CELL CYCLE
2022, VOL. 21, NO. 1, 1–11
https://doi.org/10.1080/15384101.2021.1973700

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RESEARCH PAPER

LncRNA ASB16-AS1 drives proliferation, migration, and invasion of colorectal cancer cells through regulating miR-185-5p/TEAD1 axis

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ABSTRACT

As a common malignant tumor, colorectal cancer (CRC) has a high incidence. Recent investigations have suggested that although great improvement has been achieved in the survival rate of early-stage CRC patients, the overall survival rate remains low. Mounting reports have proved that lncRNAs take part in the development of various cancers and possess the regulatory functions in cancers. For example, ASB16 antisense RNA 1 (ASB16-AS1) is a poorly researched novel lncRNA whose specific functions in CRC are still unknown. In our research, we discovered that ASB16-AS1 was with high expression in CRC cells. In addition, ASB16-AS1 silencing restrained the proliferation, migration, invasion, and stemness while accelerating cell apoptosis of CRC cells. Mechanism experiments were applied to explore the regulatory mechanism of ASB16-AS1. It turned out that miR-185-5p could interact with ASB16-AS1 and inhibited the progression of CRC cells. TEAD1 (TEA domain transcription factor1) - a major effector of the Hippo signaling was proved to serve as the target of miR-185-5p and promote CRC development. In short, ASB16-AS1 drove the progression of CRC through the regulation of miR-185-5p/TEAD1 axis.

ARTICLE HISTORY
Received 2 February 2021
Revised 15 April 2021
Accepted 20 April 2021

KEYWORDS
ASB16-as1; miR-185-5p; tead1; colorectal cancer

Background

Colorectal cancer (CRC) falls into the category of the most common cancers worldwide with a high mortality [1]. Though the incidence of CRC is low in China compared with that in the world, its incidence tends to increase in varying degrees in many regions [2]. CRC mostly occurs in the middle-aged men who are 40–70 years old [3] and the incidence ratio of male and female is about 2:1 [4]. It has been reported that the mortality rate has decreased due to the great improvement in a broad range of screening techniques and therapeutic strategies, while the overall survival rate is not satisfactory with about 40% of the patients dying of tumor recurrence [5]. Thus, it is urgent to discover new biomarkers to improve this condition .

Long non-coding RNAs (lncRNAs) are RNAs with over 200 nucleotides [6]. Also, it has been proven that lncRNAs can modulate various biological functions or affect protein activity in a direct manner [7,8]. For example, lncRNA TUG1 influences the biological behaviors of papillary thyroid cancer cell [9]. Up-regulation of lncRNA PANDAR accelerates the cell proliferation of cervical cancer [10]. Moreover, MALAT1 promotes gastric cancer metastasis by suppressing PCDH10 [11]. In recent years, existing reports have suggested that lncRNAs can exert their regulatory functions on cell progression in CRC, such as CRNDE [12], CPS1-IT1 [13] and SNHG12 [14]. LncRNA ASB16-AS1 is a novel lncRNA whose function has not been studied in various cancers. According to previous researches, lncRNA ASB16-AS1 affected the biological behaviors of glioma cells [15] and cervical cancer cells [16]. Nevertheless, there has been little research in the functions of ASB16-AS1 on CRC, and the latent mechanism of ASB16-AS1 is entirely unknown in CRC.

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Supplemental data for this article can be accessed here.

The central purpose of our research is to unveil the mechanism of ASB16-AS1 in CRC, which may offer a new idea for CRC treatment.

Materials and methods

Cell culture

Human CRC cell lines (LOVO, HCT15, HT-29, HCT116) and one human normal colorectal mucosal cell line (FHC) which were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA) were maintained in the DMEM medium added with 1% penicillin–streptomycin (Invitrogen) and 10% FBS (HyClone, Logan, UT). All these cells were cultured with 5% CO_2 at the temperature of 37°C.

Plasmid transfection

The short hairpin RNAs (shRNAs) targeting ASB16-AS1 were designed by Genechem (Shanghai, China) to silence ASB16-AS1 in LOVO and HT-29 cells employing the Lipofectamine2000 (Invitrogen). The pcDNA3.1/ASB16-AS1, pcDNA3.1/TEAD1, NC-pcDNA3.1 vectors, miR-185-5p mimics, and NC mimics were all designed by Genepharma.

Quantitative real-time PCR (RT-qPCR)

Total RNA from LOVO and HT-29 cell samples were isolated with the application of TRIzol reagent (Invitrogen) for treatment with cDNA Reverse Transcription Kit (Takara, Otsu, Japan). Quantification was conducted with SYBR green Supermix (Thermo Fisher, Waltham, MA, USA), followed by $2^{-\Delta\Delta CT}$ methods to evaluate relative gene expression.

Colony formation assay

Clonogenic cell samples of LOVO and HT-29 were seeded at 500 cells per in the 6-well plates for 14 days. Then samples were fixed and stained with 4% paraformaldehyde and 0.1% crystal violet for counting.

5-ethynyl-2′-deoxyuridine (EdU) assay

EdU assay was conducted in CRC cells of LOVO and HT-29 in light of the user manual of the Cell-light™ EdU ApolloR567 In Vitro Imaging Kit (Ribobio). Cell nuclei were observed via the DAPI staining method. Images were captured by a fluorescent microscope.

TdT-mediated dUTP Nick-End Labeling (TUNEL) assay

Using One-Step TUNEL Apoptosis Assay Kit, cell apoptosis was monitored via TUNEL assay as instructed by the supplier (Beyotime, Shanghai, China). Detection of cell nuclei was achieved after DAPI staining, followed by analysis of fluorescent microscope.

Flow cytometry analysis

Cells were fixed with 75% ethanol. After the cells were washed with PBS for three times, they were suspended in PBS containing RNase (10 mg/ml) and stained with propidium iodide (PI). Then the FACS Calibur (BD Biosciences, USA) was used to observe cell apoptosis, and the FACs Diva (BD Biosciences, USA) was utilized to analyze data.

Transwell assay

Transwell assays were implemented by use of 24-well Transwell chamber (Corning Incorporated, Corning, NY) containing 8 μm pore polycarbonate filters, with matrigel (BD Biosciences) coating or not for invasion or migration analysis. Evaluation of invasive or migratory ability was conducted via counting invading or migrating cells under microscope (magnification, ×200).

Sphere formation

Ninety-six-well ultralow attachment plates (Corning Incorporated) containing sphere medium was used to process LOVO and HT-29 cell samples (10 cells/well). After being cultured for 7 days, sphere cells with diameter >50 mm were counted.

Subcellular fractionation

Cytoplasmic and nuclear RNAs from LOVO and HT-29 cell samples were extracted and purified as per the manual of Cytoplasmic & Nuclear RNA Purification Kit (Norgen, Belmont, CA). RT-qPCR was used for determining the expression levels of isolated RNAs.

Fluorescence in situ hybridization (FISH) assay

The permeabilized cell samples were incubated with the ASB16-AS1-FISH probe (RiboBio) in a hybridization solution at 37°C all night. Samples were collected after Hoechst staining for cell nuclei and visualized under a fluorescent microscope.

RNA pull down assay

Protein extracts from CRC cell samples were harvested for mixing with the biotin-labeled miR-185-5p probes covering the WT and Mut ASB16-AS1 or TEAD1 binding sites and streptavidin agarose magnetic beads for an hour. Then, the final mixture was assayed.

Luciferase reporter assay

Recombinant luciferase reporter vectors were constructed using the Luciferase Reporter Assay System (Promega). Firstly, wild-type or mutant ASB16-AS1 fragments covering miR-185-5p binding sites were cloned to pmirGLO-vector (Promega, Madison, WI), termed ASB16-AS1-WT/Mut. Similarly, the wild-type or mutant TEAD1 3′UTR covering miR-185-5p binding sites were used to construct TEAD1 3′UTR-WT/Mut vectors. The cultured CRC cells were co-transfected with luciferase reporter vectors and miR-185-5p mimics or NC mimics for 48 h.

RNA immunoprecipitation (RIP) assay

RIP assay in CRC cell samples was implemented by employing the EZ-Magna RIP RNA Binding Protein Immunoprecipitation Kit (Millipore, Bedford, MA), along with anti-Ago2 or anti-IgG antibody. Precipitates were subjected to RT-qPCR method.

Western blot

Proteins extracted from CRC cells were placed into SDS-PAGE and transferred onto PVDF membranes (EMD Millipore). Then, membranes were then incubated with the primary antibodies at 4°C overnight. After being washed with PBS×3, membranes were cultured with secondary antibodies for 1 h at room temperature, and the proteins were detected by ECL detection kit (Invitrogen; Thermo Fisher Scientific, Inc.).

Xenograft mouse model

We constructed a xenograft mouse model in nude mouse for evaluating the influence of ASB16-AS1 on the progression of CRC. The animal experiments were permitted by the Institutional Committee of the Third Affiliated Hospital of Wenzhou Medical University and performed under the guidance of the Institutional Animal Care and Use Committee. The 6-week-old male BALB/c nude mice purchased from Slac Laboratory Animal Center (Shanghai, China) were divided into three groups (n = 5 per group). They were kept under the specific pathogen-free conditions. One hundred μl of LOVO cells (5 × 106) stably transfected with sh-ASB16-AS1#1 + pcDNA3.1/TEAD1, sh-NC, and sh-ASB16-AS1#1 were injected into the back of the mice subcutaneously. The volume of xenografts of mice in each group was measured every 4 days. After 24 days, the mice were sacrificed and tumors were dissected for further detection.

Statistical analysis

Results were displayed as the mean ± standard deviation of all experiments including three or more biological repeats. The Prism 5.0 software (GraphPad Software, Inc., La Jolla, CA) was applied for data analysis through Student's *t* test or one-way analysis of variance with post-hoc Tukey's tests. Statistics with a p value below 0.05 were considered to be statistically significant.

Results

Deficiency of ASB16-AS1 inhibits cell proliferation and accelerates cell apoptosis of CRC

First of all, ASB16-AS1 expression was detected through starBase (http://starbase.sysu.edu.cn/index.php). We found that ASB16-AS1 was with high expression in CRC tissues, while significantly down-regulated in normal tissues (Figure 1(a)). Then, we detected ASB16-AS1 expression in CRC cell lines (LOVO, HCT15, HT-29, and HCT116) and normal cells (FHC) through RT-qPCR analysis (Figure 1(b)). According to the result, ASB16-AS1 was highly expressed in CRC cells, especially in LOVO and HT-29 cells. Therefore, they were kept for the following experiments. In addition, starBase also exhibited that the overexpression of ASB16-AS1 was closely correlated to the poor overall survival outcome of CRC patients (Figure 1(c)). Then, we detected the interference efficiency of ASB16-AS1 in LOVO and HT-29 cells by transfecting sh-ASB16-AS1#1/2 (Figure 1(d)). At the same time, RT-qPCR was also adopted to detect the overexpression efficiency of ASB16-AS1 (Figure 1(e)). Subsequently, functional experiments were implemented to test cell behavior. Colony formation and EdU assays were adopted, whose results indicated that when ASB16-AS1 was silenced, cell viability and proliferation were notably impeded as the number of colonies and the percentage of EdU positive cells were both greatly decreased. On the contrary, cell viability and proliferation were dramatically enhanced when ASB16-AS1 was overexpressed (figure 1(f-g)). Then, TUNEL experiments and flow cytometry experiments were carried out to test the apoptotic rate of CRC cells (Figure 1(h-i)). The results demonstrated that after ASB16-AS1 was knocked down, cell apoptosis was observably stimulated.

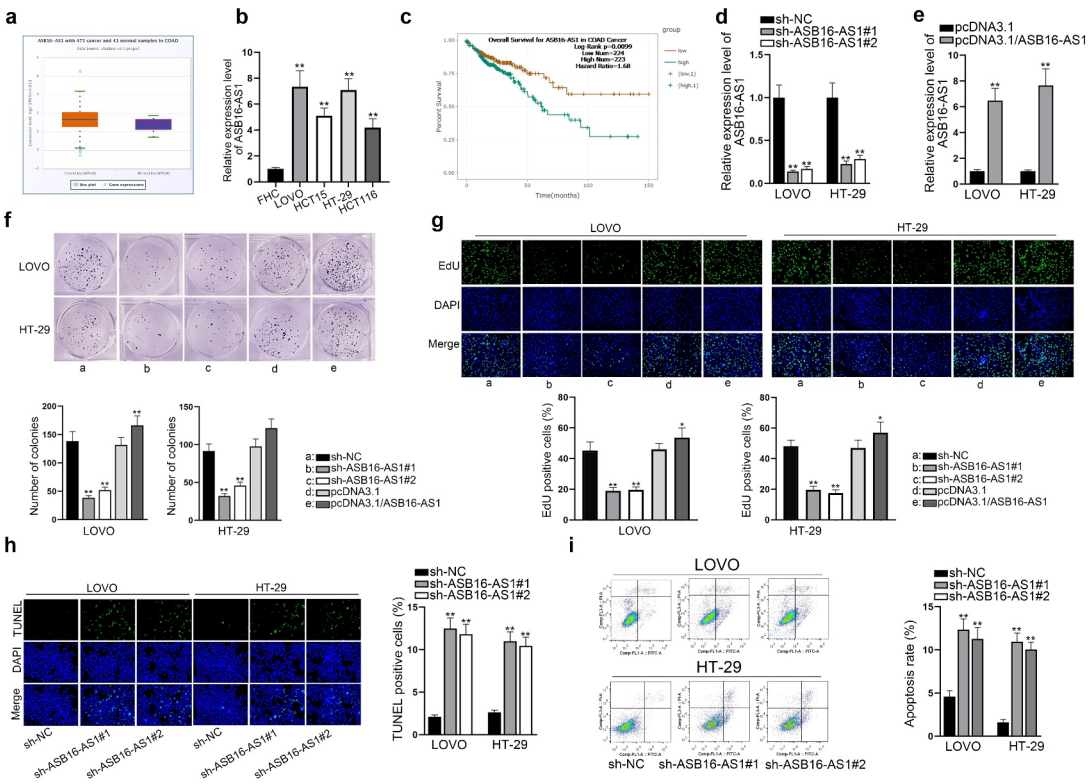

Figure 1. Deficiency of ASB16-AS1 inhibits cell proliferation and accelerates cell apoptosis of CRC. (a) starbase detected ASB16-AS1 expression in CRC tissues and normal tissues. (b) RT-qPCR analyzed ASB16-AS1 expression in CRC cells. (c) starbase predicted the overall survival rate of CRC patients under the condition of ASB16-AS1 up-regulation or down-regulation. (d) the interference efficiency of ASB16-AS1 was detected. (e) the overexpression efficiency of ASB16-AS1 was detected. (f-g) colony formation and EdU experiments evaluated cell proliferation when ASB16-AS1 was down-regulated or overexpressed. (h-i) cell apoptosis was tested after ASB16-AS1 was silenced or overexpressed. **P < 0.01.

Overall, ASB16-AS1 down-regulation alleviates CRC cell proliferation while enhancing cell apoptosis.

ASB16-AS1 inhibition hinders cell migration, invasion, and stemness in CRC

To test the influence of ASB16-AS1 on cell behavior, we conducted Transwell experiments and sphere formation experiments for verification. Firstly, the experimental results from Transwell assays indicated that the migration and invasion of cells were attenuated after the silencing of ASB16-AS1. Instead, ASB16-AS1 up-regulation raised the migratory and invasive abilities (Figure 2(a)). Then, we conducted sphere formation experiments to detect CRC cell stemness upon the ecotopic expression of ASB16-AS1. We discovered that the sphere formation efficiency was inhibited by ASB16-AS1 depletion, while it could be enhanced by ASB16-AS1 overexpression (Figure 2(b)). After that, the expression of OCT4, Nanog, and SOX2, which were cell stemness markers was detected in CRC cells after ASB16-AS1 was silenced or overexpressed. The results uncovered that the expression of the above three markers was all decreased after ASB16-AS1 was silenced, while increased after ASB16-AS1 was overexpressed (Figure 2(c-d)). Furthermore, as MMP2 and MMP9 are two proteases that link cancer cells with invasion and metastasis [17], western blot assay was adopted to verify the expression of MMP2 and MMP9 after ASB16-AS1 was silenced or overexpressed in CRC cells. Results showed that after ASB16-AS1 was down-regulated, the expression of MMP2 and MMP9 decreased, while ASB16-AS1 overexpression showed opposite results (Supplementary Figure 2D). In a word, ASB16-AS1 exerts a promoting role in cell migration, invasion, and stemness of CRC cells.

MiR-185-5p binds to ASB16-AS1

With the intention of investigating the latent regulatory network of ASB16-AS1 in CRC, subcellular fractionation and FISH assays were performed. It was shown that ASB16-AS1 mainly existed in the cytoplasm of CRC cells, indicating that it might exert regulatory functions at post-transcriptional level (Figure 3 (a-b)). Then, we applied starBase datdabase (CLIP-Data≥3) to predict potential miRNAs that could bind to ASB16-AS1 and sifted out four miRNAs (miR-2355-5p, miR-4306, miR-185-5p, and miR-4644). After that, RT-qPCR was used to detect the expression of the above four miRNAs in CRC cells, and only miR-185-5p was evidently down-regulated in CRC cells (Figure 3(c)). Thus, we selected miR-185-5p for follow-up experiments. Then, the binding capacity between miR-185-5p and ASB16-AS1 was proved by RNA pull down assay (Figure 3(d)). The two potential binding sites between ASB16-AS1 and miR-185-5p were shown in Figure 3 (e). After that, the overexpression efficiency of miR-185-5p and the expression of ASB16-AS1 after miR-185-5p overexpression were detected. It was shown that ASB16-AS1 expression was declined by miR-185-5p overexpression in LOVO and HT-29 cells (figure 3(f)). Based on the results of luciferase reporter experiments, we discovered that the luciferase activity of ASB16-AS1-WT was declined when miR-185-5p was overexpressed at the first binding site in LOVO and HT-29 cell lines, while no obvious change was found at the second binding site (Figure 3(g)). The above data demonstrated that miR-185-5p could bind to ASB16-AS1 in CRC cells. Furthermore, we adopted several functional experiments to estimate the functions of miR-185-5p in CRC. According to the results of colony formation and EdU assays, the proliferation of CRC cells was repressed after miR-185-5p overexpression (Supplementary Figure 1A-B). Similarly, the TUNEL assay and flow cytometry analysis elucidated that miR-185-5p overexpression could enhance cell apoptosis (Supplementary Figure 1 C-D). In addition, as shown by the results of the Transwell invasion and migration assays, the migration and invasion of CRC cells were weakened by miR-185-5p mimics (Supplementary Figure 1E). Moreover, the results of sphere formation assays demonstrated that miR-185-5p overexpression declined the sphere formation efficiency (Supplementary Figure 1 F). Meanwhile, OCT4, Nanog, and

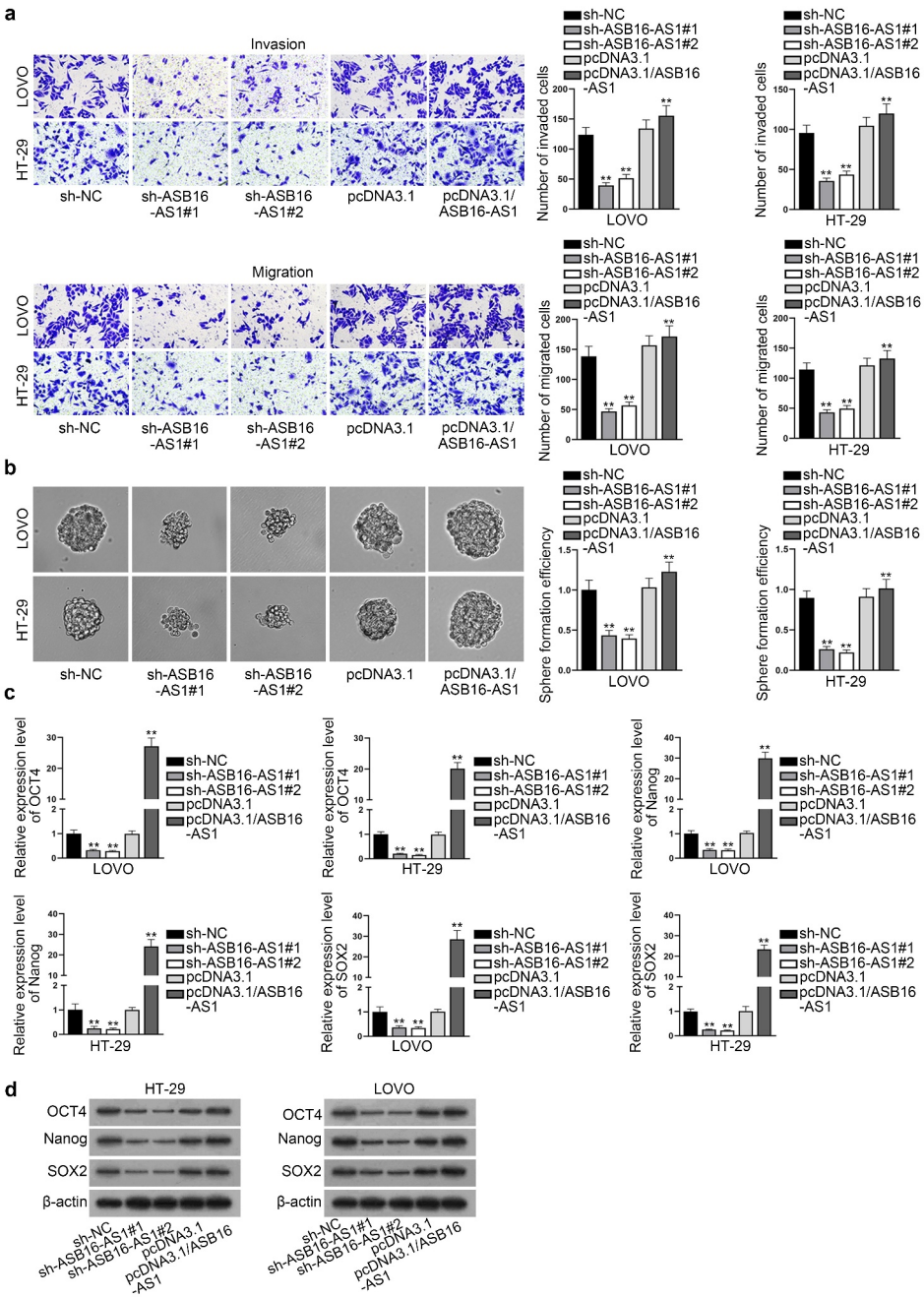

Figure 2. ASB16-AS1 inhibition hinders cell migration, invasion and stemness in CRC. (a) transwell experiments were utilized to test the migration and invasion of LOVO and HT-29 cells when ASB16-AS1 was silenced or overexpressed. (b) Sphere formation experiments were adopted to test cell stemness under the situation of ASB16-AS1 up-regulation or ASB16-AS1 inhibition. (c-d) RT-qPCR and western blot analysis were utilized to examine the expression of OCT4, Nanog and SOX2 when ASB16-AS1 was overexpressed or silenced. **P < 0.01.

SOX2 expressions at mRNA level and protein level were all reduced after the overexpression of miR-185-5p (Supplementary Figure 1 G-H). We also conducted a western blot analysis to examine the expression of MMP2 and MMP9 upon miR-185-5p overexpression in CRC cells. Related results showed that the protein levels of MMP2 and MMP9 were declined after miR-185-5p overexpression (Supplementary Figure 2E). In summary, miR-185-5p is sequestered by ASB16-AS1 and suppresses CRC cell progression.

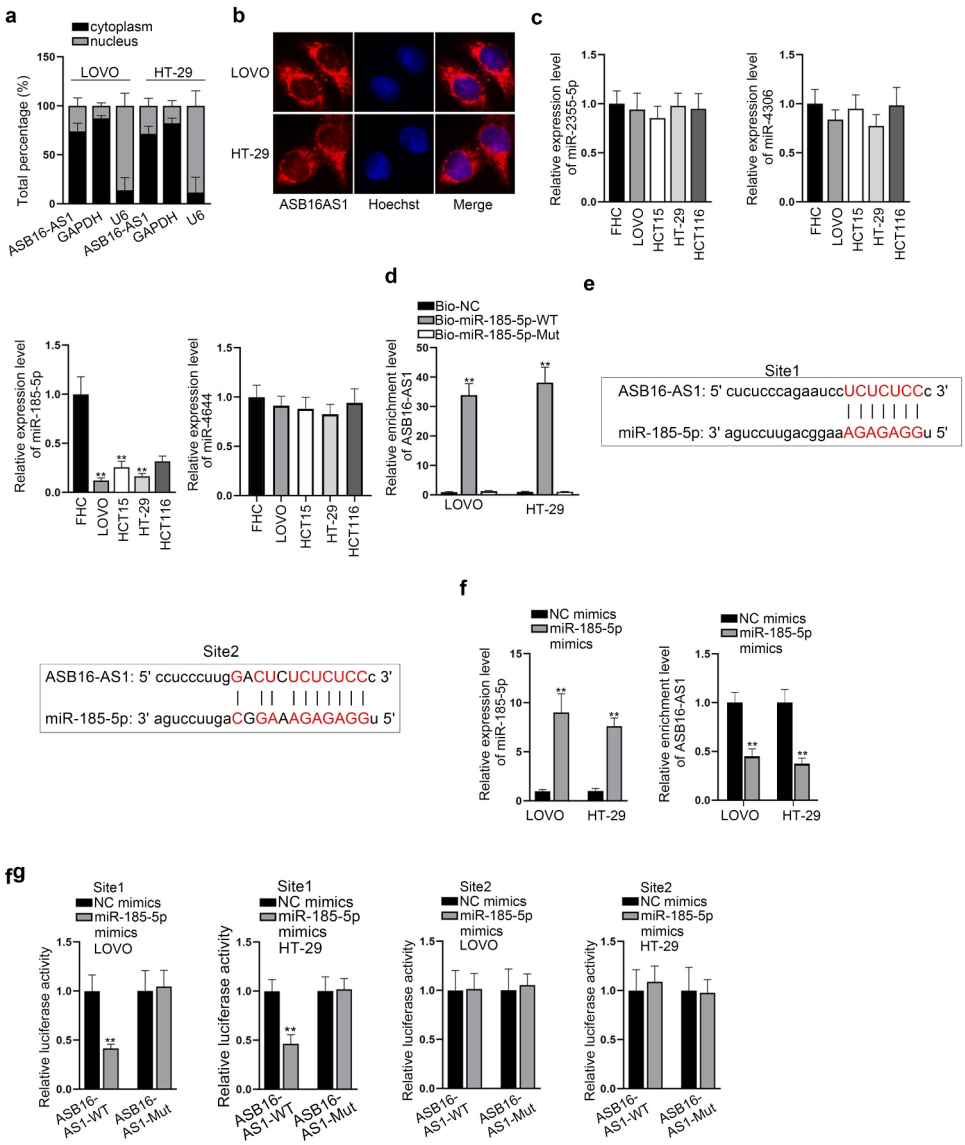

Figure 3. MiR-185-5p binds to ASB16-AS1. (a-b) the distribution of ASB16-AS1 in CRC cells was tested by subcellular fractionation and FISH experiments. (c) the expression of miR-2355-5p, miR-4306, miR-185-5p and miR-4644 was detected in CRC cells. (d) The binding sites between miR-185-5p and ASB16-AS1 were validated by RNA pull down assay. (e) the binding sites of miR-185-5p and ASB16-AS1. (f) the overexpression efficiency of miR-185-5p and the expression of ASB16-AS1. (g) luciferase reporter assay verified the binding ability between miR-185-5p and ASB16-AS1. **P < 0.01.

TEAD1 is the target gene of miR-185-5p in CRC

We utilized starBase (pan-Cancer ≥10; programNum ≥5) and sifted out 17 potential mRNAs which could be the target gene of miR-185-5p in line with microT, miRmap, PITA, PicTar, and TargetScan databases. We detected the expression of the mRNAs above after the transfection of miR-185-5p mimics in LOVO and HT-29 cells (Figure 4(a)). Among them, TEAD1 and PBX1 were down-regulated evidently when miR-185-5p was overexpressed. To further determine which mRNA was the target gene of miR-185-5p, RT-qPCR was adopted to detect the expression of TEAD1 and PBX1 in cells transfected with sh-ASB16-AS1#1/2. It was found that TEAD1 expression was decreased by ASB16-AS1 down-regulation, while PBX1 expression was not influenced by ASB16-AS1 (Figure 4(b)). Hence, we chose TEAD1 for further studies. Then, the expression of TEAD1 in human CRC cell lines

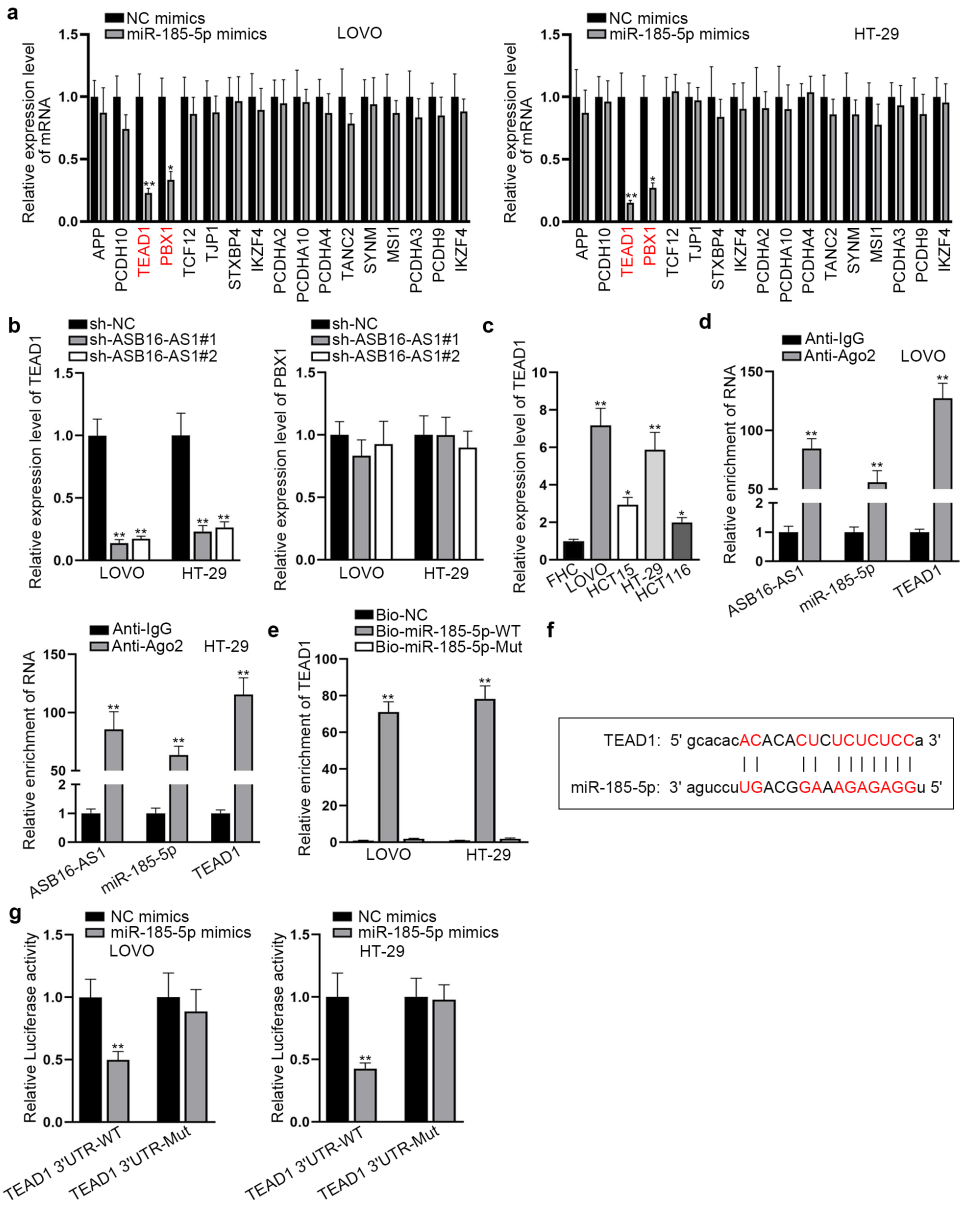

Figure 4. TEAD1 is the target gene of miR-185-5p in CRC. (a) relative expression levels of mRNAs were tested upon miR-185-5p overexpression. (b) RT-qPCR detected TEAD1 and PBX1 expression after ASB16-AS1 was silenced. (c) TEAD1 expression was examined in CRC cells. (d) RIP assay was adopted to test the binding situation among ASB16-AS1, miR-185-5p and TEAD1. (e) the interaction between miR-185-5p and TEAD1 was verified by RNA pull down assay. (f-g) the binding sites were shown and luciferase reporter experiments were utilized to prove the binding situation between miR-185-5p and TEAD1. *P < 0.05, **P < 0.01.

(LOVO, HCT15, HT-29, and HCT116) and human normal colorectal mucosal cell line (FHC) were detected and we found that TEAD1 expression was visibly high in LOVO and HT-29 cells (Figure 4(c)). After that, the binding relationship among ASB16-AS1, miR-185-5p, and TEAD1 was testified by the RIP assay (Figure 4(d)). As shown by the result, ASB16-AS1, miR-185-5p, and TEAD1 were all enriched in the Anti-Ago2 group, which demonstrated that there existed a binding relationship between them. Also, the interaction between miR-185-5p and TEAD1 was further confirmed by RNA pull down assay (Figure 4(e)). The binding site was displayed in figure 4(f) after searching on the starBase. Then, luciferase reporter experiments were carried out and indicated that the luciferase activity of TEAD1 3′UTR-WT was declined by miR-185-5p overexpression (Figure 4(g)). Taken together, TEAD1 is the target gene of miR-185-5p in CRC.

ASB16-AS1 promotes CRC development through enhancing TEAD1 expression

To confirm whether ASB16-AS1 accelerated CRC procession by modulating the expression of TEAD1, a series of rescue experiments were carried out in LOVO cells. First of all, we tested the overexpression efficiency of TEAD1 (Figure 5(a)). The results of colony formation and EdU assays indicated that ASB16-AS1 silencing hampered cell proliferation, while this effect could be restored by the co-transfection of pcDNA3.1/TEAD1 (Figure 5 (b-c)). Conversely, the enhanced apoptotic rate of LOVO cells caused by ASB16-AS1 reduction was rescued by TEAD1 up-regulation (Figure 5(d-e)). As expected, the overexpression of TEAD1 could offset the suppressed migration and invasion of LOVO cells caused by ASB16-AS1 silencing (figfigure 5(f)). We then carried out sphere formation experiments to test cell stemness in different transfection groups. It turned out that the sphere formation efficiency decreased after ASB16-AS1 was silenced and could be reversed by the co-transfection of pcDNA3.1/TEAD1 (Figure 5(g)). In addition, the decrease of OCT4, Nanog, and SOX2 expression caused by ASB16-AS1 inhibition was counteracted by TEAD1 up-regulation (Figure 5(h-i)). In addition, *in vivo* assays demonstrated that ASB16-AS1 silencing could inhibit the growth of tumor size, tumor volume, and tumor weight, while such result could be partially reversed by the co-transfection of sh-ASB16-AS1 and pcDNA3.1/TEAD1 (Supplementary Figure 2A-C). According to the results shown by western blot assay, we found that the declined expression of MMP2 and MMP9 caused by ASB16-AS1 inhibition was partially recovered by TEAD1 up-regulation (Supplementary Figure 2 F). Collectively, ASB16-AS1 contributes to CRC progression via up-regulating TEAD1.

Discussion

CRC is a common malignant tumor. Since its early symptoms are not obvious, most of the patients are in the advanced stage when they are diagnosed which leads to a lower overall survival rate. Therefore, exploring effective biomarkers is of great significance to improve the therapeutic effect

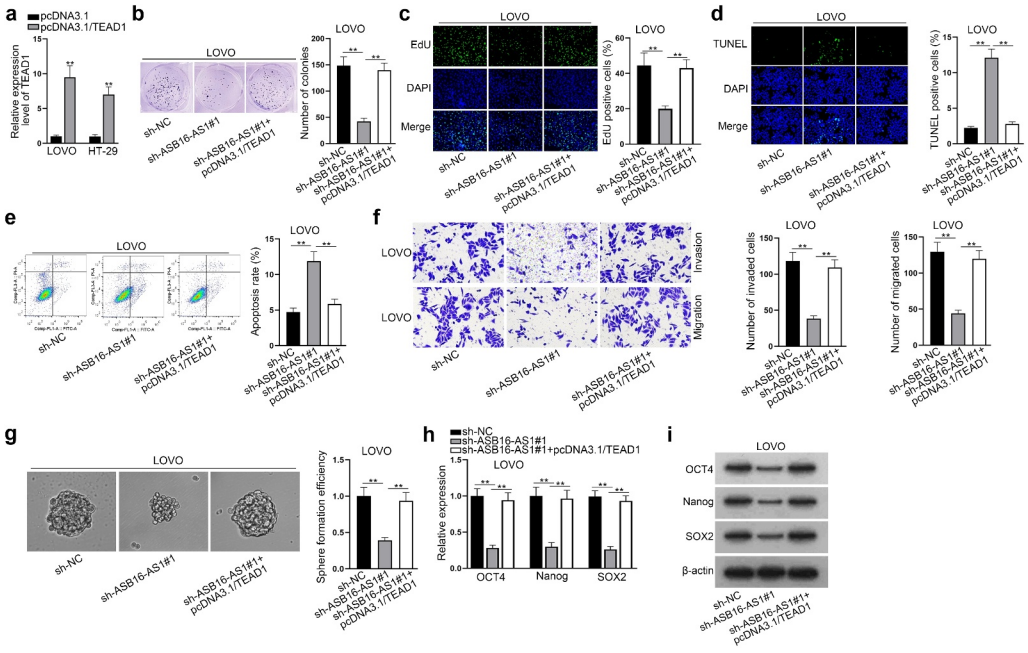

Figure 5. ASB16-AS1 promotes CRC development through enhancing TEAD1 expression. (a) TEAD1 overexpression efficiency was detected in LOVO and HT-29 cells. (b-c) Cell proliferation experiments were adopted to test the proliferative ability of CRC cells in different groups. (d-e) cell apoptosis assays were carried out for testing cell apoptosis in different groups. (f) transwell experiments were adopted to estimate cell migration and invasion in different groups. (g) sphere formation experiment was utilized to test cell stemness under different transfection conditions. (h-i) the expression of OCT4, Nanog and SOX2 was detected in different groups. **P < 0.01.

on CRC treatment. Long lncRNAs could influence the progression of cancers, and the importance of lncRNAs in regulating biological process has been identified [23], in which they act as tumor-promoters or suppressors. For example, MALAT1 promotes the proliferation and metastasis of epithelial ovarian cancer [18]. UCA1 accelerates the proliferation and cisplatin resistance of oral squamous cell carcinoma cells via sequestering miR-184 [19]. What is more, LINC00319 could promote ovarian cancer progression via miR-423-5p/NACC1 pathway [20]. MEG3 inhibits the proliferation and metastasis of gastric cancer [21]. Recently, ASB16-AS1 has been found to be a novel lncRNA, and it has been unveiled that ASB16-AS1 can regulate adrenocortical carcinoma cell proliferation [22]. What is more, ASB16-AS1 can increase HDGF expression by sponging miR-760, thereby exerting cancer-promoting roles in osteosarcoma [23]. Here, it was unveiled that ASB16-AS1 was with high expression in CRC. Silencing of ASB16-AS1 restrained the proliferation, migration, invasion, and stemness while accelerating the apoptosis of CRC cells. All of our experimental outcomes indicated the oncogenic role of ASB16-AS1 in CRC progression.

MicroRNAs (miRNAs) have been validated to regulate the progression of cancers [24]. More importantly, lncRNAs have been validated to control the roles of miRNAs through the ceRNA mechanism. For example, miR-185-5p promotes the apoptosis of prostate cancer cells [25]. FOXD2-AS1 promotes glioma progression by regulating miR-185-5p/HMGA2 axis [26]. LncRNA PDIA3P participates in oral squamous cell carcinoma progression by interacting with miR-185-5p [27]. Nevertheless, the internal connection between miR-185-5p and ASB16-AS1 on CRC is not clear yet. Here, we unveiled that miR-185-5p was down-regulated in CRC cells. What is more, miR-185-5p could bind to ASB16-AS1 and inhibit the progression of CRC. TEAD1 has been proved to be an oncogene in various cancers like gastric cancer [28], prostate cancer [29] and osteosarcoma [30]. In this study, it was revealed that TEAD1 served as the target of miR-185-5p and was negatively modulated by miR-185-5p in CRC cells. At the same time, TEAD1 up-regulation offset the suppressive functions of silencing ASB16-AS1 on CRC progression.

In summary, ASB16-AS1 exerted carcinogenic effects on cell proliferation, migration, invasion, and stemness while playing a suppressive role in CRC cell apoptosis, and it accelerated CRC progression through modulation of miR-185-5p/TEAD1 axis, which might offer a new insight into the treatment of CRC in the future.

Acknowledgments

We are grateful for the help from all lab personnel in this research.

Disclosure Statement

No potential conflict of interest was reported by the author(s).

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