Long non-coding RNA CASC15 promotes HCC progression via modulation of Six1 targeted by miR-2355-5p

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Abstract

Background: Long-chain non-coding RNA (LncRNA) plays a key role in the biological processes of tumors. LncRNA CASC15 has been shown to be involved in the development of a variety of tumors. The study aimed to elucidate the mechanism of LncRNA CASC15 in the progression of hepatocellular carcinomas (HCC).

Methods: qRT-PCR was used to detect the expression levels of CASC15, miR-2355-5p and Six1 mRNA in HCC tissues and cells. Six1 protein expression levels were detected by Western Blot. CCK-8 experiment, colony formation experiment, Edu staining and Transwell experiment analysis were used to analyze the effects of CASC15, miR-2355-5p and Six1 on cell proliferation, cell invasion and migration. The relationship between CASC15, miR-2355-5p and Six1 was analyzed using bioinformatics analysis and Luciferase.

Result: CASC15 was raised in HCC tissues and HCC cells. Down-regulation of CASC15 inhibited the growth, migration, invasion and tumor growth of HCC cells. The expression level of miR-2355-5p was reduced in HCC tissues. In addition, miR-2355-5p inhibitor induced the growth, migration and invasion of HCC cells. MiR-2355-5p was predicted to be a downstream target of CASC15. The expression level of miR-2355-5p was negatively correlated with CASC15 in HCC tumor tissues. Six1 was predicted to be a downstream target of miR-30a-5p. In vitro and in vivo results showed that CASC15/miR-2355-5p can regulate Six1.

Conclusion: LncCASC15 regulated the proliferation and invasion of Six1 by binding with miR-2355-5p in HCC, suggesting that CASC15 may be a potential target for HCC.

Introduction

Primary liver cancer is one of the major malignant diseases in the world[1]. Hepatocellular carcinoma (HCC) accounts for about 70%-90% of primary liver cancer[2]. More about 80% of HCC patients delay treatment because of the early symptoms are not obvious, and most of them have poor prognosis[3]. Because of the high recurrence rate after operation and the poor response of anticancer drugs and radiotherapy, the 5-year survival rate of HCC is also relatively low[4]. Previous studies have found that the HCC increases with age, and its occurrence and development are also related to various risk factors[5, 6]. However, the existing research still cannot elaborate the specific mechanism of the development of HCC. Therefore, it is urgent to find new specific biomarkers and therapeutic targets to provide a new direction for treatment of HCC.

Studies show that non-coding RNA (ncRNA) is a key element of cell function, and it can play an crucial role in the process of post-translational modification and chromatin remodeling[7]. Among them, ncRNA with a length of more than 200 NT is called long-chain non coding RNA (LncRNA), which has limited or no protein coding ability[8]. Studies have found that abnormally expressed LncRNAs are closely related to the pathological process of human disease, such as tumors[9]. In the development of tumor, Lnc RNAs

can play the role of tumor promotor or suppressor through a variety of mechanisms, such as regulating the expression of adjacent genes, or affecting the expression of related genes as transcription regulators or molecular scaffolds[10]. Studies have found that there is a wide range of lncRNAs imbalances in HCC, which can play a regulatory role in tumor formation in a variety of ways, thereby affecting the occurrence, metastasis and prognosis of HCC[11]. With the continuous deepening of research on lncRNAs, lnc RNA is expected to become an effective biomarker for the diagnosis of tumor progression and even a new target for the treatment of HCC[12]. lncRNA CASC15, a new discovered tumor-related lncRNA, have expression significance in a variety of malignant tumors, and can regulate the biological processes of related tumors [13, 14]. For example, lncRNA CASC15 was found to be a risk factor for the prognosis of gastric cancer[14]. However, its functions and mechanisms in HCC have not been studied.

It has been found that lncRNA can regulate the expression of miRNA and play a role in tumorigenesis[15]. MiRNAs play a key role in the regulation of biological processes, including tumor progression[16]. miRNAs have the role of oncogenes or tumor suppressor genes in hepatocellular carcinoma cells. With in-depth research on miRNAs, it has been found that certain specific miRNAs have has a regulatory role in hepatocellular carcinoma[17]. It has been found that low expression miRNA-122, miRNA-145, miRNA-16 and high expression miRNA-222 and miRNA-21 play a key role in the progress of HCC[18, 19]. These miRNAs mainly play a role in cell cycle, apoptosis, DNA damage or transduction pathway. MiR-2355-5p is a newly discovered miRNA that participates in the occurrence and development of some diseases[20]. For example, miR-2355-5p is up-regulated in endothelial colony-forming cells of coronary heart disease[21]. At present, the research on target genes downstream of miRNA is in a relatively mature stage, but there are still many target gene sites not found. Six1 homeobox homolog 1 (SIX1) is a tumor-promoting factor and can be used as a transcription factor to participate in the formation and development of a variety of tissues[22]. Studies have found that SIX1 in normal mature tissues is low, while the expression level in malignant tissues is significantly increased[23]. Some studies have suggested that SIX1 can regulate the overexpression of transforming growth factor signaling pathways, activate vascular endothelial growth factor, and effectively regulate tumor interstitial angiogenesis. At the same time, it can inhibit the adhesion of epithelial cells and the degree of cell-to-cell binding, and promote tumor proliferation, invasion, and metastasis[24]. It was speculated that lncRNA CASC15 regulated the progression of HCC by miR-2355-5p. The main aim of this study was to explore the mechanism of lncRNA CASC15 in regulating HCC.

Materials And Methods

2.1 Tissue sample

The cancerous tissue and adjacent non-cancerous tissues of 50 patients with primary HCC surgery were obtained from the clinical sample bank of the First Affiliated Hospital of Zhengzhou University. The collection of human specimens was approved by the Biomedical Ethics Committee of the First Affiliated Hospital of Zhengzhou University. Patients had signed informed consent. The standard was to perform a curative hepatectomy between 201602-201806. HCC was diagnosed pathologically by two senior

pathologists and no adjuvant treatment was performed before the operation. All specimens were collected, and were stored at -80 °C.

2.2 Cell culture and transfection

Human liver cancer cell lines (HepG2, MHCC97H, SK-Hep1, HUH7, HepG2, LO2) were obtained from the cell bank of the Chinese Academy of Sciences (Shanghai, China). HCCLM3, MHCC97H and cells LO2 were obtained from Fenghuishengwu(Wuhan,China).The cells were cultured in RPMI 1640 medium supplemented with 10% FBS. All cells were incubated at 37 ° C in a cell incubator containing 5% CO₂.

Lipofectamine 2000 (Invitrogen, Waltham, MA, USA) was used to transfect si-CASC15, sh-CASC15,miR-2355-5p mimics, miR-2355-5p agomir and corresponding controls (GenePharma, Shanghai, China) into cells. The human cDNA sequence of CASC15 was cloned into the pcD-ciR vector to construct CASC15 over-expression plasmid. The vector was purchased from Genesee Biotech Co., Ltd. (Guangzhou, China), and oligonucleotides were transfected into cells by Lipofectamine 2000 (Invitrogen, Carlsbad, CA).

2.3 Cell proliferation detection

Cells were seeded in a 96-well plate at a density of 5,000 cells per well. 100 µLCCK8 solution (Liji, Shanghai, China) was added. After 4 h, the absorbance value was analyzed by a microplate reader (Bio Tek Instruments, USA) at 450nm.

2.4 EdU staining

HCC cells concentration were adjusted to 1×10^5 / ml, and inoculated in a 96-well for 24 hours. After culture treatment, EdU with a final concentration of 50 µmol / L was added and the cells were incubated for 2 h. Then 4% polyoxymethylene fixing solution was added for 30min, 0.5% Triton X-100 was added for 20min, 100 µl of Apollo reaction solution was add to each well to incubate for 30min, and Hoechst33342 staining solution was added for 30min. The cells were observed under fluorescence microscope.

2.5 Colony formation

The cells were made into a 1000 cell / mL cell suspension, and placed in a 6-well culture plate. The original culture solution was discarded after 48 hours of culture. Then the methanol fixing solution was taken out and fixed for 10 min. After repeated experiments, the colony formation rate was observed under a microscope. A cell cluster composed of more than 50 cells was counted as 1 colony.

2.6 Transwell analysis

Transwell chambers with 8 µm pores were purchased from Corning (Corning, NY). The transfected cells with a concentration of 1×10^6 cells in 100 µl were resuspended in RPMI 1640 medium without FBS and, afterward, seeded into the upper chambers of the 24-well plate. For another, the lower chambers were full of 600 µl RPMI 1640 medium added with 10% FBS. Cells were incubated for a whole night. Finally, cells

which migrated into the opposite side of the transwell membrane were fixed by methanol, stained using crystal violet, and counted under a light microscope. For the invasion assay, 30 µl Matrigel (BD Biosciences, Heidelberg, Germany) was used to cover the transwell membrane and proceeded similarly as described above.

2.7 Luciferase reporter assay

Total RNA was extracted from the cells, and CASC15 was amplified using WT and MUT primers. The fragment of CASC15 was inserted into the pGL3-Basic luciferase reporter vector named CASC15-WT-Luc. The mutant plasmid CASC15-Mut-Luc was formed by mutagenesis in the binding region of CASC15 and miR-2355-5p. CASC15-WT-Luc and CASC15-Mut-Luc were co-transfected into cells with miR-30a-5p mimic and pRLTK. After 6 h, Luciferase activity was analyzed by the DualLuciferase Reporter Assay Kit (Promega, Madison, WI).

2.8 Quantitative reverse transcription-PCR (qRT-PCR)

Total RNA in tissues and cells was extracted by TRIzol reagent (Biosntech, Beijing, China). SYBR-Green (Takara Biotechnology, Co., Lt. (Dalian, China) was used in qRT-PCR reaction. Amplification was performed by ABI 7,500 real-time PCR system. A qScript microRNA cDNA synthesis kit (Quantabio, Beverly, California, USA) was used for cDNA synthesis. The expression levels of RSU1P2 and miR-202-5p were analysed by the $2^{-\Delta\Delta CT}$ method. The expression levels of miRNA were standardized by U6. The expression levels of lncRNA were standardized by GAPDH. The primer sequences were as follows:

lncRNA CASC15: forward 5'- CTTATTCACTTGCCGGGAG-3',

lncRNA CASC15: reverse 5'- CGCTTAGATCCCAAGGG-3'.

miR-2355-5p: forward: 5'- CTGATTGTGAAGAGAATGT-3',

miR-2355-5p: reverse: 5'-GTTCTTCGACATCCGGGCCG-3'.

Six1: forward: 5'-CCACAGAATCCGCGAACCT-3',

Six1: reverse: 5'-GAGTTCATTACAGCGTTGGC-3'.

GAPDH: forward: 5'-CGAGAGAATCCGCGGACAT-3',

GAPDH: reverse: 5'-TTGTGCAATACAGCGTGGAC-3'.

U6: forward: 5'-GACAGATTCGGTCTGTGGCAC-3',

U6: reverse: 5'-GATTACCCGTCGGCCATCGATC-3'.

2.9 Western blot

The transfected cells were collected, the total proteins were extracted, and the protein concentration was quantified by the BCA Protein Assay Kit. Next, the loading buffer was added to the protein, and then 50 µg per well was loaded. Proteins were separated using 10% polyacrylamide gel electrophoresis, transferred to PVDF membrane, and blocked with 5% BSA for 1 h. Then the membrane was incubated with anti-Six1 (1: 1000, Amyjet, Wuhan, China), TGF- β (1: 1000, Amyjet, Wuhan, China) and anti-GAPDH The antibody (1: 1000, Amyjet, Wuhan, China) overnight. Then it was incubated with anti-rabbit secondary antibody (1: 1000, Amyjet, Wuhan, China) for 1 h [25].

2.10 In vivo tumor growth assay

To examine the effects of CASC15 and miR-2355-5p on the growth of HCC cells in vivo, sh-CASC15 or miR-2355-5p inhibitor were stably transfected into luciferase-labeled HCC cells. Tumors were inoculated into nude mice. A total of 3×10^6 transfected HCC cells were subcutaneously injected into six-week-old male nude mice ($n = 5$ per group). The tumor volume was measured with a caliper. 4 weeks after tumor formation, euthanize nude mice, remove tumor samples, collected tumors in mice and measure tumor weight. After 4 weeks of tumor formation, the nude mice were euthanized, and tumor samples were taken out to collect tumor and measure tumor weight. The experiment was approved by the First Affiliated Hospital of Zhengzhou University.

2.11 Statistical methods

The monitoring data were analyzed by SPSS19.0 statistical software. The results of data analysis were represented as mean $\pm$ standard deviation (mean $\pm$ SD). Multigroup data analysis was founded on one-way ANOVA. LSD test was used for subsequent analysis. $P < 0.05$ indicated significant difference.

Results

3.1 LncRNA CASC15 was significantly overexpressed and predicted poor prognosis in HCC

The expression level of CASC15 in liver cancer tissues was significantly raised contrasted with the normal group ($P < 0.05$, Fig.1A) ($n = 50$). And the results showed that CASC15 expression levels were highest in most HCC tissues (Fig. 1B, $P < 0.05$) ($n = 50$). Contrasted with LO2 cells, the expression levels of CASC15 in HCC cell lines (PLC, HepG2, HCCLM3, SK-Hep1, Huh7 and Hep3B) were significantly increased (Figure 1C, $P < 0.05$). In addition, HCC tissues were divided into two groups based on the average expression level of CASC15. The overall survival was significantly reduced in the CASC15 overexpression group ($P < 0.05$, Fig.1D). These data suggested a potential carcinogenic effect of CASC15 in HCC.

3.2 Silencing of LncRNA CASC15 inhibited HCC growth in vitro

Contrasted with the si-NC group, the expression level of CASC15 in the si-CASC15 group in Huh7 and HepG2 cells were significantly reduced ($P < 0.05$), indicating successful transfection (Fig.2A). And si-CASC15 significantly reduced the viability of Huh7 and HepG2 cells ($P < 0.05$, Fig.2B-2D). Transwell

results showed that si-CASC15 was significantly inhibited invasion and migration of Huh7 and HepG2 cells ($P < 0.05$, Fig.2E). The results showed that si-CASC15 inhibited HCC cell growth, invasion and apoptosis.

3.3 LncRNA CASC15 served as a sponge of miR-2355-5p

Next, the potential mechanism of CASC15 regulating HCC progress was explored. MiR-2355-5p was identified as the potential target of CASC15 through online prediction tool Starbase v2.0 (<http://starbase.sysu.edu.cn>) (Fig.3A). Contrasted with the miR-NC group, the expression level of miR-2355-5p in the miR-2355-5p mimic group was significantly raised ($P < 0.05$), indicating successful transfection (Fig.3B). The luciferase reporter assay showed that miR-2355-5p mimic and CASC15-WT co-transfected cells had significantly reduced luciferase activity ($P < 0.05$), but there was no significant change in luciferase activity of CASC15-MUT (Fig.3C). Contrasted with the si-NC group, the expression level of miR-2355-5p in the siCASC15 group was significantly increased ($P < 0.05$). The expression level of miR-2355-5p was significantly reduced in the CASC15 group Fig.3B the NC group ($P < 0.05$, Fig.3D). Contrasted with the Normal group, the expression level of miR-2355-5p was significantly reduced in liver cancer tissues ($P < 0.05$, Fig.3E) ($n = 50$). The expression of CASC15 in HCC tissues was negatively correlated with the expression of miR-2355-5p ($R^2 = 0.572$, Fig.3F).

3.4 miR-2355-5p knockdown effectively reversed si-CASC15-induced inhibition of HCC progression migration and invasion

Next, whether CASC15 regulated HCC progress through miR-2355-5p was analyzed. Contrasted with the si-NC group, si-CASC15 inhibited the proliferation of HCC cells, and the miR-2355-5p inhibitor induced cell proliferation ($P < 0.05$), while co-transfection of si-CASC15 with miR-2355-5p inhibitor eliminated the effect of si-CASC15 on cell proliferation ($P < 0.05$, Fig.4A-4C). As shown in Fig.4D, si-CASC15 inhibited the migration and invasion of HCC cells, and the miR-2355-5p inhibitor was able to induce cell migration and invasion ($P < 0.05$), while the co-transfection of si-CASC15 with miR-2355-5p inhibitor eliminated the the effect of si-CASC15 on cell migration and invasion ($P < 0.05$). These results demonstrated that CASC15 had a biological effect on HCC cells through miR-2355-5p ($P < 0.05$).

3.5 LncRNA CASC15circNASP sponged and sequestered miR-2355-5p to upregulate Six1 expression

We predicted with online prediction tool Starbase v2.0 and Six1 was identified as a potential target of miR-2355-5p (Fig.5A). Luciferase activity in the cells co-transfected with miR-2355-5p mimic and Six1-WT was significantly reduced ($P < 0.05$), but the luciferase activity of Six1-MUT did not change significantly (Fig.5B). Contrasted with the miR-NC group, the mRNA and protein expression levels of Six1 in the miR-30a-5p inhibitor group were significantly reduced ($P < 0.05$), and the mRNA and protein expression levels of Six1 in the miR-30a-5p inhibitor group were significantly reduced ($P < 0.05$). The mRNA and protein expression levels of Six1 were significantly raised in the Six1 overexpression group, while co-transfection of miR-30a-5p inhibitor with Six1 reversed the effect of miR-30a-5p inhibitor on Six1 expression level ($P < 0.05$, Fig.5C and 5D). Contrasted with the normal group, the expression level of Six1 was significantly

increased in liver cancer tissues ($P < 0.05$, Fig.5E) ($n = 50$). The expression of Six1 in HCC tissues was negatively correlated to that of miR-30a-5p ($R^2 = 0.614$, Fig.5F). These data demonstrated that CASC15 enhanced the level of Six1 by regulating miR-2355-5p.

3.6 The effects of miR-2355-5p mimic on cell growth, invasion and migration was mediated by Six1

Contrasted with the si-NC group, miR-2355-5p mimic significantly reduced cell viability ($P < 0.05$), while co-transfection of miR-2355-5p mimic with pcDNA-Six1 reversed the effect of miR-2355-5p mimic on cell proliferation ($P < 0.05$, Fig.6A-6C). And miR-2355-5p mimic significantly reduced cell invasion and migration ($P < 0.05$), while co-transfection of miR-2355-5p mimic and pcDNA- Six1 was able to reverse the effect of miR-2355-5p mimic on cell invasion and migration (Fig.6D, $P < 0.05$).

3.7 LncRNA CASC15circNASP - miR-2355-5p axis regulated glioma cell tumor growth in vivo

Finally, whether CASC15 knockdown can inhibit HCC progression in vivo was analyzed. As shown in Fig.7A-7C, compared to the Ctrl group, shCASC15 significantly reduced tumor growth rate, tumor volume and weight ($P < 0.05$), and miR-2355-5p inhibitor significantly raised tumor growth rate, tumor volume and weight ($P < 0.05$), while co-transfection of shCASC15 with miR-2355-5p inhibitor reversed the effect of shCASC15 on tumor growth. As shown in Fig.7D, shCASC15 significantly reduced the protein expression level of Six1 ($P < 0.05$), and miR-2355-5p inhibitor significantly increased the protein expression level of Six1 ($P < 0.05$), while co-transfection of shCASC15 with miR-2355-5p inhibitor reversed the effect of shCASC15 on Six1 protein expression level ($P < 0.05$).

Discussion

The incidence of liver cancer ranks fifth among male malignancies, and the mortality rate ranks second. Among women, its incidence is ninth among all malignant tumors [26]. Although the diagnosis and treatment technology of liver cancer is constantly updated, the survival prognosis of patients with HCC is still poor[27]. At present, studies have confirmed many risk factors play a key role the progress of HCC, including alcoholic cirrhosis, excessive intake of hepatotoxic substances, nonalcoholic liver disease (NAFLD) and genetic factors[28]. Among them, the study of the molecular biological mechanism related to liver cancer can more clearly reveal the root cause of the formation and development of liver cancer, and can also design targeted drugs for clinical use according to the corresponding key regulatory factors, so it is of great significance for the early diagnosis and prevention of liver cancer.

At present, LncRNA is a research hotspot in the field of tumors[29]. It has been found that there are a wide range of LNC RNAs disorders in HCC, which can play a regulatory role in tumor formation through a variety of ways, thus affecting the occurrence, metastasis and prognosis of HCC[30]. For example, studies have found that the expression of LncRNA MEG-3 in liver cancer tissues is down-regulated and over-expression of LncRNA MEG-3 inhibited the proliferation of Huh7 cells by negatively regulating miRNA-664, thus reducing the growth and invasion of tumors in the in-situ HCC model[31]. Other studies have also confirmed that HULC can advance the proliferation of HCC cells and is positively related to the

invasion of liver cancer cells[32]. LncRNA CASC15 has been found abnormally expressed in a variety of malignant tumors[33]. This study found that the expression level of CASC15 in HCC was increased. SiCASC15 inhibited tumor proliferation, migration, invasion and the growth of tumors in mice. Therefore, siCASC15 can control the development of HCC.

MiRNAs and lncRNAs can interact with each other and affect the behavior of cells. This interaction has become a relatively popular research area in recent years[34]. MiRNA is closely related to hepatocyte proliferation, cytoskeleton rearrangement and apoptosis, and then induces a series of pathological and physiological changes in the body[35]. Experimental evidence shows that mi RNAs can be used as oncogenes or tumor suppressor genes in HCC[36]. For example, miR-21 is used as a cancer-promoting factor in HCC, which is highly expressed in HCC and induces cell proliferation, invasion and chemotherapy resistance[37]. In contrast, miR-122, as a tumor suppressor, is lowly expressed in HCC[38]. MiR-2355-5p is a kind of miRNA recently discovered, which has abnormal expression in many malignant diseases. For example, miR-2355-5p has a negative regulatory effect on ERFFI1 and can inhibit the inhibitory effect of ERFFI1 on NP cell inflammation[39]. In this study, we screened miR-2355-5p as a target for CASC15. The expression level of miR-2355-5p in HCC tissues was reduced. The expression of CASC15 and miR-2355-5p were negatively correlated. In addition, miR-2355-5p inhibitor significantly induced tumor proliferation, migration and invasion. And co-transfection of miR-2355-5p inhibitor with si-CASC15 reversed the effects of si-CASC15 on cell proliferation, migration, invasion and tumor growth. These results suggested that CASC15 can demonstrate the growth of HCC by miR-2355-5p.

Six1 is involved in many biological processes as a transcription factor, including cancer. Six1 expression has been detected in a variety of tumor cell lines and tumor tissues, indicating that Six1 is essential for tumor cell proliferation and growth[40]. The abnormal increase of Six1 was found in many cancer, such as esophageal squamous cell carcinoma and pancreatic cancer[41]. Studies have shown that Six1 helps tumorigenesis, and its increased expression promotes angiogenesis, invasion and metastasis[42]. In pancreatic cancer cells, down-regulation of Six1 expression reduces MMP-2, MMP-9 and VEGF expression, thereby inhibiting angiogenesis, invasion and migration[43]. In addition, studies have reported that Six1's function in tumorigenesis can be regulated by miRNAs. For example, down-regulated miR-370 promotes proliferation and cell aging by up-regulating Six1 in acute myeloid leukemia (AML)[44]. Studies have shown that Six1 plays a key role of advanced HCC[45]. This study found that Six1 was a target gene of miR-2355-5p. The expression level of Six1 in HCC tissues was raised. The expression of Six1 and miR-2355-5p was negatively correlated. Co-transfection of miR-2355-5p mimic with pcDNA-Six1 reversed the effect of miR-2355-5p mimic on cell proliferation, migration, and invasion. And miR-2355-5p inhibitor raised the expression level of Six1, and co-transfection of shCASC15 with miR-2355-5p inhibitor reversed the effect of shCASC15 on the expression level of Six1.

Conclusion

CASC15 promoted the proliferation and invasion of HCC by Six1 through miR-2355-5p, suggesting that CASC15 may be a potential oncogene of HCC.

Abbreviations

HCC

Hepatocellular carcinoma

ncRNA

non-coding RNA

LncRNA

long-chain non coding RNA

SIX1

Sineoculis homeobox homolog 1

NAFLD

Nonalcoholic liver disease

Declarations

Author contribution statement

CS and TQ conceived and designed research. XX and XZ conducted experiments and contributed new reagents or analytical tools. QF, JY and MX analyzed data. HH wrote the manuscript. All authors read and approved the manuscript.

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Availability of data and materials

The data appeared in this article are already publicly available in the literature.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Ethical clearance was obtained through the Ethics Review Committee, Shanghai Eastern Hepatobiliary Surgery Hospital and Prevention the informed consent was obtained from all participants. Data collected

from participants were kept confidential and were accessible only to the researchers.

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Figures

Figure 1

LncRNA CASC15 expression levels in HCC tissues and cell lines. (A, B) Detection of CASC15 expression levels in liver cancer tissues and paired normal tissue (n = 50). (C) CASC15 expression level in HCC cell lines. (D) Kaplan-Meier survival analysis of CASC15 expression. n=3. ** P <0.01, *** P <0.001.

Figure 2

LncRNA CASC15 knockdown inhibited the growth of HCC cells. (A) CASC15 mRNA expression levels transfected with siRNA CASC15. (B, C) The effect of CASC15 on cell proliferation. (C) The effect of CASC15 on the number of cell colonies. (D) Edu staining was used to analyze the effect of CASC15 on cell proliferation. (E) The effect of CASC15 on cell migration and invasion. scale bar = 20μb. n=3 ** p < 0.01 and *** p < 0.001.

Figure 3

miR-2355-5p was a target of CASC15. (A) Targeting sites for CASC15 and miR-2355-5p are predicted by StarBase v. 2.0. (B) mRNA expression level of miR-2355-5p. (C) Analysis of luciferase activity in HCC cells co-transfected with miR-2355-5p mimic and CASC15-WT or CASC15-Mut vector. (D) mRNA expression level of miR-2355-5p. (E) Expression level of miR-202-5p in HCC tissues and adjacent normal tissues (n = 50). (F) Pearson correlation analysis of CASC15 and miR-202-5p in HCC tissues (n = 50) ($r = -0.641$, $P < 0.01$). n=3. ** $P < 0.01$, *** $P < 0.001$.

Figure 4

miR-2355-5p knockdown reversed the inhibitory effect of si-CASC15 on HCC. (A) CCK-8 analysis of cells co-transfected with si-CASC15 or miR-2355-5p inhibitor. (B) Colony formation assay of cells co-transfected with si-CASC15 or miR-2355-5p inhibitor. (C) Edu analysis of cells co-transfected with si-CASC15 or miR-2355-5p inhibitor. scale bar = 50µm. (D) Transwell assay of cells co-transfected with si-CASC15 or miR-2355-5p inhibitors. Scale bar = 20 µm. n=3. * Vs si-NC group, #vs si- RSU1P2 group. ** P < 0.01, #P < 0.05.

Figure 5

LncRNA CASC15 up-regulated Six1 expression through miR-2355-5p. (A) Six1 was predicted to be a target gene of miR-2355-5p. (B) Luciferase activity analysis in HCC cells co-transfected with Six1-WT or Six1-Mut vectors. (C) mRNA expression level of Six1. (D) Protein expression level of Six1. (E) Expression level of Six1 in HCC tissues. (F) Pearson correlation analysis of Six1 and miR-2355-5p in HCC tissues. (n = 50). N=3. * vs miR-NC group, #vs miR-2355-5p inhibitor group. ** P < 0.01, #P < 0.05.

Figure 6

The effects of miR-2355-5p mimics on cell growth are mediated by Six1. (A) CCK-8 analysis in cells co-transfected with miR-2355-5p mimics or pcDNA-6-Si1. (B) Colony formation assay of cells co-transfected with miR-2355-5p mimic or pcDNA-Six1. (C) Edu assay of cells co-transfected with miR-2355-5p mimic or pcDNA-Six1 (D) Cell invasion and migration of cells co-transfected with miR-2355-5p mimic or pcDNA-Six1. Scale bar = 20 μ m. n=3. * Vs si-NC group, #vs miR-2355-5p mimic group. ** P < 0.01, #P < 0.05.

Figure 7

Effect of lncRNA CASC15 on the growth of HCC tumor cells in vivo. (A) Measurement of tumor volume in nude mice weekly. (B) Comparison of tumor weight in nude mice. (C) Representative images of three groups of subcutaneous tumors. (D) Six1 protein expression level in tumor tissues. * $P < 0.05$, ** $P < 0.01$, $n = 5$.