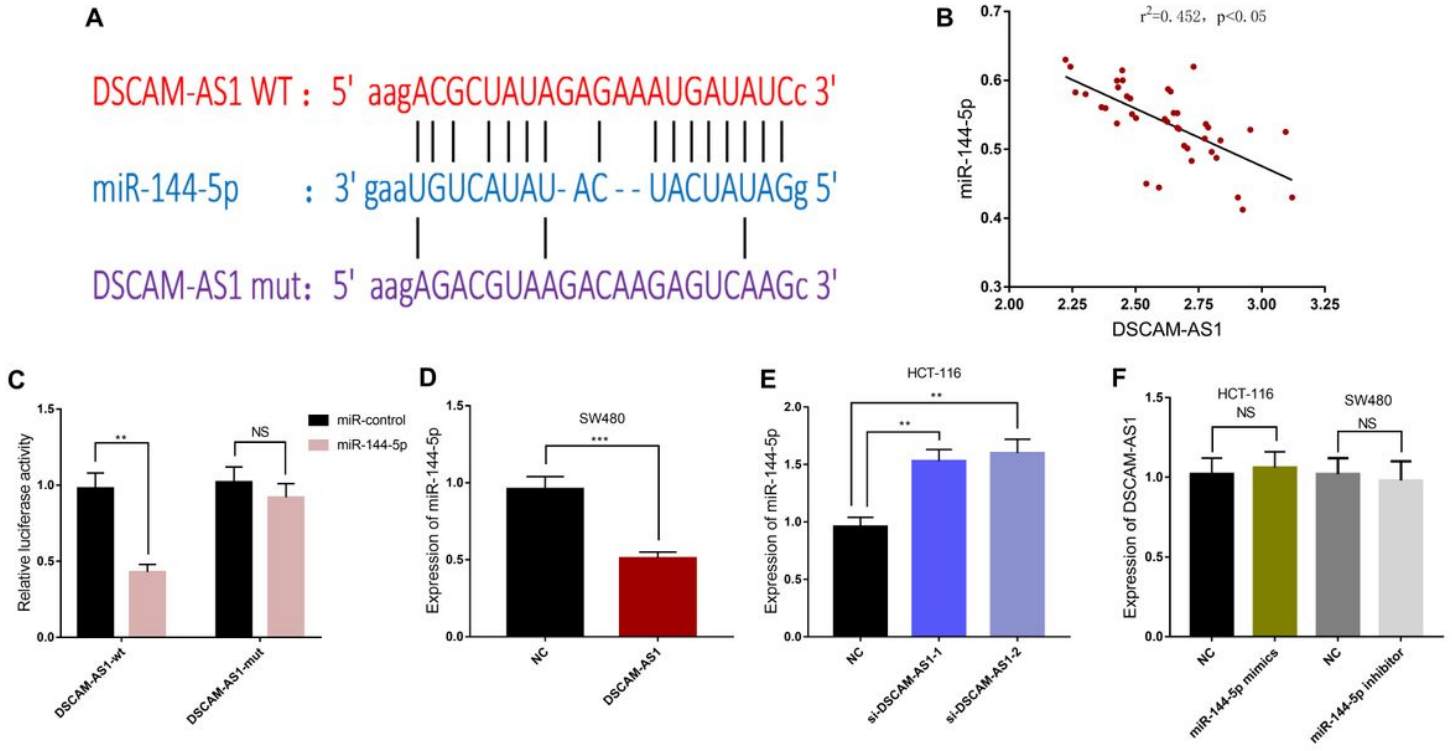

Figure 3

MiR-144-5p can directly bind to DSCAM-AS1 at the recognition site of miRNA (A) StarBase library showed that DSCAM-AS1 contained miR-144-5p target site. (B) qRT-PCR was carried out to detect the expressions of DSCAM-AS1 and miR-144-5p in the same tissue respectively, and the results showed that their expression were negatively correlated. (C) miR-144-5p reduced the luciferase activity of wild-type DSCAM-AS1, but not mutant DSCAM-AS1. (D-E) qRT-PCR was conducted to detect miR-144-5p expression after DSCAM-AS1 knock-down and DSCAM-AS1 overexpression, respectively. (F) qRT-PCR was conducted to detect DSCAM-AS1 expression after miR-144-5p knock-down and miR-144-5p overexpression, respectively. (Compared with Normal or NC group, *P<0.05; **P<0.01; ***P<0.001; NS P>0.05)

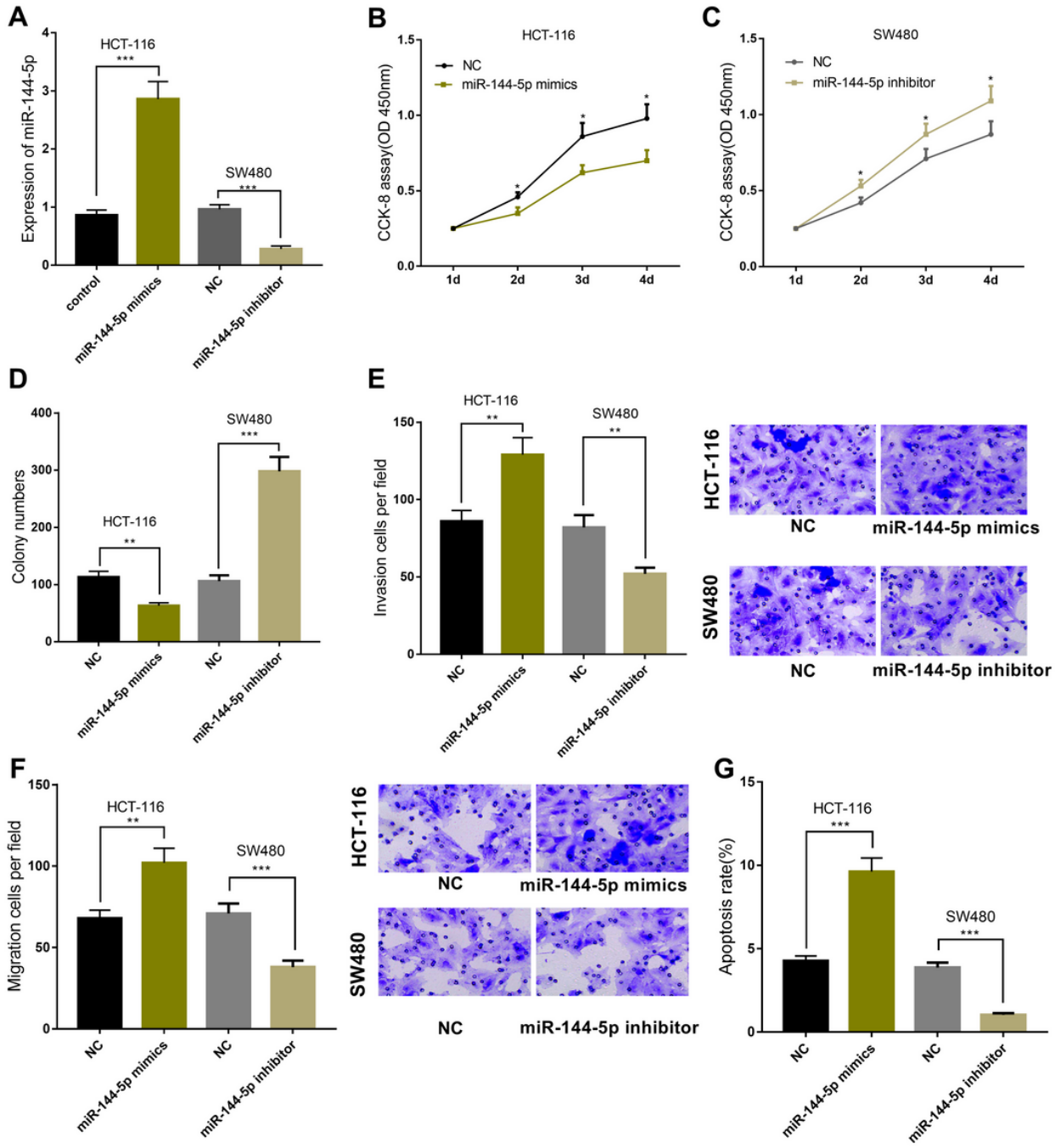

Figure 4

Low expressed miR-144-5p can promote the proliferation, migration and invasion of CRC cells (A) After the cell lines were transfected with miR-144-5p mimics and miR-144-5p inhibitor, the expression level of miR-144-5p was detected by qRT-PCR. (B-D) CCK-8 assay and colony formation assay were conducted to detect the cell proliferation after the overexpression of miR-144-5p in HCT-116 cell line and miR-144-5p inhibition in SW480 cell line. (E-F) The invasion and migration of HCT-116 cell line transfected with miR-144-5p mimics and SW480 transfected with miR-144-5p inhibitors were monitored via transwell assay. (G) Flow cytometry was applied to detect the apoptosis of SW480 cells after overexpression or inhibition of miR-144-5p. (Compared with Normal or NC group, *P<0.05; **P<0.01; ***P<0.001; NS P>0.05)

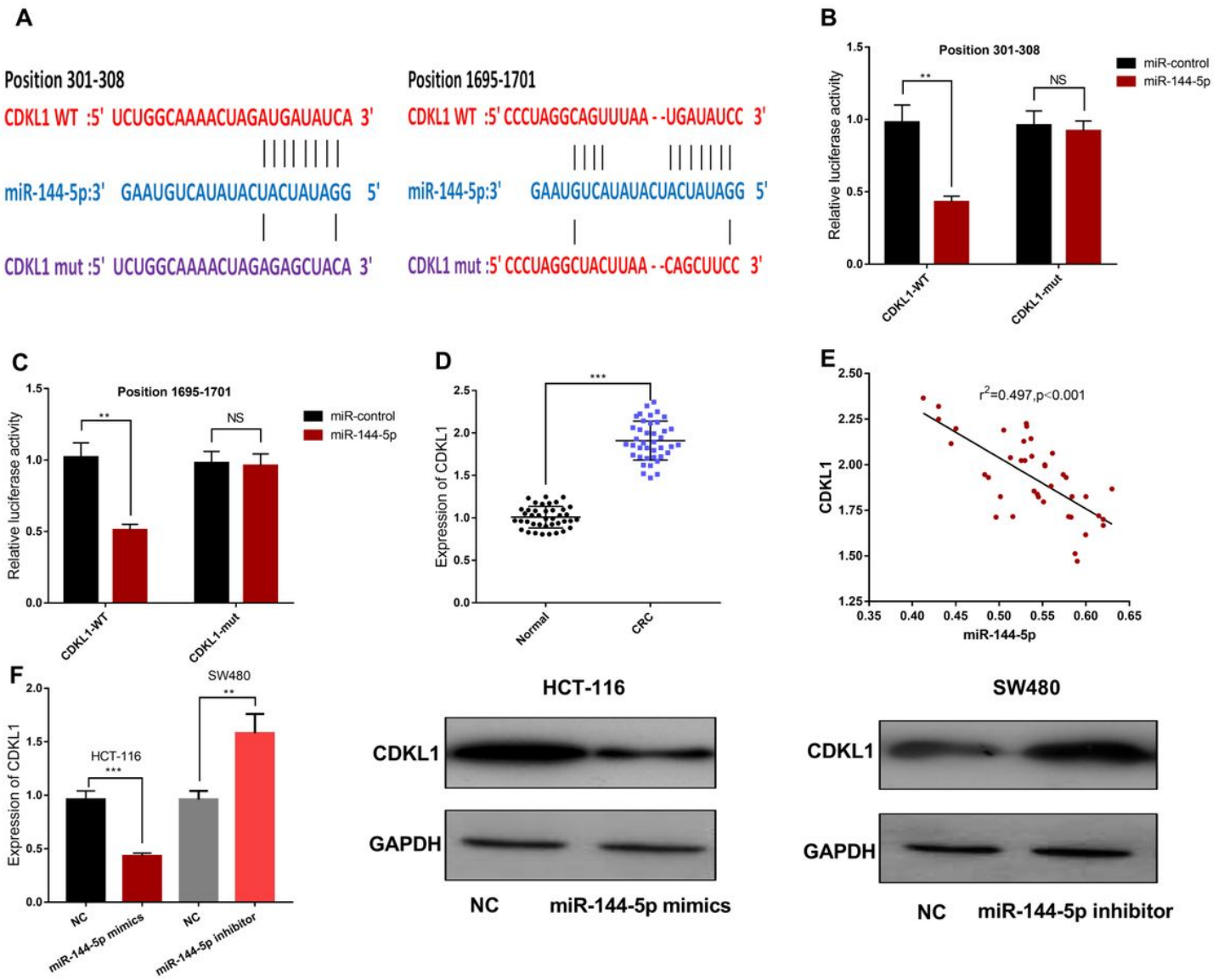

Figure 5

MiR-144-5p can directly bind to CDKL1 at the recognition site of miRNA (A) Targetscan informed us that miR-144-5p and CDKL1 had two binding sites. (B-C) MiR-144-5p reduced the luciferase activity of wild-type CDKL1, but not mutant CDKL1. (D) CDKL1 expressions in CRC tissues and normal tissues adjacent to cancer in 40 patients were analyzed by qRT-PCR. (E) qRT-PCR was carried out to detect the expressions of CDKL1 and miR-144-5p in the same tissue respectively, and the results showed that their expression were negatively correlated. (F) qRT-PCR and western blot were used to detect CDKL1 expression after miR-144-5p overexpression or inhibition, respectively. (Compared with Normal or NC group, *P<0.05; **P<0.01; ***P<0.001; NS P>0.05)

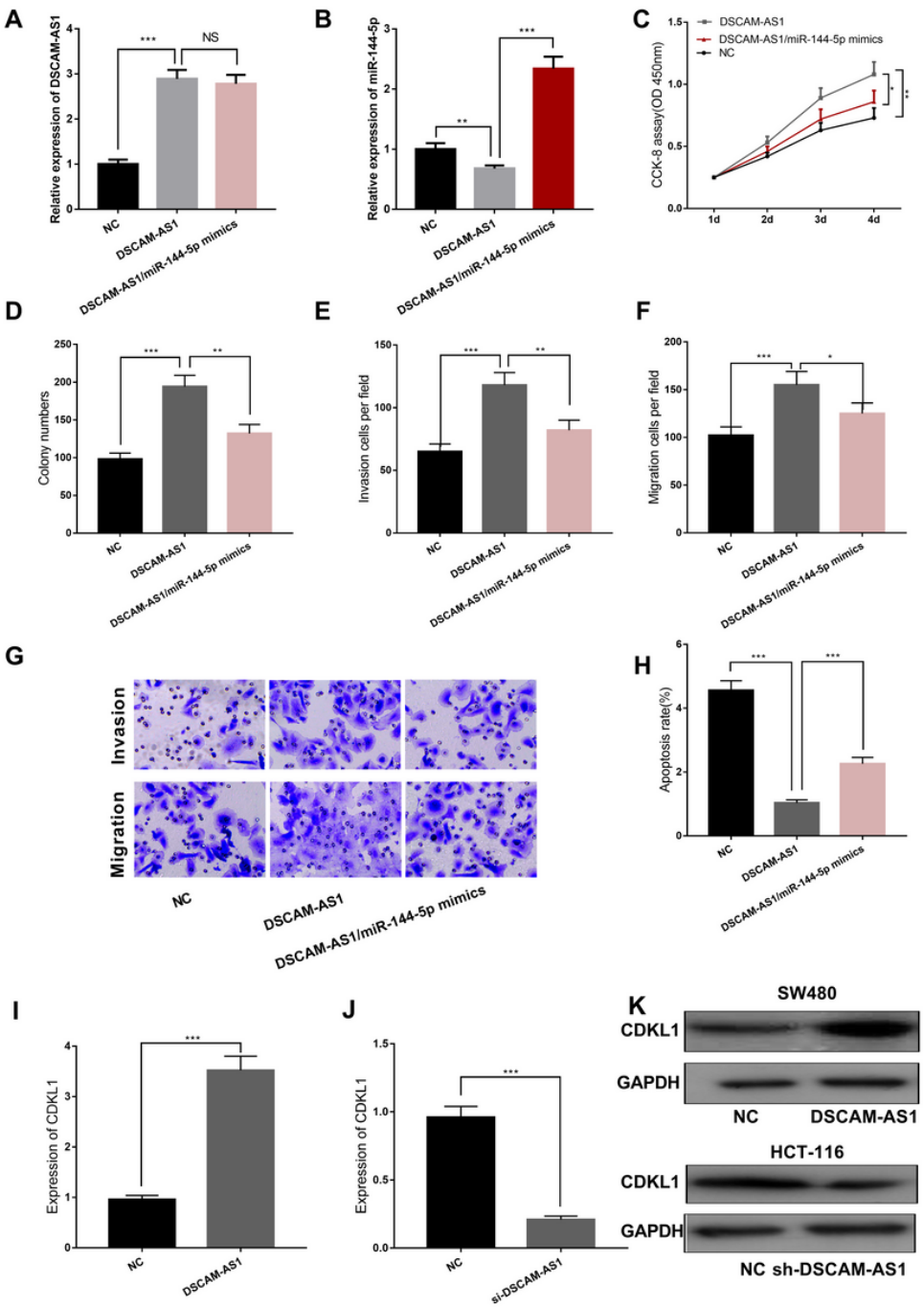

Figure 6

DSCAM-AS1 promotes the proliferation, migration and invasion of CRC cells via regulating miR-144-5p and CDKL1 (A-B) qRT-PCR was used to detect the expression level of DSCAM-AS1 in SW480 cells from DSCAM-AS1 overexpression group, DSCAM-AS1/miR-144-5p group and control group. (C-D) CCK-8 assay and cell colony formation assay were conducted to detect the proliferation of SW480 cells from DSCAM-AS1 overexpression group, DSCAM-AS1/miR-144-5p group and control group. (E-G) The invasion and migration of SW480 cells from DSCAM-AS1 overexpression group, DSCAM-AS1/miR-144-5p group and control group were monitored via transwell assay. (H) Flow cytometry was applied to detect the apoptosis of SW480 cells from DSCAM-AS1 overexpression group, DSCAM-AS1/miR-144-5p group and control group. (I-K) qRT-PCR and western blot were performed to detect CDKL1

expression after the overexpression and knockdown of DSCAM-AS1. (Compared with Normal or NC group, *P<0.05; **P<0.01; ***P<0.001; NS P>0.05)

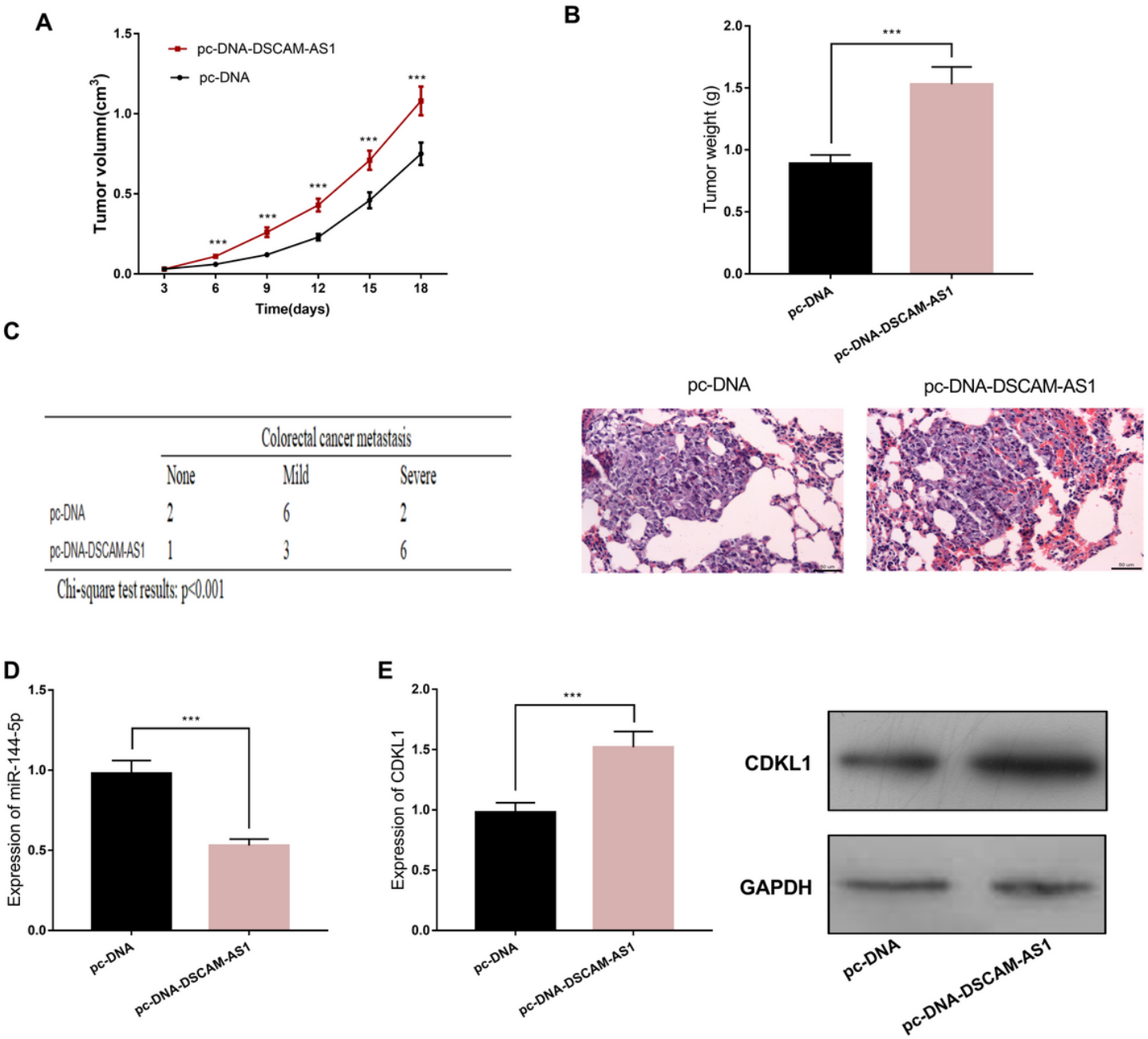

Figure 7

DSCAM-AS1/miR-144-5p/CDKL1 signal path can facilitate the growth of CRC. (A) The tumor volume of mice was measured every three days. The tumor volume in DSCAM-AS1 overexpression group was higher than that of control group. (B) 18 days later, the mice were killed and the tumor tissues were isolated to measure its weight. (C) After the mice were killed, the lung metastasis of mice was observed and evaluated. (D) miR-144-5p expression in tumor tissue of mice was measured by qRT-PCR method. (E) CDKL1 Expression in tumor tissue of mice was measured by qRT-PCR and western blot. (Compared with Normal or NC group, *P<0.05; **P<0.01; ***P<0.001; NS P>0.05)