Am J Transl Res 2022;14(10):7324-7335
www.ajtr.org /ISSN:1943-8141/AJTR0142030

Original Article

lncRNA EPS15-AS1 affects the biological behavior of liver cancer stem cells by regulating EPS15 expression

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Received January 26, 2022; Accepted July 8, 2022; Epub October 15, 2022; Published October 30, 2022

Abstract: Objective: To investigate whether long non-coding RNA (lncRNA) EPS15-antisense RNA (EPS15-AS1) affects the biological behavior of liver cancer stem cells (LCSCs) by targeting EPS15. Methods: The expression of EPS15 in liver cancer was analyzed based on TCGA database. The expression of EPS15-AS1 and EPS15 in LCSCs was detected by real-time quantitative PCR (RT-qPCR). MTT method, flow cytometry, and Transwell assay were used to detect the effects of EPS15-AS1 and EPS15 expression on the biological behavior of LCSCs. Results: EPS15 was highly expressed in liver cancer tissues in TCGA, and EPS15 was closely related to the survival and prognosis of liver cancer patients ($P<0.05$). EPS15 was highly expressed in LCSCs, while lncRNA EPS15-AS1 was lowly expressed in LCSCs ($P<0.05$). After silencing lncRNA EPS15-AS1, the proliferation, invasion, and EPS15 protein expression of LCSCs were promoted ($P<0.05$) while apoptosis was suppressed ($P<0.05$). After overexpression of lncRNA EPS15-AS1, the proliferation, invasion, and EPS15 protein expression of LCSCs were suppressed while the apoptosis ability was promoted. However, simultaneous overexpression of lncRNA EPS15-AS1 and EPS15 attenuated the effect of lncRNA EPS15-AS1 overexpression alone on proliferation and apoptosis of LCSCs. Conclusion: lncRNA EPS15-AS1 overexpression can inhibit proliferation and invasion but promote apoptosis of LCSCs by down-regulating the expression of EPS15.

Keywords: EPS15, liver cancer stem cells, proliferation, invasion, apoptosis

Introduction

Hepatocellular carcinoma (HCC) is a type of tumor with high morbidity and malignancy. According to the data, the number of new HCC cases worldwide was about 930,000, and the number of deaths was about 850,000 in 2018 [1]. The incidence of HCC in China exceeds 40% of the global incidence, and the mortality rate ranks second in systemic tumors [2]. Risk factors associated with HCC include chronic HBV and HCV infection, alcoholic liver disease and non-alcoholic fatty liver disease [3]. Despite the presence of risk factors, cirrhosis is not detected in most patients until HCC is definitively diagnosed; and in only a minority of cases, HCC is diagnosed on physical examination. At present, HCC is diagnosed mainly based on pathological examination, imaging examination and laboratory detection of tumor markers [4-6]. As we all know, pathological examination is currently the "gold standard" for diagnosing HCC. However, pathological biopsy has certain difficulty and risks in obtaining specimens, which seriously hinders the implementation of "early detection, early diagnosis, and early treatment" of HCC [7]. The development of HCC is a complex multistep process involving various factors at the molecular, cellular, and tissue levels, as well as persistent inflammatory damage, including hepatocyte necrosis and regeneration, and fibrotic deposition [8]. After developing liver cirrhosis, the risk of HCC increases with progressive liver damage. In addition to epigenetic modifications, HCC is the result of the accumulation of alterations in passenger and driver genes in the genome of somatic cells [9]. Unfortunately, none of the molecular-level theoretical knowledge of HCC proposed so far could well-predict the disease progression or recurrence.

With the deepening of tumor research, scientists have found that not all tumor cells have

the ability to proliferate indefinitely. In 1983, Mackillop et al. [10] proposed a new hypothesis on the pathogenesis of tumors, that is, tumor tissue is composed of cells in different stages of differentiation, in which there are very few cells with stem cell properties. These very few cells have unlimited proliferation and multidirectional differentiation potential, and play a major role in tumor recurrence and drug resistance; this group of cells is called "cancer stem cells" (CSCs) [11]. This hypothesis was first confirmed in hematological tumors [12], followed with verification in various solid tumors. Studies have confirmed that liver cancer stem cells (LCSCs) also exist in HCC, which are involved in the occurrence, development, recurrence, and drug resistance of HCC [13].

With the in-depth study in tumor-related mechanisms, the role of non-coding RNA regulatory networks has been gradually unveiled. For example, Epidermal Growth Factor Receptor (EGFR) has been identified as an oncogenic driver of NSCLC [14]. In particular, the activation of EGFR mutations and the inhibition of specific tyrosine kinase inhibitors (TKIs) can produce significant tumor responses and play a key role in tumor progression [15]. EGFR protein tyrosine kinase substrate 15 (EPS15) is a protein tyrosine kinase substrate of EGFR involved in the endocytosis and secretion of EGFR, and a multifunctional adaptor protein that regulates intracellular transport [16]. Receptor tyrosine kinases (RTKs) are mainly composed of EGFR, and the development of many malignancies is associated with the overexpression of RTKs [17]. Termination of growth factor signaling pathways is primarily affected by downregulation of active growth factor receptor complexes [18]. Dysregulation of EGFR signaling is an important feature in the progression of different types of cancers [19]. The abundance of EGFR is partly regulated by its ubiquitination, and EGFR deubiquitination may inhibit its recruitment, thereby inhibiting endocytosis [20]. Therefore, EPS15 may regulate EGFR and is associated with clinical outcomes in certain tumors. For the past few years, abnormal EPS15 expression has been found in some tumor cells and tissues, which may be related to the formation and progression of tumors [21].

Antisense lncRNA (AS-lncRNA) refers to RNA molecules that are transcribed from the antisense strand of a gene (usually a protein-coding gene) and overlap with the mRNA of the gene [22]. Studies have found that about 70% of genes have AS-lncRNAs [23]. AS-lncRNAs are often correlated with the expression of their sense-strand genes (SS-gene), indicating that AS-lncRNAs may be extensively involved in the expression regulation of protein-coding genes. Regarding the mechanism of AS-lncRNAs affecting the gene expression of the sense-strand, there are mainly three categories: 1) The transcription process of AS-lncRNA inhibits the transcription of the SS-gene and regulates the expression of the gene. 2) AS-lncRNA binds DNA or histone modifying enzymes to regulate the epigenetics of the gene locus where it is located, thereby affecting the expression of SS-gene. 3) AS-lncRNA binds to SS-gene through complementary base pairing, which affects the alternative splicing of mRNA [24]. The lncRNA EPS15-AS1 is the antisense-strand of EPS15, which may regulate the expression of EPS15, thereby affecting the progression of tumors, but the relationship between the two has not been reported in LCSCs.

Therefore, the purpose of this study was to analyze the expression of lncRNA EPS15-AS1 and EPS15 in LCSCs, and to determine whether lncRNA EPS15-AS1 has the ability to regulate the participation of EPS15 in the development of LCSCs, and to provide potential therapeutic targets for LCSCs therapy.

Materials and methods

Analysis of EPS15 expression in liver cancer by TCGA

The UALCAN website (http://ualcan.path.uab.edu/index.html) was used to observe EPS15 expression in liver cancer (LIHC), and the fold difference method (Log_2FC) was used to compare EPS15 expression levels between liver cancer tissue samples and adjacent normal tissue samples.

Culture of hepatoma cells

Hepatoma cell lines (HepG2, Huh7) were bought from the American Standard Biological Collection (ATCC) and cultivated in MEM medium (Item No. 211095080, Gibco-BRL, Grand Island) with 10% FBS in a 37°C incubator with 5% CO_2 saturated humidity. When the cells

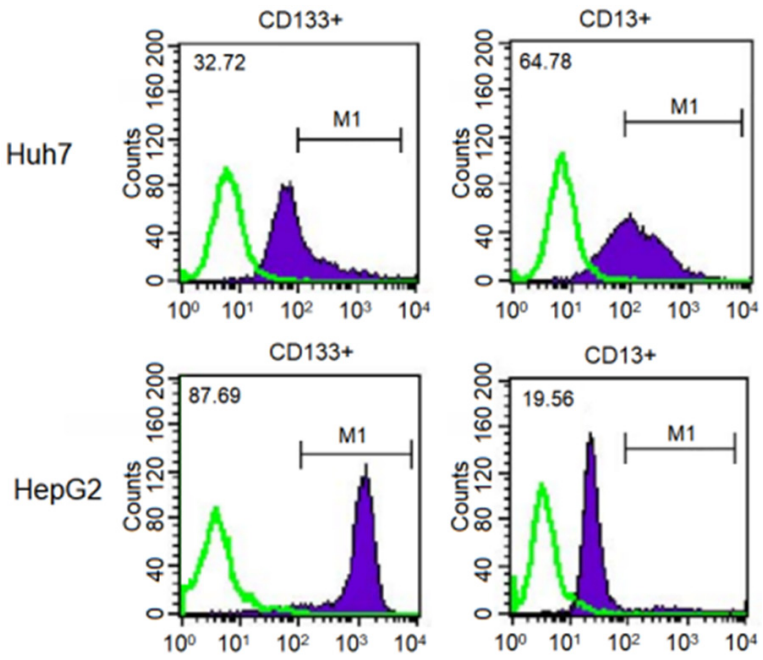

Figure 1. Identification of CD13+ CD133+ cells.

grew to about 80% confluence, 0.25% trypsin (Item No. 25200056, Gibco-BRL, Grand Island) was added for digestion. After 3 min, when the cells were observed to be round and partially floating, 2 additions of fresh medium with 10% FBS (Item No. C0235, Gibco-BRL, Grand Island) were added to terminate the digestion. The mixture was centrifuged at 1,000 rpm for 5 min and the supernatant was discarded to collect the pelleted cells. The cells were resuspended in MEM complete medium containing 10% FBS to make a single cell suspension and then passaged or seeded.

Culture of liver cancer stem cells

HCC cell lines were incubated with DMEM supplemented with 10% FBS in a humid incubator at 37°C and 5% CO_2. When the degree of cell fusion reached 80%, the cells were isolated with 0.25% trypsin to prepare a single cell suspension. The cell suspension was transferred into a 1.5 mL EP tube. Then 5 μL CD133 (dilution ratio 1:300, Abcam, ab19898) and CD13 (dilution ratio 1:100, Abcam, ab108310) were added and incubated at 4°C for 30 min away from light. Centrifugation was performed at 700 RPM for 5 min, and the supernatant was discarded. Accuri C6 flow cytometry (Becton Dickinson, Franklin Lakes, NJ, USA) was used to separate CD13+CD133+ cells which were identified as LCSCs (**Figure 1**). The positive rates of CD13 and CD133 in HepG2 were 19% and 87%, respectively. The positive rates of CD13 and CD133 in Huh7 were 65% and 32%, respectively.

Plasmid construction and cell transfection

The silenced LncRNA EPS15-AS1 shRNA plasmid and LncRNA EPS15-AS1 cDNA high expression recombinant vector were constructed. The silenced EPS15-AS1 group was named EPS15-AS1-KD, and the sequence was as follows: 5'-ACCTCGAAATACTGATCCGCCATTAGATTCAAGAGATCTAATGGCGGATCAGTATTTCTT-3'. The negative control group was named EPS15-AS1-NC with the following sequence: 5'-ACCTCGAAATACTGATCCGCCATTAGATTCAAGAGATCTAATGGCGGATCAGTATTTCTT-3'. The EPS15-AS1 sequence (GenBank: XR_007066080.1) was synthesized from the full-length EPS15-AS1 sequence (based on the EPS15-AS1 sequence) and cloned into pcDNA3.1 vectors, which was the EPS15-AS1 overexpression group (named EPS15-AS1-OE). The primer sequences were designed and synthesized by Hanyin Biotechnology (Shanghai) Co., Ltd. All transfections were performed using Lipofectamine 2000 reagent (Invitrogen; Thermo Fisher Scientific Inc.) according to the manufacturer's protocol.

RT-qPCR detection of lncRNA EPS15-AS1 and EPS15 expression

Total RNA of LCSCs was extracted with TRIzol reagent (Item No. 15596026, Invitrogen, Carlsbad), 200 ng of total RNA was reverse transcribed into cDNA with reverse transcription kit (Item No. 18080093, Invitrogen, Carlsbad), and RT-qPCR was performed with SYBR qPCR Mix (Item No. BL698A, Biosharp, China). GAPDH was used as endogenous regulatory genes, and the relative expression levels of lncRNA EPS15-AS1 and EPS15 were detected by $2^{-\Delta\Delta Ct}$ method. qRT-PCR reaction system: SYBR Green Master Mix 10 μL/well, forward and reverse primers

0.8 μL/well, cDNA 1 μL/well, ddH_2O supplemented to 20 μL; reaction conditions: 95°C for 2 min, 95°C for 15 s, 60°C for 30 s, and 72°C for 30 s, a total of 40 cycles.

EPS15 primer sequence: F primer: 5'-ACCTTCACTTAGGCCCCTGT-3'. R primer: 5'-CCCTTACCCTCACTCAACCA-3'.

EPS15-AS1 primer sequence: F primer 5'-ACCCCAAAGCCTCTTGATTT-3'. R primer: 5'-CGTCTCCTCAGACGGTTCTC-3'.

GAPDH primer sequence: F primer 5'-CATCACTGCCACCCAGAAGACTG-3', R primer 5'-ATGCCAGTGAGCTTCCCGTTCAG-3'.

Cell proliferation detected by MTT assay

LCSCs (2×10^5/mL) in each group were seeded in 96-well plates (100 μL/well) for 24 h, and then MTT solution (20 μL/well; Item No. M1020, Beijing Soleibao Technology, China) was added to each well to incubate for another 4 h. Then the culture solution was removed. Then, DMSO solution (Item No: D8418, Sigma, USA) was added to each well (150 μL/well). After incubation in the dark at 22°C for 5 min, the absorbance value (OD 490 nm) of each well was detected, and the cell viability = OD value of experimental group/OD value of control group × 100%.

Cell apoptosis detected by flow cytometry

The LCSCs were rinsed with pre-cooled PBS, and the supernatant was discarded. Next, 500 μL of binding buffer was added to the cell pellet, and 5 μL of Annexin V-FITC (Item No. CA1020, Sigma, USA) and 5 μL of PE (Item No. CA1020, Sigma, USA) were added to the suspended cells respectively. The apoptosis rate was detected by flow cytometry.

Cell invasion assay

BD BioCoat™ BD Matrigel™ Invasion Chamber (Item No. 354234, BD, USA) was used for cell invasion experiments. Transwell Chambers were added with 300 μL of serum-free medium. Cells were rinsed 3 times with PBS and trypsinized. 0.05%-0.2% BSA (Item No: A8010, Solarbio, China) serum-free medium was added to terminate the digestion. The cell suspension density was adjusted to 1×10^5/mL, and 200 μL of the cell suspension was inoculated into the Transwell Chamber, and 500 μL of complete medium (Item No. 11269016, Gibco-BRL, Grand Island) with 10% FBS was added to the lower chamber. The chamber was place in a 37°C, 5% CO_2 incubator for 36-48 hours. It was rinsed 3 times with PBS and fixed with formaldehyde solution (Item No. G2162, Solarbio, China) for 20 minutes. After drying in the air, the cells were stained with 0.1% crystal violet solution (Item No. C8470, Solarbio, China) for 30 minutes, rinsed with running water and dried. 6-10 randomly selected fields were observed under a 100 × microscope, and the number of cells passing through the chamber was recorded.

The targeting relationship between lncRNA EPS15-AS1 and EPS15 was detected using dual-luciferase reporter gene assay

LncBase v.2 prediction showed the existence of binding sites for lncRNA EPS15-AS1 and EPS15. The binding site was mutated by gene mutation technology, and the wild-type vector WT-EPS15-AS1 containing the binding site and the mutant vector MUT-EPS15-AS1 containing the mutation site were constructed. miR-NC and EPS15 mimics were co-transfected into LCSCs with WT-EPS15-AS1 and MUT-EPS15-AS1, respectively. After co-incubation for 24 h, the cells were collected and the luciferase activity of cells was detected by dual-luciferase reporter gene detection system. Dual-luciferase reporter vectors (WT-FOXF1-AS1, MUT-FOXF1-AS1) were obtained from Shanghai Shengong Company.

Protein expression detected by Western blotting

Total protein from LCSCs were extracted using 500 μL RIPA lysis buffer (Item No. P0013C, Beyotime, China). When the protein concentration met the determination conditions, the loading buffer (Item No. D0071, Beyotime, China) was added and placed in boiling water for 10 min before protein denaturation. The denatured protein was transferred to a PVDF membrane (Item No. ISEQ00010, Millipore, USA) and blocked for 2 h. Anti-EPS15 antibody (1:1000; Abcam, Item No. ab174291) was added and incubated at 4°C for 12 h. After washing with TBST (Item No. ST677, Beyotime, China), the secondary antibody (1:1000; Abcam, Item No. ab6702) was added and incubated at 22°C for 1 h. After washing with TBST, ECL developing solution (Item No. 180-

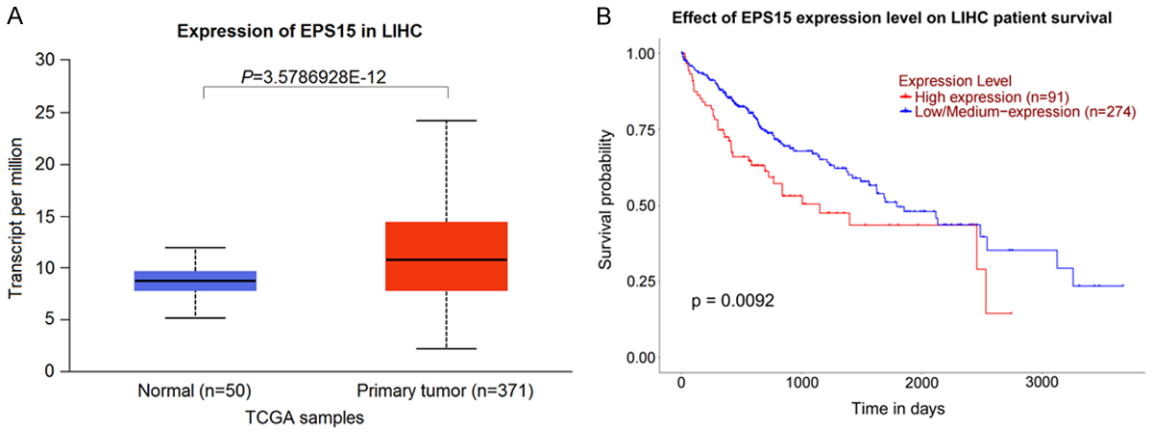

Figure 2. EPS15 expression in TCGA-LIHC. A. Expression level of EPS15 in LIHC tumor samples and paired normal tissues. B. KM survival curves of patients with high and low EPS15 expression.

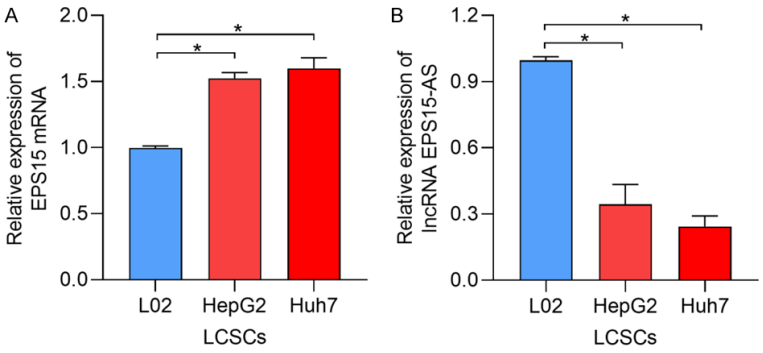

Figure 3. RT-qPCR detection of the expression of lncRNA EPS15-AS1 (A) and EPS15 (B) in two LCSCs (HepG2, Huh7) and normal hepatocyte (L02).

501, Tanon, China) was added dropwise, and the gray value was observed by ImageJ software.

Statistical processing

SPSS21.0 software was used for analysis, measurement data were expressed as ($\bar{x} \pm s$), independent sample *t* test was used for comparison between two groups. ANOVA followed with SNK-q test was adopted for data analysis among multiple groups or multiple time points. P<0.05 was considered statistically significant.

Results

Analysis of EPS15 expression in liver cancer based on the TCGA database

We first investigated the expression of EPS15 in liver cancer using the TCGA database. By analyzing the expression profile of EPS15 in 421 samples (371 cancer samples and 50 normal samples) in the TCGA-LIHC, we found that EPS15 expression was higher in liver cancer tissues (P<0.05, **Figure 2A**). In addition, we also observed that the survival rate of patients with high-EPS15 expression was lower than the patients with medium/low-EPS15 expression (log-rank P= 0.0092, **Figure 2B**). Thus it was preliminarily shown that EPS15 is significantly related to the occurrence and development of liver cancer.

Expression of lncRNA EPS15-AS1 and EPS15 in LCSCs

RT-qPCR was used to detect the expression of lncRNA EPS15-AS1 and EPS15 in two LCSCs (HepG2, Huh7) and normal liver cell line L02. As shown in **Figure 3**, the expression of EPS15 in HepG2 and Huh7 cells was significantly higher than that in normal liver L02 cells (all P<0.05), while the expression of lncRNA EPS15-AS1 was significantly lower (all P<0.05).

Effects of silencing lncRNA EPS15-AS1 on the proliferation and apoptosis of LCSCs

MTT assay was performed to detect the proliferation of transfected LCSCs, and the growth curve was plotted (**Figure 4A**, **4B**). The OD values of HepG2-NC, HepG2-EPS15-AS1-KD and

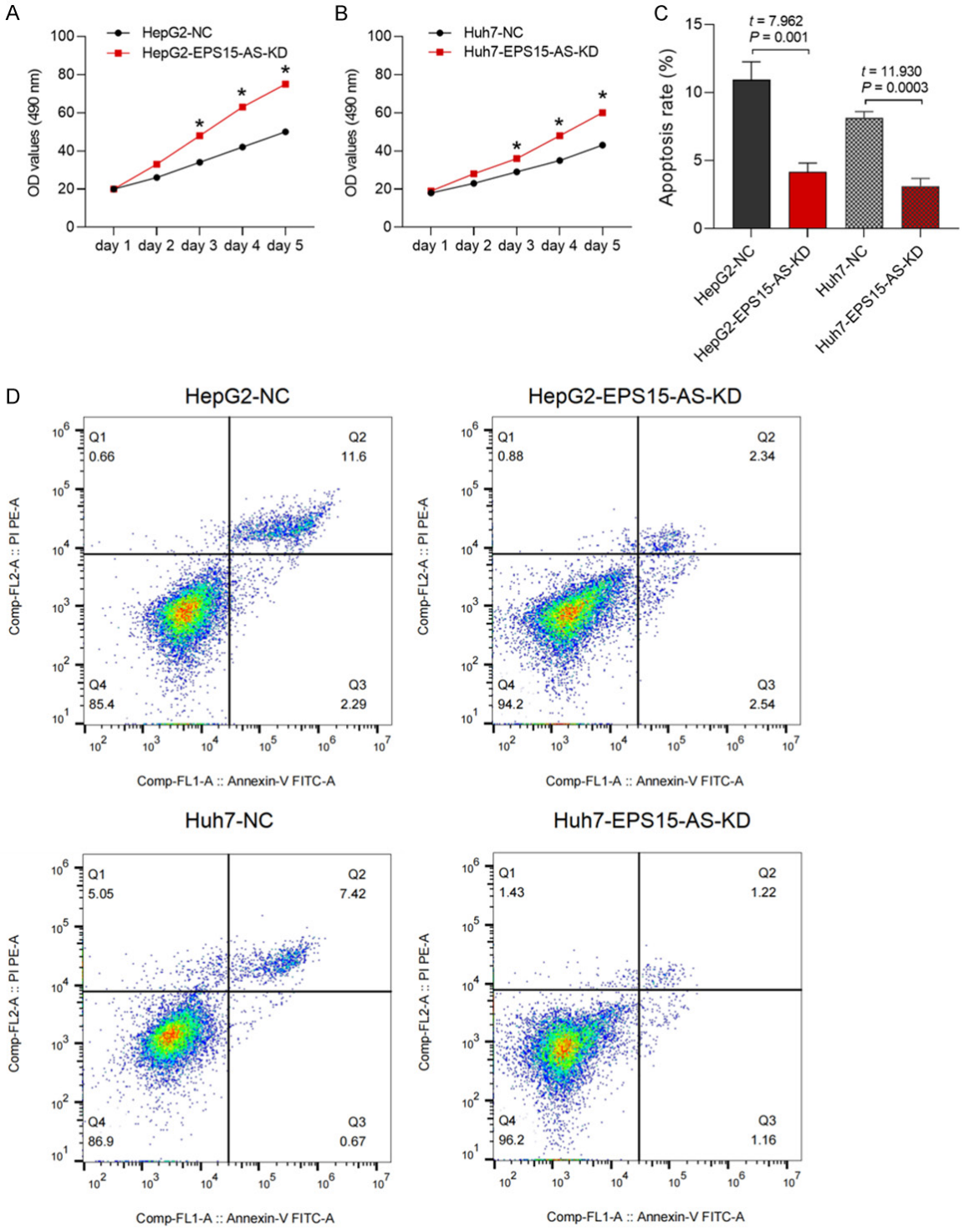

Figure 4. Effects of silencing lncRNA EPS15-AS1 on the proliferation and apoptosis of LCSCs. A, B: Cell proliferation rate; C: Apoptosis rate. D: Flow cytometry for apoptosis. *P<0.05, comparison with control group.

Huh7-NC, Huh7-EPS15-AS1-KD cells were detected after passage 1d, 2d, 3d, 4d, and 5d. The results showed that after 3d, 4d, and 5d, the proliferation of LCSCs after silencing lncRNA EPS15-AS1 was increased as compared with control group (all P<0.05). The effect of

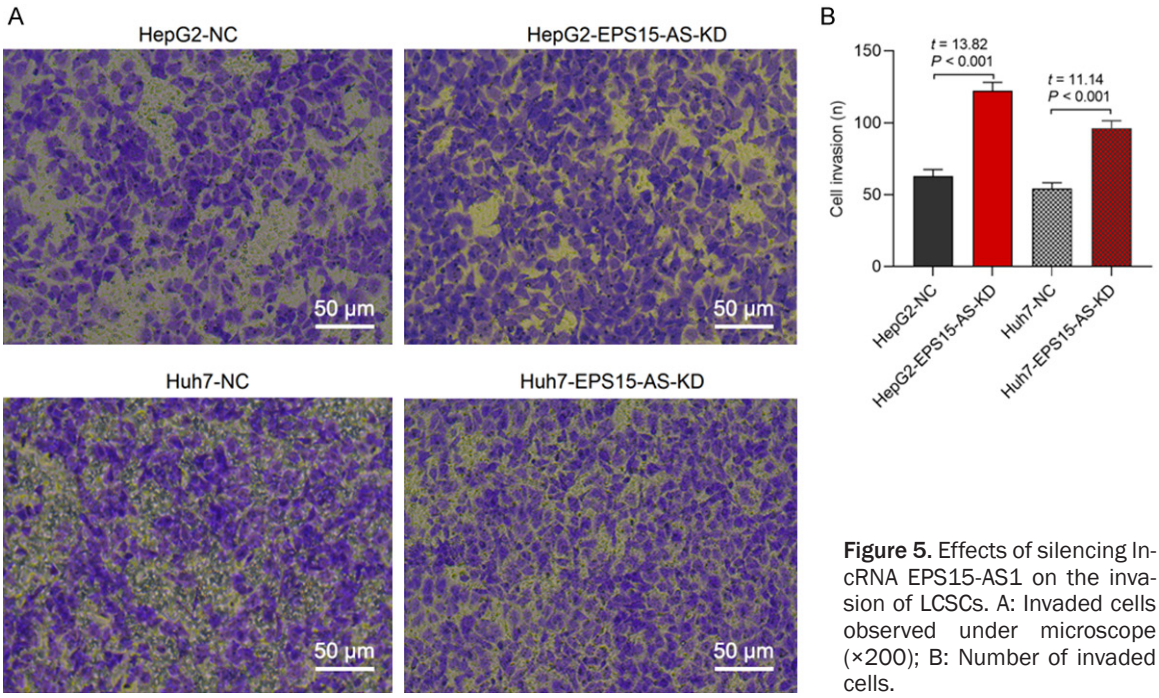

Figure 5. Effects of silencing lncRNA EPS15-AS1 on the invasion of LCSCs. A: Invaded cells observed under microscope (×200); B: Number of invaded cells.

silencing EPS15-AS1 on apoptosis of LCSCs was analyzed by flow cytometry. Compared with control cells (HepG2-NC, Huh7-NC), the apoptosis level of cells with silenced EPS15-AS1 expression (HepG2-EPS15-AS1-KD, Huh7-EPS-15-AS1-KD) was significantly decreased as compared with control cells (all P<0.05, **Figure 4C**).

The effect of silencing lncRNA EPS15-AS1 on the invasion of LCSCs

Compared with the control cells (HepG2-NC, Huh7-NC), the cells with silenced EPS15-AS1 (HepG2-EPS15-AS1-KD, Huh7-EPS15-AS1-KD) showed significantly enhanced invasion ability (all P<0.05, **Figure 5**).

Effects of overexpression and silencing of lncRNA EPS15-AS1 on EPS15 mRNA expression in LCSCs

In LCSCs, silencing lncRNA EPS15-AS1 increased the mRNA and protein expression of EPS15, while overexpressing lncRNA EPS15-AS1 decreased the mRNA and protein expression of EPS15 (P<0.05, **Figure 6**).

Targeting relationship between LncRNA EPS15-AS1 and EPS15

As shown in **Figure 7**, overexpression of EPS15 significantly reduced the luciferase activity of the wild-type vector WT-EPS15-AS1 (P<0.05), but had no significant effect on the luciferase activity of the mutant vector MUT-EPS15-AS1.

Overexpression of EPS15 reduced the effect of lncRNA EPS15-AS1 on the proliferation and apoptosis of LCSCs

As shown in **Figure 8**, overexpression of lncRNA EPS15-AS1 inhibited cell proliferation, and the addition of EPS15 partially antagonized the inhibitory effect of lncRNA EPS15-AS1 overexpression on the proliferation of LCSCs (P< 0.05). Overexpression of lncRNA EPS15-AS1 promoted the apoptosis of LCSCs, and the addition of EPS15 partially antagonized the promotive effect of lncRNA EPS15-AS1 overexpression on the apoptosis of LCSCs (P<0.05).

Overexpression of EPS15 reduced the effect of lncRNA EPS15-AS1 on the invasion of LCSCs

As shown in **Figure 9**, overexpression of lncRNA EPS15-AS1 inhibited cell invasion (P<0.05), while adding EPS15 partially offset the inhibitory effect of lncRNA EPS15-AS1 overexpression on LCSCs cell invasion (P<0.05).

Discussion

HCC is a highly aggressive, and most of the HCC patients are in the III-IV stages at the time of diagnosis, and thus have a poor prognosis

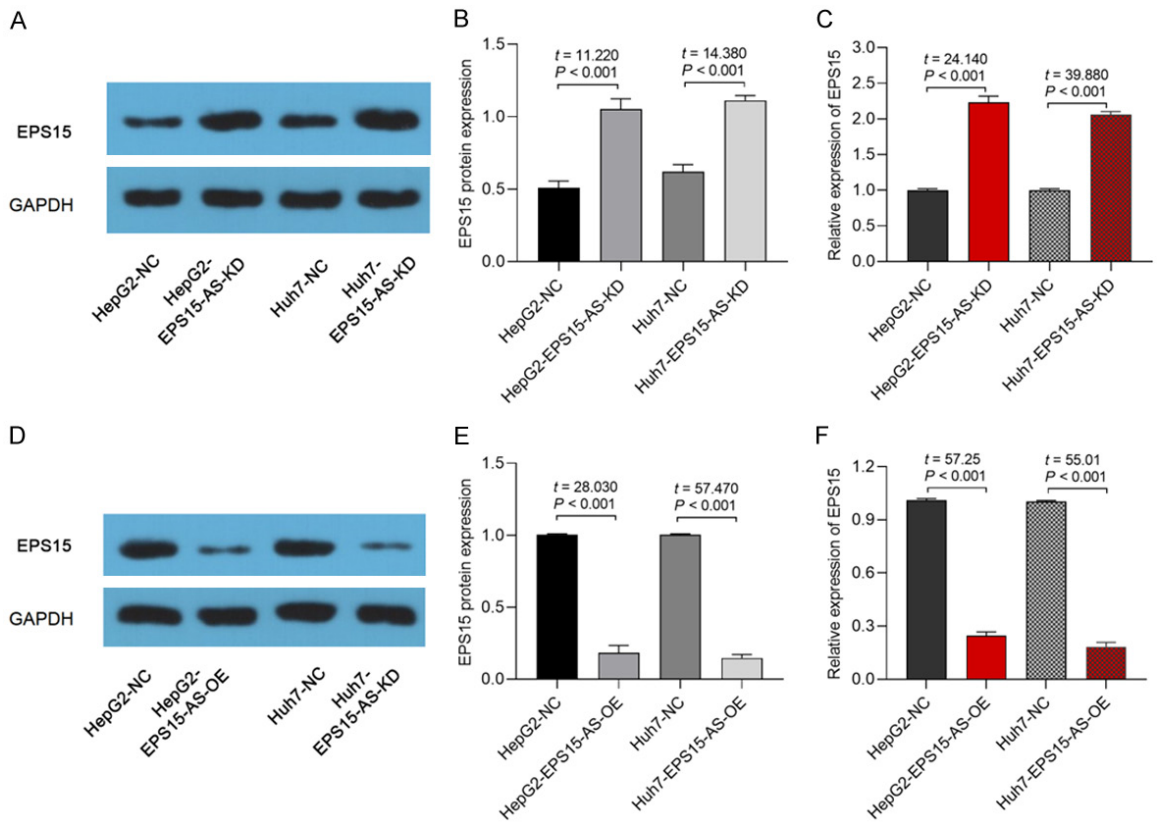

Figure 6. Effects of overexpression and silence of lncRNA EPS15-AS1 on EPS15 expression in LCSCs. A, B: Protein expression of EPS15 after silencing EPS15-AS1 detected by WB; C: mRNA expression of EPS15 after silencing EPS15-AS1 detected by PCR; D, E: Protein expression of EPS15 after EPS15-AS1 overexpression detected by WB; F: mRNA expression of EPS15 after EPS15-AS1 overexpression detected by PCR.

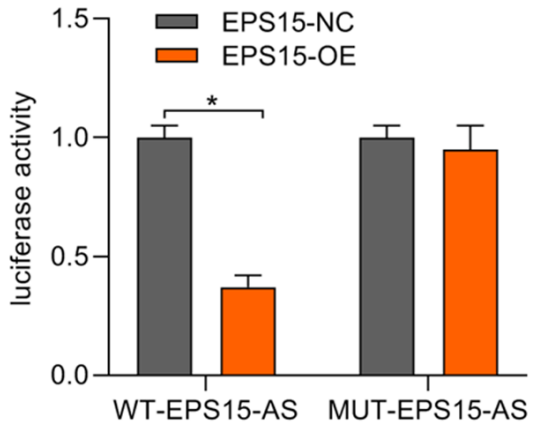

Figure 7. Luciferase activity detected by Dual-luciferase reporter assay. *P<0.05.

with a 5-year survival rate of under 10% [25]. In this paper, the role and mechanism of lncRNA EPS15 targeting EPS15 in regulating the proliferation, apoptosis, and invasion of LCSCs was discussed to provide theoretical reference for finding new HCC diagnostic or prognostic targets, guiding clinical treatment decisions, and improving patient prognosis.

In the beginning, we analyzed EPS15 expression in liver cancer tissues and adjacent tissues by using TCGA database and found that EPS15 was highly expressed in liver cancer tissues, and EPS15 was closely related to the survival and prognosis of liver cancer. Moreover, Yu et al. [26] demonstrated that the prognosis of osteosarcoma patients with low EPS15 homology domain 1 expression was better than that of patients with high expression. In addition, *in vitro* experiments confirmed that EPS15 was highly expressed in LCSCs, while the expression of lncRNA EPS15-AS1 was lowly expressed in LCSCs. Based on previous studies, we speculated that the expression level of lncRNA EPS15-AS1 and EPS15 were closely related to the occurrence and development of

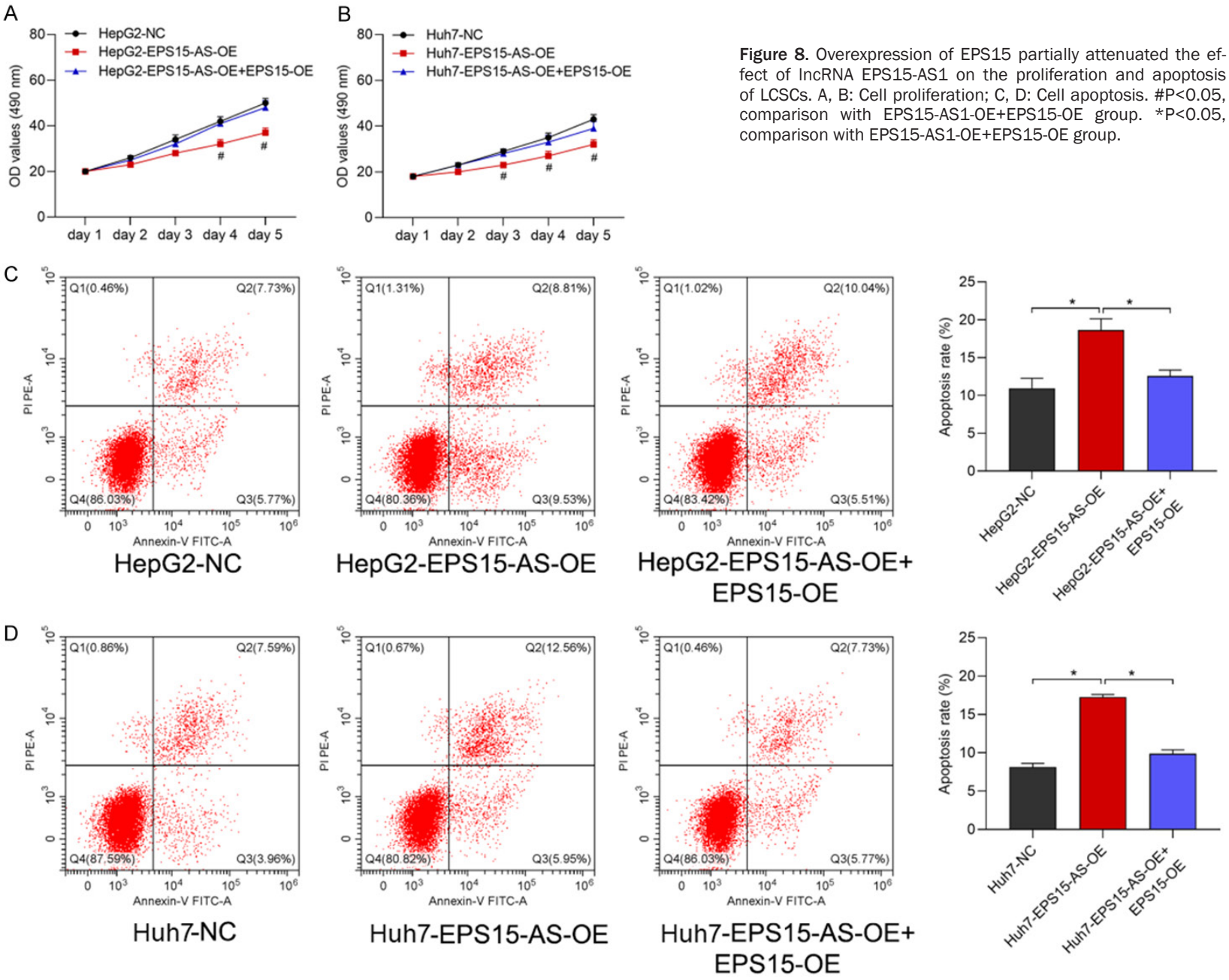

Figure 8. Overexpression of EPS15 partially attenuated the effect of lncRNA EPS15-AS1 on the proliferation and apoptosis of LCSCs. A, B: Cell proliferation; C, D: Cell apoptosis. #P<0.05, comparison with EPS15-AS1-OE+EPS15-OE group. *P<0.05, comparison with EPS15-AS1-OE+EPS15-OE group.

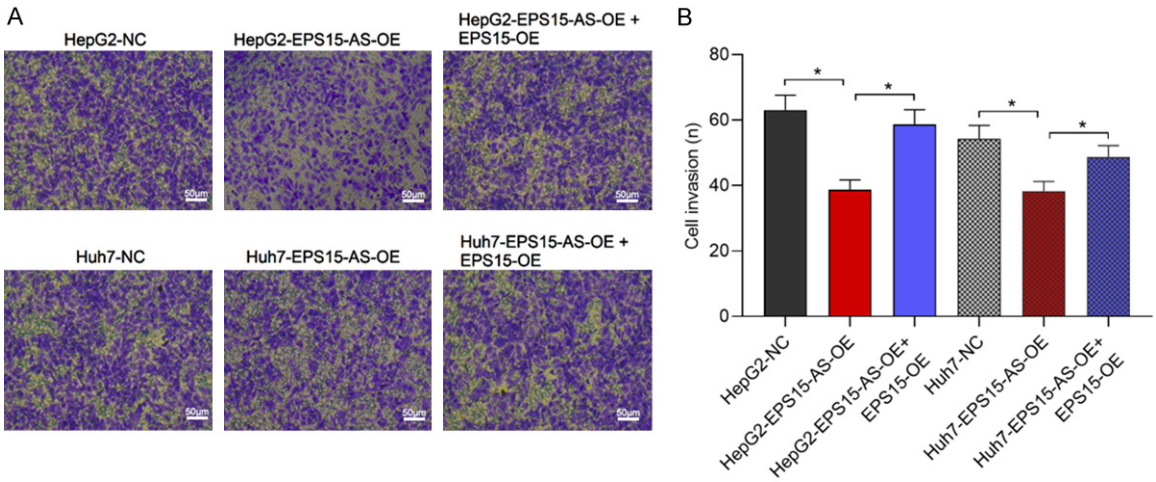

Figure 9. Overexpression of EPS15 reduced the effect of lncRNA EPS15-AS1 on the invasion of LCSCs. A: Invaded cells observed under microscope (× 200); B: Number of invaded cells; *P<0.05.

liver cancer, and there might be a regulatory relationship between lncRNA EPS15-AS1 and EPS15, but there is no research report yet.

In order to explore the biological effect of lncRNA EPS15-AS1 on liver cancer, we established a stable knockdown of LncRNA EPS15-AS1 in LCSCs, and observed the effect of silencing LncRNA EPS15-AS1 on LCSCs. MTT method, flow cytometry and Transwell method were used to analyze cell proliferation, apoptosis, and invasion of LCSCs. The results all demonstrated that compared with the control group, cells with lncRNA EPS15-AS1 knockdown exhibited increased proliferation and invasion ability and decreased apoptosis ability. This suggests that lncRNA EPS15-AS1 can inhibit the proliferation, and promote apoptosis of LCSCs.

To explore whether lncRNA EPS15-AS1 can target and regulate EPS15 expression, we used Western blot detection to verify that lncRNA EPS15-AS1 targeted EPS15 at the protein level. The results showed that EPS15 protein expression in LCSCs increased when lncRNA EPS15-AS1 was silenced, and EPS15 protein expression decreased when lncRNA EPS15-AS1 was overexpressed. In addition, we performed dual-luciferase reporter gene assay and verified that lncRNA EPS15-AS1 could target and regulate the expression of EPS15 in LCSCs.

To further study the effect of lncRNA EPS15-AS1 targeting EPS15 on the biological behaviors of LCSCs, we performed functional recovery experiments. The results showed that lncRNA EPS15-AS1 overexpression suppressed the proliferation and invasion but promoted the apoptosis of LCSCs. However, simultaneous overexpression of lncRNA EPS15-AS1 and EPS15 abrogated the effect of lncRNA EPS15-AS1 overexpression alone on the proliferation, invasion and apoptosis of LCSCs, suggesting that overexpression of EPS15 can offset the effect of lncRNA EPS15-AS1 overexpression on liver cancer stem cells. There are also related studies that EPHA2 antisense RNA (EPHA2-AS) is an important regulator of EPHA2 in breast cancer, and EPHA2-AS1/2 plays a key role in regulating EPHA2 mRNA levels, thereby producing EPHA2 protein (EPHA2 is a key oncogenic receptor tyrosine kinase that contributes to tumorigenesis of breast cancer cells) [27]. Similarly, Ma et al. found in liver cancer that LncRNA BSG-AS1 could positively regulate BSG by maintaining the stability of BSG mRNA, thereby promoting the proliferation of HCC [28]. Wang et al. found that MCM3AP-AS1 directly binds to miR-194-5p and acts as a competitive endogenous RNA (ceRNA) to promote the expression of miR-194-5p target gene FOXA1 in HCC cells [29]. Another study showed that MAPKAPK5-AS1 upregulated PLAG1 like zinc finger 2 (PLAGL2) expression by acting as an endogenous competing RNA (ceRNA) to sponge miR-154-5p, thereby activating EGFR/AKT signaling [30].

This study demonstrated that lncRNA EPS15-AS1 targets and regulates EPS15. lncRNA EPS15-AS1 may serve as a potential target for liver cancer treatment. In this study, there is no

further study on whether lncRNA EPS15-AS1 also regulates some microRNAs, which needs to be explored in future research. This study didn't conduct *in vivo* research, and the complex *in vivo* environment may lead to some differences in research conclusions, which needs further investigation.

Conclusion

lncRNA EPS15-AS1 is lowly expressed, while EPS15 is highly expressed in LCSCs. lncRNA EPS15-AS1 can competitively bind to EPS15. Promoting the expression of lncRNA EPS15-AS1 can inhibit the proliferation, and invasion but promote apoptosis of LCSCs. Overexpression of EPS15 can antagonize the effects of lncRNA EPS15-AS1 on the biological behaviors of LCSCs. lncRNA EPS15-AS1 may serve as a potential target for liver cancer treatment, and it can also lay an experimental foundation for further revealing the pathogenesis of liver cancer.

Disclosure of conflict of interest

None.

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