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LncRNA ST8SIA6-AS1 promotes colorectal cancer cell proliferation, migration and invasion by regulating the miR-5195/PCBP2 axis

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Abstract. – OBJECTIVE: Long non-coding RNAs (lncRNAs) have been reported to play a vital role in the development and progression of various cancers, including colorectal cancer (CRC). Although the dysregulation of lncRNA ST8SIA6-AS1 participates in the development of multiple malignancies, the underlying molecular mechanisms of ST8SIA6-AS1 in regulating CRC progression remain to be fully discovered.

PATIENTS AND METHODS: The expression level of lncRNA ST8SIA6-AS1 was examined in the tumor tissues and paracancerous tissues of CRC patients. Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was utilized to examine the expression levels of ST-8SIA6-AS1, miR-5195, and Poly-(C) Binding Protein 2 (PCBP2). The protein expression level of PCBP2 was detected by Western blotting. MTT assay was performed to measure the proliferation of HCT-116 and SW480 cells. Cell migration and invasion abilities were measured by transwell assay. Luciferase reporter assay was used to examine the interaction between miR-5195 and ST8SIA6-AS1 or PCBP2.

RESULTS: This study revealed that lncRNA ST8SIA6-AS1 was upregulated in CRC tissues and cells. Knockdown of ST8SIA6-AS1 inhibited proliferation, migration, and invasion of CRC cells. Moreover, ST8SIA6-AS1 was proved to inhibit miR-5195 expression by directly targeting miR-5195. In addition, it was demonstrated that overexpression of miR-5195 inhibited CRC progression. Furthermore, PCBP2 was shown to enhance sh-ST8SIA6-AS1 and miR-5195 mimics-attenuated cell proliferation, migration, and invasion by directly binding to miR-5195.

CONCLUSIONS: Our study revealed that ST-8SIA6-AS1 promoted CRC progression *via* the miR-5195/PCBP2 axis. This study may provide an improved understanding of the pathogenesis of CRC.

Key Words:
Colorectal cancer, ST8SIA6-AS1, MiR-5195, PCBP2.

Introduction

Colorectal cancer (CRC) is the third most common cause of cancer-associated mortality in the world, which leads to more than one million deaths every year[1,2]. The occurrence and progression of CRC are caused by multiple factors[3,4]. Although great advances have been made in CRC therapeutic methods, such as surgery, irradiation, chemotherapy, and combined therapy[5,6], the 5-year survival rate of CRC patients is still unsatisfactory[7]. Therefore, it is urgent to look for better diagnostic or therapeutic biomarkers for the diagnosis and treatment of CRC.

Long noncoding RNAs (lncRN As) are transcripts longer than 200 nts in length without protein-coding ability[8,9]. Although lncRNAs account for less than 2% of all transcripts, differently expressed lncRNAs have great powers in regulating cellular processes of human tumors. LncRNA UCA1 promoted non-small cell lung cancer progression by targeting miR-193a-3p[10]. LncRNA XIST facilitated pancreatic cancer proliferation through miR-133a/EGFR axis[11]. Aurora A/PLK1 associated lncRNA (APAL; also known as ST-8SIA6 Antisense RNA 1, ST8SIA6-AS1) has been reported to play an oncogenic role in various tumor cells[12,13]. However, the exact mechanisms of ST8SIA6-AS1 in CRC remain unclear.

MicroRNAs (miRNAs), another type of endogenous noncoding RNAs (ncRNAs) with a length of ~22 nts[14], exert various functions during tumor occurrence and development. MiR-328-3p suppressed the initiation of bladder cancer by the inhibition of ITGA5 and PI3K/AKT pathway[15]. MiR-23a promoted migration and proliferation of colorectal cancer cells through targeting MARK1[16]. MiR-5195 has been reported to be involved in the development and progression

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of several types of cancers, such as osteosarcoma, triple-negative breast cancer, and ovarian cancer[17-19]. However, there is yet no research on miR-5195-mediated CRC progression.

Several mRNAs, such as PFKFB4, YY1, POLR1B, and TBX2, have been reported to be deeply associated with cancer progression[20-23]. Poly-(C) Binding Protein 2 (PCBP2) was reported to play an oncogenic role in various cancers. Chen et al[24] demonstrated that PCBP2 boosted the viability of gastric cancer cells by regulating CDK2. Ye et al[25] reported that PCBP2 promoted the progression of esophageal squamous cell carcinoma by modulating cell proliferation and apoptosis.

In this current study, it was demonstrated that ST8SIA6-AS1 upregulated PCBP2 expression through absorbing miR-5195 in CRC. This finding may provide a new perspective for the tumorigenesis of CRC.

Patients and Methods

Tissue Samples

A total of 22 pairs of CRC and adjacent normal tissues were obtained from Suqian First Hospital. All patients did not receive chemotherapy, radiotherapy or other anti-tumor therapies. All tumor specimens had been confirmed by histopathological analysis. The control group specimens were from paracancerous tissues of the same patient (at least 3 cm away from the surgical margin), and no cancer cells were found after pathological examination. Each patient has signed the written informed consent and this research was conducted following the Declaration of Helsinki. This investigation was approved by the Ethics Committee of Suqian First Hospital. After surgery, these samples were stored at -80°C for further analysis.

Cell Culture

Human normal colon mucosal epithelial cell line NCM460, four CRC cell lines (HCT-116, HT-29, SW480 and LoVo) and 293T cells were obtained from Shanghai Institute of Biological Sciences (Shanghai, China). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Hyclone, Logan, UT, USA) with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and penicillin-streptomycin (penicillin: 100 UI/mL and streptomycin: 100 μg/mL; Thermo Fisher Scientific, Waltham, MA, USA). All the cell lines were maintained at 37°C in a humidified atmosphere with 5% CO_2.

Cell Transfection

The shRNA specific to ST8SIA6-AS1 (sh-ST8SIA6-AS1) with its negative control (sh-NC) and miR-5195 mimics with its negative control (NC mimics) were synthesized by GenePharma (Shanghai, China). PCBP2 was subcloned into pcDNA3.1 (GenePharma, Shanghai, China) to overexpress PCBP2 levels with empty pcDNA3.1 serving as the control. The transfection was conducted using Lipofectamine 2000 reagent (Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's instructions.

Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR)

Total RNA of tissues and cells was extracted using TRIzol reagent (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and cDNA was synthesized utilizing PrimeScript RT Reagent kit (TaKaRa, Dalian, Liaoning, China). Then, the mRNA expression was measured by SYBR Green PCR Kit (TaKaRa, Dalian, China). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and U6 served as internal controls. The relative expression was calculated by applying the $2^{-\Delta\Delta Cq}$ method. The primer sequences used were as follows: ST8SIA6-AS1 forward, 5'-CGAGCTGCGTGCGTCCTGTC-3' and reverse, 5'-TCGCTGCCCGTGAGTCTGT-3'; miR-5195 forward, 5'-CAGATACAGCCCATCCTTCCAAG-3' and reverse, 5'-CTGTCCGCTCTTACTCCCTC-3'; PCBP2 forward, 5'-ACCAATAGCACAGCTGCCAGTAGA-3' and reverse, 5'-AGTCTCCAACATGACCACGCAGAT-3'; GAPDH forward, 5'-TGCACCACCAACTGCTTAGC-3' and reverse, 5'-GGCATGCACTGTGGTCATGAG-3'; U6 forward, 5'-GCTTCGGCAGCACATATACTAAAAT-3' and reverse, 5'-CGCTTCACGAATTTGCGTGTCAT-3'.

MTT Assay

The cell viability of CRC cells was evaluated by MTT assay kit (Beyotime, Shanghai, China). Briefly, cells (2×10^3 cells for each well) were seeded into 96-well plates. Next, 10 μL MTT (5 mg/ml; Bioswamp, Wuhan, China) was added and cells were then incubated for 4-6 h. Subsequently, dimethyl sulfoxide (~100 μl; Bio-Swamp, Wuhan, China) was added to the wells. The optical density at 450 nm was detected *via* a microplate reader (Bio-Rad, Hercules, CA, USA).

Transwell Assay

The migration and invasion abilities were assessed through using transwell chambers (8.0 μm

pore size; EMD Millipore, Billerica, MA, USA) and Matrigel (Corning Inc., Corning, NY, USA). For migration assay, transfected cells (1×10^5) and 200 μL Roswell Park Memorial Institute-1640 (RPMI-1640; HyClone, South Logan, UT, USA) were placed in the upper chamber. 600 μL RPMI-1640 medium containing 10% FBS was added in the lower chamber. The cells were cultured for 48 hours, and the cells in the lower chamber were fixed with methanol and dyed with crystal violet (Sigma-Aldrich, St. Louis, MO, USA). For invasion assay, the insert membranes were coated with Matrigel but were cultured under the same conditions. Finally, migrated and invaded cells were counted under an inverted microscope and photographed.

Luciferase Reporter Assay

ST8SIA6-AS1-Wt (wild-type), PCBP2-Wt (wild-type), ST8SIA6-AS1-Mut (mutant type), and PCBP2-Mut (mutant type) reporters with wild or mutant miR-5195 binding sites were purchased from Hanbio Biotechnology Co., Ltd. (Shanghai, China). Next, miR-5195 mimics and NC mimics were transfected into 293T cells, which had been transfected with wild-type or mutant fragments. Luciferase activity was detected by a Dual-Luciferase reporter assay kit (Promega, Madison, WI, USA).

Western Blot

To evaluate the PCBP2 protein expression, Western blot analysis was performed. Proteins were extracted from CRC cells using radioimmunoprecipitation assay buffer (RIPA; Beyotime Institute of Biotechnology, Haimen, China), followed by separation of denatured proteins by sodium dodecyl sulfate and polyacrylamide gel (SDS-PAGE) and transfer to polyvinylidene difluoride (PVDF) membranes. Next, the membrane was subjected to blocking by Tris-buffered saline (TBS) with 5% skim milk for about 2 h. The diluted primary antibody against PCBP2 or GAPDH (1:1000; Abcam, Cambridge, MA, USA) was added for overnight incubation at 4°C. Then, the membranes were rinsed thrice through TBS containing Tween-20 (TBS-T), and cultured again with the horseradish peroxidase (HRP)-labeled secondary antibody (1:5000; Beyotime Institute of Biotechnology, Haimen, China) for an extra 2 h and washed three times with TBS-T. Lastly, protein bands were quantified *via* employing enhanced chemiluminescence (ECL; Keygentec, Nanjing, China) and observed by ChemiDoc™ XRS systems (Bio-Rad, Hercules, CA, USA).

Statistical Analysis

All experiments involved in this current study were repeated 3 times. Statistical analysis was conducted using SPSS 19.0 (IBM, Armonk, NY, USA) and the data were expressed as means ± standard deviation (SD). Comparisons of parameters between two groups were analyzed by a paired Student's *t*-test. Comparisons among multiple groups were performed using one-way ANOVA followed by Tukey's test. Data with p-value < 0.05 were statistically significant.

Results

ST8SIA6-AS1 Was Highly Expressed In CRC Tissues and Cells

To determine whether ST8SIA6-AS1 was involved in CRC, the expression level of ST8SIA6-AS1 in CRC tissues and cells was measured by qRT-PCR. As presented in Figure 1A, RT-qPCR revealed that ST8SIA6-AS1 was upregulated in CRC tissues compared with that in adjacent normal tissues. Likewise, the expression level of ST8SIA6-AS1 in CRC cells (HCT-116, HT-29, SW480 and LoVo) was higher than that in normal colon mucosal epithelial cell line (NCM460) (Figure 1B). In summary, these results indicated that ST8SIA6-AS1 was upregulated in CRC tissues and cells.

ST8SIA6-AS1 Promoted CRC Cell Proliferation, Migration, and Invasion

To explore the effect of ST8SIA6-AS1 on CRC progression, HCT-116 and SW480 cells were transfected with sh-ST8SIA6-AS1. The transfection efficiency was confirmed by qRT-PCR (Figure 2A). MTT assay demonstrated that the proliferation of HCT-116 and SW480 cells was significantly inhibited after the silencing of ST8SIA6-AS1 (Figure 2B). Moreover, transwell assays revealed that cell migration and invasion abilities were dramatically repressed in CRC cells transfected with sh-ST8SIA6-AS1 (Figure 2C and D). Taken together, knockdown of ST8SIA6-AS1 inhibited proliferation, migration, and invasion of CRC cells.

ST8SIA6-AS1 Was a Molecular Sponge of MiR-5195

StarBase database predicted the interplaying between ST8SIA6-AS1 and miR-5195 (Figure 3A). Then, we detected the expression of miR-5195 in

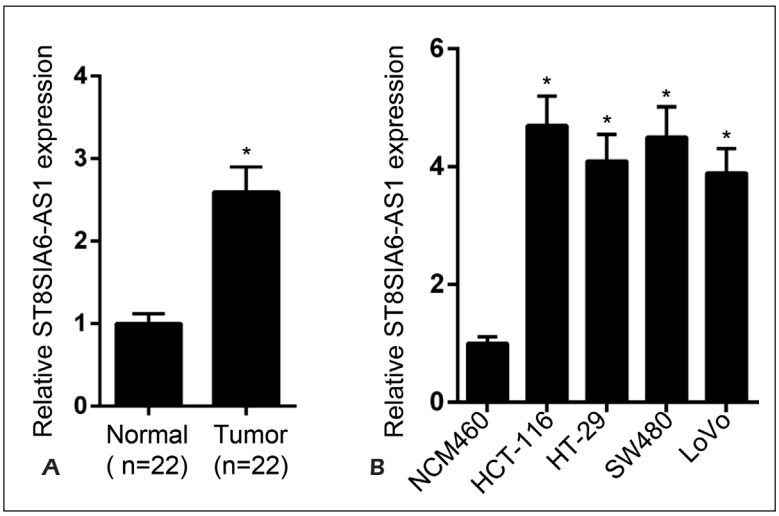

Figure 1. High expression of ST8SIA6-AS1 in CRC tissues and cells. **A**, qRT-PCR analysis of ST8SIA6-AS1 in CRC tissues and adjacent normal tissues (n=22). **B**, qRT-PCR detection of ST8SIA6-AS1 in CRC cells (HCT-116, HT-29, SW480, LoVo) and normal colon mucosal epithelial cell line NCM460. *p<0.05.

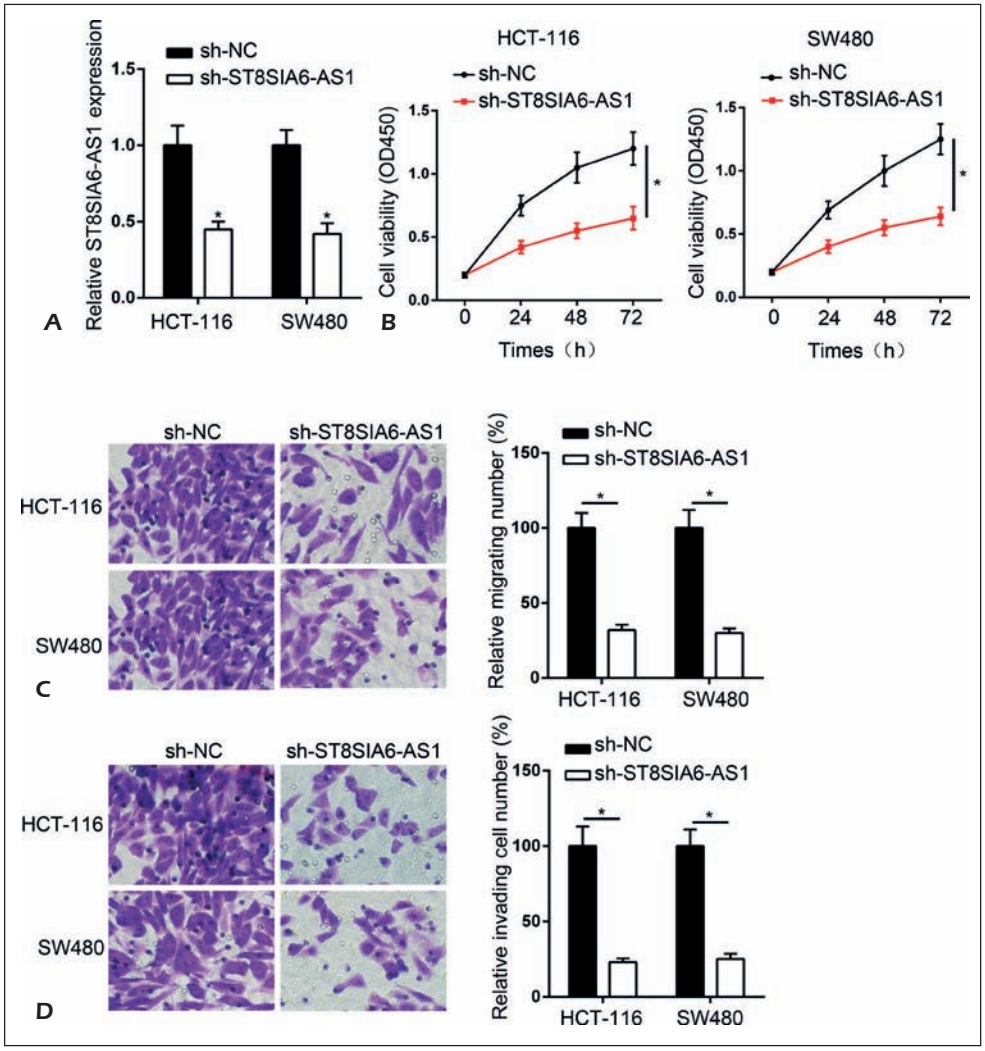

Figure 2. Oncogenic role of ST8SIA6-AS1 in cell proliferation and motility of CRC cells. **A**, ST8SIA6-AS1 expression after transfection of sh-NC or sh-ST8SIA6-AS1 in HCT-116 and SW480 cells was examined through RT-qPCR. **B**, MTT assay evaluated the cell viability of two CRC cells transfected with sh-NC or sh-ST8SIA6-AS1. **C-D**, The migration and invasion abilities of sh-NC or sh-ST8SIA6-AS1-transfected CRC cells were assessed via transwell experiments (magnification x 40). *p<0.05.

CRC tissues and cells. RT-qPCR indicated that miR-5195 expression was markedly reduced in CRC tissues and cells compared with that in normal tissues and cells (Figure 3B and C). Moreover, knockdown of ST8SIA6-AS1 significantly increased the expression of miR-5195 (Figure 3D). In addition, Luciferase reporter assay demonstrated that overexpression of miR-5195 decreased the Luciferase activity of wild-type ST8SIA6-AS1 but had no effect on that of mutant ST8SIA6-AS1 (Figure 3E). These findings indicated that ST8SIA6-AS1 sponged miR-5195 in CRC cells.

MiR-5195 Inhibited CRC Proliferation, Migration and Invasion

To explore the biological role of miR-5195 in CRC, HCT-16 and SW480 cells were transfected with miR-5195 mimics and NC mimics. The transfection efficiency was confirmed by RT-qPCR (Figure 4A). MTT assay disclosed that the upregulation of miR-5195 inhibited proliferation, migration, and invasion of CRC cells (Figure 4B-D). All these findings demonstrated the repressive role of miR-5195 in CRC cells.

ST8SIA6-AS1/miR-5195 Axis Promoted CRC Progression Via PCBP2

We investigated whether PCBP2 mediated ST8SIA6-AS1/miR-5195-regulated CRC phenotypes. The mRNA and protein expression of PCBP2 was significantly decreased in HCT-16 cells transfected with sh-ST8SIA6-AS1 (Figure 5A and B). Also, overexpression of miR-5195 dramatically decreased PCBP2 mRNA and protein expression (Figure 5C and D). Through starBase database, PCBP2 was assumed to be a target of miR-5195 (Figure 5E). Luciferase reporter assay revealed that the Luciferase activity of wild-type PCBP2 was decreased after the upregulation of miR-5195. However, the Luciferase activities in other groups had no changes in 293T cells (Figure 5F). Moreover, overexpression of PCBP2

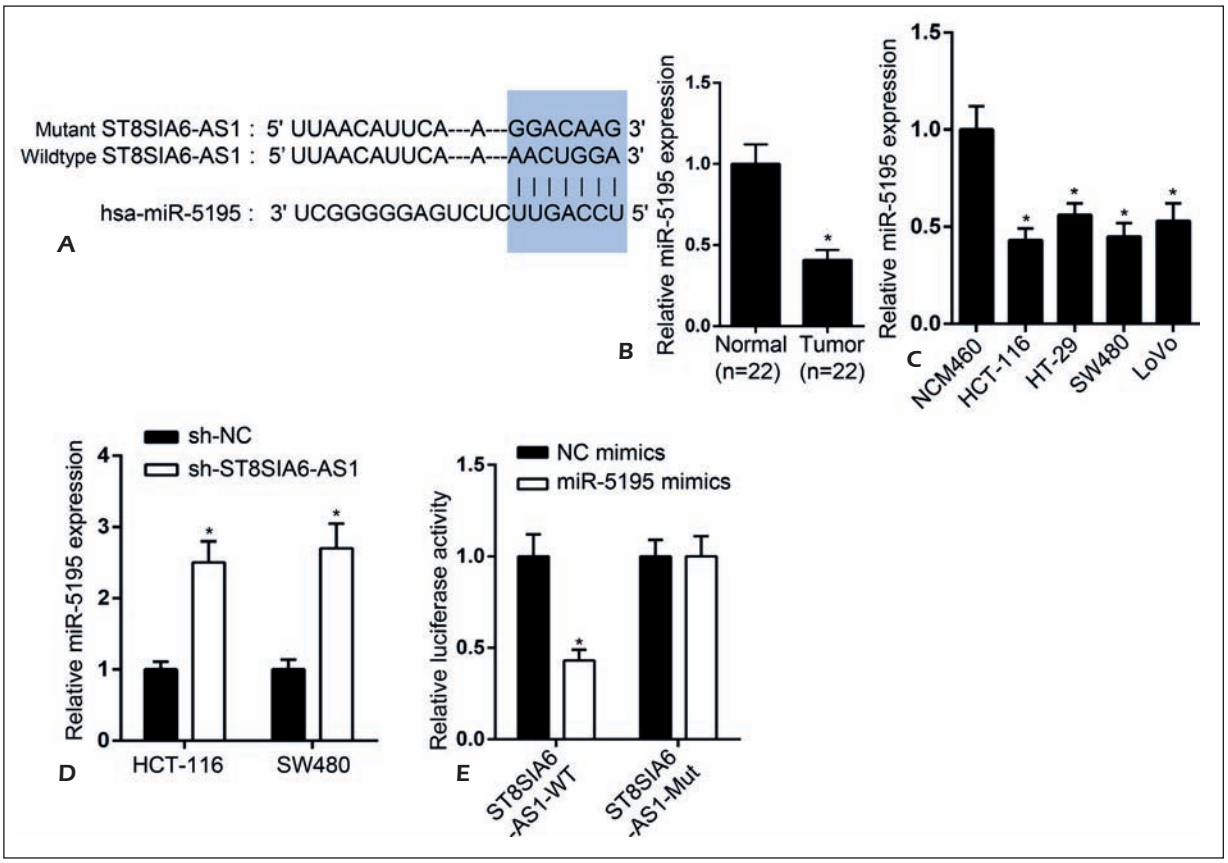

Figure 3. ST8SIA6-AS1 acted as a molecular sponge of miR-5195. **A**, StarBase prediction of ST8SIA6-AS1 as a binding partner of miR-5195. **B**-**C**, MiR-5195 expression level in CRC and normal tissues or cells was explored through RT-qPCR (n=22). **D**, The influence of ST8SIA6-AS1 on miR-5195 was determined through RT-qPCR. E, Luciferase reporter assay investigated the relationship between ST8SIA6-AS1 and miR-5195. *$p<0.05$.

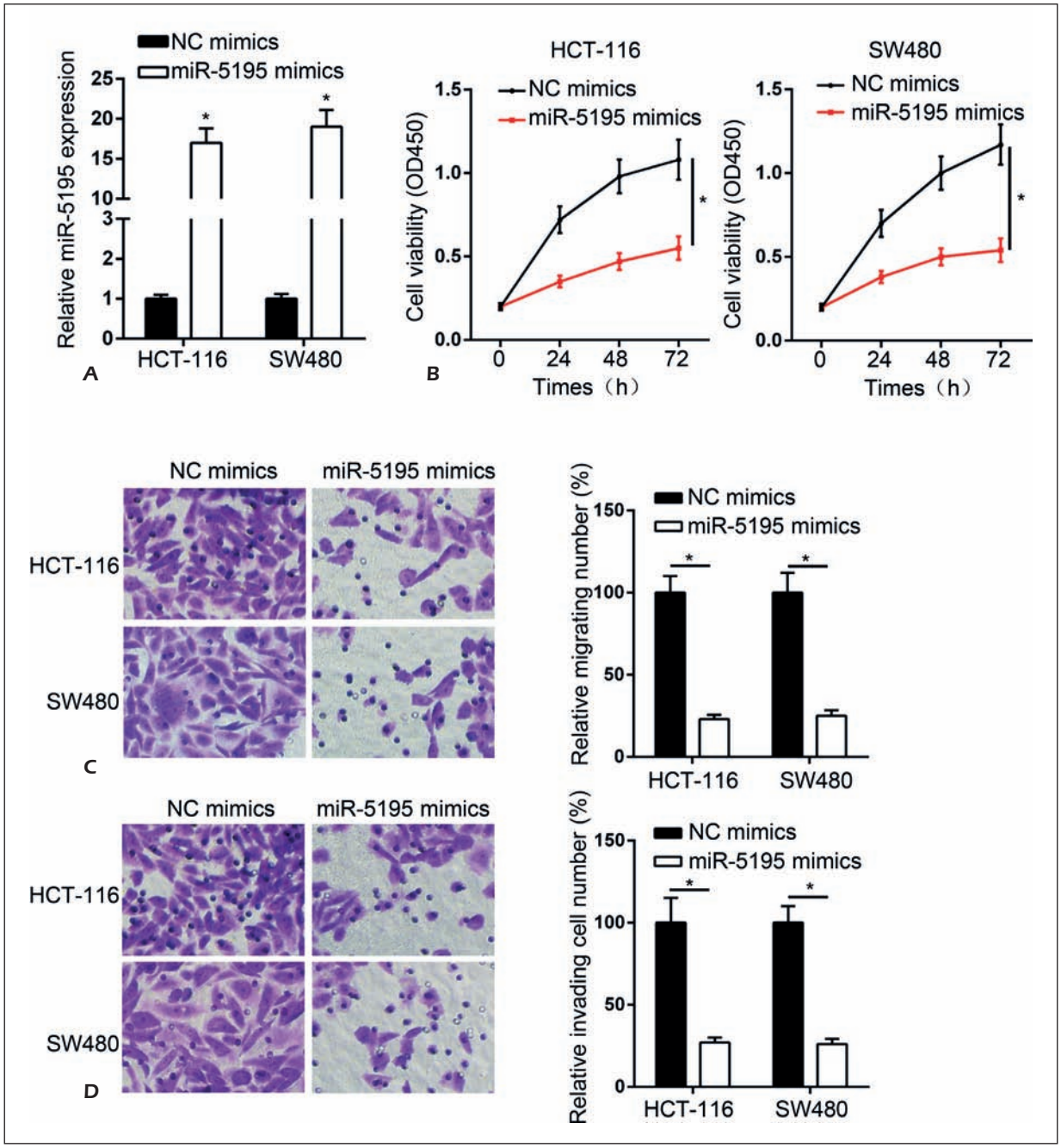

Figure 4. Repressive role of miR-5195 in cell proliferation and motility of CRC cells. **A**, Overexpression efficacy of miR-5195 mimics in HCT-116 and SW480 cells, as evaluated by RT-qPCR. **B**, Cell viability was assessed via MTT assays in two cells under the transfection of NC mimics or miR-5195 mimics. **C**-**D**, Transwell experiments were conducted to test cell migration and invasion in transfected CRC cells (magnification x 40). *$p<0.05$.

significantly abolished the inhibitory effects of ST8SIA6-AS1 knockdown or miR-5195 mimics on the cell viability, migration, and invasion of CRC cells (Figure 5G-L). In conclusion, the ST8SIA6-AS1/miR-5195/PCBP2 axis promoted cell proliferation and motility of CRC cells.

Discussion

We found that ST8SIA6-AS1 was upregulated in CRC tissues and cells, and ST8SIA6-AS1 sponged miR-5195 to promote CRC progression through upregulating PCBP2.

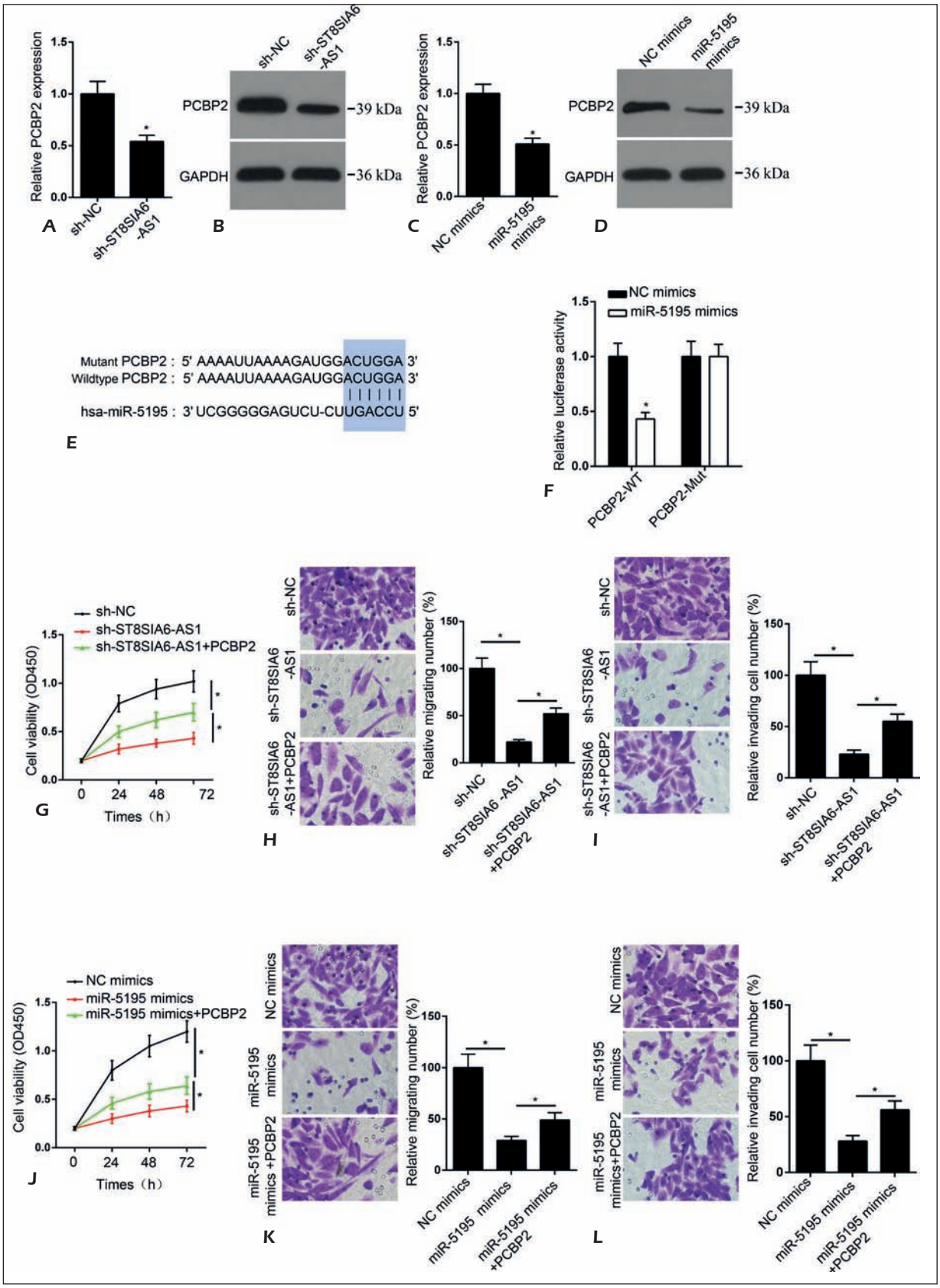

Figure 5. ST8SIA6-AS1 enhanced PCBP2 expression by miR-5195. **A-B**, PCBP2 mRNA and protein expression after ST-8SIA6-AS1 silencing in HCT-116 cells were tested by RT-qPCR and Western blotting. **C-D**, The impact of miR-5195 promotion on PCBP2 mRNA and protein levels in two cells were affirmed through RT-qPCR and Western blot. **E**, PCBP2 was predicted to interact with miR-5195 by straBase. **F**, The binding between miR-5195 and PCBP2 was confirmed through Luciferase reporter experiment. **G**, Cell viability was assessed via MTT assays in HCT-116 cells under the transfection of sh-NC or sh-ST8SIA6-AS1 and sh-ST8SIA6-AS1+ PCBP2. **H**, and **I**, Transwell experiments were conducted to test cell migration and invasion in transfected HCT-116 cells (magnification x 40). **J**, Cell viability was assessed via MTT assays in HCT-116 cells under transfection of NC mimics or miR-5195 mimics and miR-5195 mimics + PCBP2. **K**, and **L**, Transwell experiments were conducted to test cell migration and invasion in transfected HCT-116 cells (magnification x 40). *p<0.05.

LncRNA plays a vital role in the pathological activities of cancerous cells, such as proliferation, apoptosis, migration, and invasion[26-28]. ST8SIA6-AS1 was shown to promote the progression of breast cancer through the p38 MAPK signaling pathway[12]. In the present study, we found that ST8SIA6-AS1 was markedly upregulated in CRC tissues and cells, and knockdown of ST8SIA6-AS1 inhibited proliferation, migration, and invasion of CRC cells.

LncRNAs interact with miRNAs by acting as competitive endogenous RNA (ceRNA) to regulate the proliferation and migration of tumor cells. Of note, HOXA11-AS promoted the tumorigenesis of non-small cell lung cancer through sponging miR-148a-3p and upregulating expression of DNMT1[29]. Regarding CRC, Jin et al[30] reported that LINC00520 promoted the progression of CRC by serving as a ceRNA of miR-577. Through StarBase database, miR-5195 was predicted as a downstream gene of ST8SIA6-AS1, suggesting that miR-5195 might be a key effector for ST8SIA6-AS1-mediated tumorigenesis of CRC. Ebrahimi et al[19] reported that miR-5195 contributed to suppressing ovarian cancer, and the downregulation of miR-5195 was associated with increased cell proliferation and invasion. Jiang et al[31] demonstrated that miR-5195 repressed proliferation and invasion of bladder cancer cells by targeting KLF5. In the present study, it was confirmed that miR-5195 was downregulated in CRC tissues and cells, and ST8SIA6-AS1 inhibited miR-5195 expression by direct interaction. The absorbing effect of ST8SIA6-AS1 on miR-5195 in CRC was first discovered. Furthermore, the inhibitory function of miR-5195 in CRC cells was validated. Then, we decided to excavate the downstream factor under the ST8SIA6-AS1/miR-5195 axis. PCBP2 is an oncogene in multiple carcinomas[32,33]. Thus, the role of PCBP2 under the regulation of the ST8SIA6-AS1/miR-5195 axis in CRC was investigated. The experimental data illustrated that the ST8SIA6-AS1/miR-5195/PCBP2 axis promoted proliferation and motility of CRC cells.

Conclusions

Our results demonstrated that ST8SIA6-AS1 upregulated PCBP2 expression through sponging miR-5195 and accelerated CRC progression. The effects of ST8SIA6-AS1 on the pathogenesis of CRC suggest that it may be a potential therapeutic target for CRC patients.

Conflict of Interests

The authors declare that they have no conflict of interests.

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