European Review for Medical and Pharmacological Sciences 2018; 22: 6784-6792

lncRNA HOXB-AS3 promotes hepatoma by inhibiting p53 expression

X.-M. ZHANG[1,2], H. CHEN[1], B. ZHOU[1], Q.-Y. ZHANG[3], Y. LIAO[1], J.-S. WANG[1], Z.-H. WANG[1]

[1]Department of Hepatobiliary Surgery, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou, China
[2]The Second School of Medicine, Wenzhou Medical University, Wenzhou, China
[3]Department of Hepatobiliary Surgery, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China

Abstract. – OBJECTIVE: We investigate the mechanism of HOXB-AS3 in promoting the development of hepatocellular carcinoma.

PATIENTS AND METHODS: The expression of HOXB-AS3 in tumor tissues and adjacent tissues of hepatocellular carcinoma was detected by quantitative real time-polymerase chain reaction (qRT-PCR), and the relationship between the expression of HOXB-AS3 and tumor tissues was analyzed. The effects of HOXB-AS3 and p53 on cell proliferation, cell cycle and apoptosis were detected by plate cloning experiment and flow cytometry. The binding relationship between HOXB-AS3 and DNMT1 and the regulation mechanism of DNMT1 on p53 were tested by RNA immunoprecipitation (RIP) and chromatin immunoprecipitation (ChIP) experiments, respectively. Western blot was used to detect the expression of p53 after knockdown of HOXB-AS3. The torsion experiment was performed to assess whether HOXB-AS3 regulated the proliferation and apoptosis of hepatoma cells by inhibiting p53 expression.

RESULTS: The results of qRT-PCR showed that the expression of HOXB-AS3 was significantly higher in cancerous tissues of patients with hepatocellular carcinoma than in adjacent tissues. The expression of HOXB-AS3 in patients in stage III and IV was significantly higher than that in stage I and II. Inhibition of HOXB-AS3 expression in liver cancer cells including Hep3B and LM3 could promote cell proliferation, inhibit cell apoptosis and induce cell cycle arrest in the G0/G1 phase. The results of RIP and ChIP experiments showed that HOXB-AS3 inhibited the expression of p53 by binding to DNMT1, and overexpression of p53 in Hep3B cells could partially reverse the changes in cell proliferation and apoptosis induced by HOXB-AS3.

CONCLUSIONS: Highly expressed HOXB-AS3 was confirmed to promote the proliferation of hepatocellular carcinoma cells and inhibit apoptosis, and the mechanism was related to the regulation role of HOXB-AS3 in p53 expression by binding to DNMT1.

Key Words:
HOXB-AS3, Hepatocellular carcinoma, Cell proliferation, Apoptosis.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies. In recent years, it has become the fastest growing malignant tumor in China. Hepatocellular carcinoma usually progressed very rapidly, with a high degree of malignancy, poor prognosis, and a high mortality rate[1,2]. Cirrhosis and liver fibrosis caused by chronic hepatitis virus infection are considered to be the major risk factors for HCC at present. However, the exact pathogenic mechanism of HCC is still not fully elucidated. It may be a combination of genetic and environmental factors that lead to abnormal expression of key genes. It also involves a number of signal transduction disorders in the process of cell growth, differentiation and apoptosis, eventually leading to the malignant transformation of hepatocytes[3,4]. Therefore, it has great clinical value to thoroughly explore the genes involved in the pathogenesis of HCC.

Long non-coding RNAs (lncRNAs) are endogenous RNAs that cannot encode protein, which account for about 80% of ncRNAs[5,6]. Their transcripts are more than 200 nt in length and the tail often has a polyA structure. Current studies have demostrated that lncRNAs can participate in cell physiological activities through various ways such as modification of chromosomes, shear splicing, transcriptional activation, or protein degradation and translation regulated

Corresponding Author: Zhaohong Wang, MD; e-mail: wangzhaohong55@163.com

by mRNA. The abnormal expression of lncRNAs was closely related to pathogenesis, progression and prognosis of multiple diseases such as liver cancer, gastric cancer, prostate cancer, breast cancer, and glioma[7-10]. lncRNA HOXB-AS3 has been confirmed to have a crucial role in multiple tumors, but its mechanism in the pathogenesis and progression of hepatocellular carcinoma remains to be studied.

At present, p53 is one of the most deeply studied genes that has been discovered. The function of p53 is closely related to tumorigenesis, tumor cell growth and apoptosis. It can mediate cell growth at DNA replication level and involve in a variety of cell signal pathways in the process of cell growth, differentiation, deterioration and apoptosis. P53 can induce cell apoptosis by transcription-dependent or non-dependent manner mediated by the mitochondrial FAS pathway or KILLER/DR5 pathway[11-13]. DNA methyltransferases (DNMTs) are an important family of enzymes that catalyze and maintain DNA methylation in epigenetics. They are divided into three families in mammals: DNMT1, DNMT2 and DNMT3[14, 15], among which DNMT1 is the key enzyme for DNA replication repair and maintenance of its normal methylation. Researches have demonstrated that DNMT1 was associated with abnormal methylation of DNA, and both of them are closely related to the occurrence and development of tumors.

The aim of this study was to elucidate the effect of HOXB-AS3 on the proliferation and apoptosis of hepatocellular carcinoma cells, and to verify whether HOXB-AS3 can bind to DNMT1 and inhibit the expression of p53.

Patients and Methods

Research Objects and Sample Collection

A total of 36 fresh liver cancer tissues and normal adjacent tissues were collected from the patients diagnosed with liver cancer and undergoing surgical treatment in The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University from July 2012 to August 2017. All patients did not receive any treatment before surgery and had no family history. They were diagnosed with liver cancer by pathological examination. All patients voluntarily participated in the study and signed written informed consent. This study has been approved by the Ethics Committee of the Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University. After radical surgery, the collected specimens were stored in liquid nitrogen.

Cell Culture

Hepatoma cells including HepG, PLC, Hep3B and LM3 were all purchased from American Type Culture Collection (ATCC) (Manassas, VA, USA). The cells were seeded with Dulbecco's modified eagle medium (DMEM) medium (Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA) along with 1% penicillin and streptomycin, and were placed in a 37°C, 5% CO_2 incubator. The medium was changed every other day. Until grown to 70-80% confluency, the cells were digested with 0.25% trypsin and passaged at a ratio of 1:2 or 1:3.

Cell Transfection

The cells in the logarithmic growth phase with good viability were seeded in culture plate and then transfected with HOXB-AS3 siRNA, pcDNA-p53 and the negative controls according to Lipofectamine 2000 instructions. Both HOXB-AS3 siRNA and pcDNA-p53 were designed and synthesized by GenePharma (Shanghai, China). After 48 hours of transfection, cells were collected for other experiments.

RNA Extraction

The cells and tissues required for the experiment were collected, and were lysed by 1 mL of TRIzol (Invitrogen, Carlsbad, CA, USA). After 250 μL of chloroform was added, the cell precipitate were mixed for 30 s after shaking, and then centrifuged at 4°C. The aqueous phase was aspirated and an equal volume of pre-chilled isopropanol was added. After centrifugation, the precipitate was gently washed with 75% ethanol. Thus, the extracted RNA was dissolved in 20 μL of diethyl pyrocarbonate (DEPC) water. After the concentration was measured using a spectrophotometer, the RNA were placed in a refrigerator at -80°C until use.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

The reverse transcription system was prepared on ice using the kit PrimeScript RT reagent Kit (TaKaRa, Otsu, Shiga, Japan). Complementary Deoxyribose Nucleic Acid (cDNA) was obtained when the reaction completed. PCR

amplification conditions were: pre-denaturation at 94°C for 5 min, followed by 40 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min and 30 s. The primer sequences were: HOXB-AS3 (F: 5'-CCCTCCAAGTCCAGTAAGAAGT-3', R: 5'-AGATCCTAAGAGGTGCGAGTTTA-3'), p15 (F: 5'-CGGCGGTCAACCTGGAGGACTCC-3', R: 5'-CCAGTGCAGGGTCCGAGGTAT -3'), p21 (F: 5'-CGACGCGTCGTTGTAATAAAGCCTCCAG-3', R: 5'- GACTAGTCGTTTTCATTTCAATCGTAG-3'), p27 (F: 5'- TGGAAGACTAGTGATTTTGTTGT-3', R:5'-TACTGGCACCACTGGAAACC-3'), p53 (F:5'-GGACTCTGCCCTGCCACCATTTA-3', R: 5'-CTTGTGCCCTGTGAGGTCGTTGA-3'), DNMT1 (F: 5'-TGGGAACTATATCTCTCGCTTGC-3', R: 5'-GGGTGAGACAGAACCGTCT-3'), GAPDH (F: 5'-AGCCACATCGCTCAGACAC-3', R: 5'-GCCCAATACGACCAAATCC-3'), U6 (F: 5'-CTCGCTTCGGCAGCAGCACATATA-3', R: 5'-AAATATGGAACGCTTCACGA 3').

Plate Cloning Experiments

The Hep3B and LM3 cells in logarithmic growth phase were collected. The cell suspension concentration was adjusted to 1×10^4 cells/mL, and 2000 cells were seeded in a six-well plate. After culturing for 3 to 4 days in a 37°C incubator, 4% formaldehyde was used for fixation for 30 min. After stained with 0.1% crystal violet for 30 min, the cloned cells were counted and photographed.

Cell Apoptosis

The cells were digested with pancreatin containing no Ethylene Diamine Tetraacetic Acid (EDTA), and 1×10^5 cells/mL of suspended cells were collected. 100 μL of 1 × Annexin buffer was added to suspend cells and Annexin V-FITC was used for marking. 5 μL of Annexin V and 1 μL of Propidium Iodide (PI) were added to the cells for staining, then mixed and incubated for about 15 min at room temperature. 400 μL of 1× buffer was added to the cells and apoptosis was then detected.

Cell Cycle

The cells were collected and the concentration of each group was adjusted to 1×10^5/mL. After fixing with 1 mL of pre-chilled 75% ethanol, cells were placed in a refrigerator at 4°C overnight. The next day, the fixative was washed with phosphate-buffered saline (PBS) twice, and the supernatant was discarded. After adding 100 μL of RNaseA, the cells were placed in a 37°C water bath and protected from light for 30 min. The absorbance was measured at the excitation wavelength of 488 nm, and red fluorescence was used to detect the cell cycle by flow cytometry. The experiment was repeated three times.

Western Blot

The cell lysate containing the protease inhibitor phenylmethylsulfonyl fluoride (PMSF) was added into cells, which were scraped and lysed on ice. The lysate was aspirated and centrifuged at 12 000 r/min for 20 min. Then the supernatant was taken and the total protein concentration was measured by the bicinchoninic acid (BCA) method (Pierce, Rockford, IL, USA). 50 ug of total protein were taken from each sample and loaded on sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel for electrophoresis. Then the membranes were transferred and blocked in skimmed milk for 2 hours. After eluting with Tris-buffered saline and Tween (TBST) 6 times for 10 min each, the membrane was incubated with specific primary antibodies overnight at 4°C. On the next day, corresponding secondary antibody was used to incubate for 1h after the membrane was washed with TBST, and finally exposure was performed.

Nuclear Plasma Separation

After the number of cells reached to 1×10^6, 200 μL of Lysis Buffer J was added to the culture flask to lyse the cells. After centrifugation, cytoplasmic RNAs were contained in the supernatant, and the nuclear RNAs were contained in remaining liquid. Then the supernatant was transferred. Buffer SK and absolute ethanol were added to the liquid containing cytoplasmic RNA and nuclear RNA, respectively. At last, cytoplasmic RNAs and nuclear RNAs were eluted using column centrifugation.

RNA Binding Protein Immunoprecipitation (RIP)

After cell lysis, the detection antibody was added and the working concentration of the antibody was 8 μg per reaction system. After overnight incubation on a shaker at 4°C, the plate was warmed for 1 h at room temperature. Protein G beads were added to capture the complexes. After washing with the buffer, the RNAs were extracted. And RNA expression levels were detected by fluorescence quantitative PCR.

Chromatin Immunoprecipitation (ChIP)

After sonicated and cross-linked, the cells were incubated with DNMT1 antibody. After incubation overnight at 4°C in a shaker, the DNA was captured again and extracted. The binding of DNMT1 on the promoter region of p53 gene was detected by PCR.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 statistical software (IBM, Armonk, NY, USA) was used for statistical analysis. Measured data were expressed as mean±standard deviation. Two-sample *t*-test statistical methods were used for comparison between two groups. $p < 0.05$ was considered statistically significant. GraphPad Prism 7 software (La Jolla, CA, USA) was used to perform related statistical charting.

Results

High Expression of HOXB-AS3 in Hepatocellular Carcinoma

We detected the expression of HOXB-AS3 in hepatic carcinoma tumor tissues and corresponding paracancerous tissues of 36 cases by fluorescence quantitative real-time PCR. The results demonstrated that HOXB-AS3 was highly expressed in tumor tissues and was remarkably higher in advanced hepatocellular carcinoma tissues than in early ones (Figure 1A, 1B). Subsequently, we examined the expression of HOXB-AS3 in hepatocellular carcinoma cell lines including HepG, PLC, Hep3B and LM3. The results demonstrated that HOXB-AS3 was highly expressed in Hep3B and LM3 cells (Figure 1C). Three HOXB-AS3 siRNAs (si-HOXB-AS3 1#, si-HOXB-AS3 2#and si-HOXB-AS3

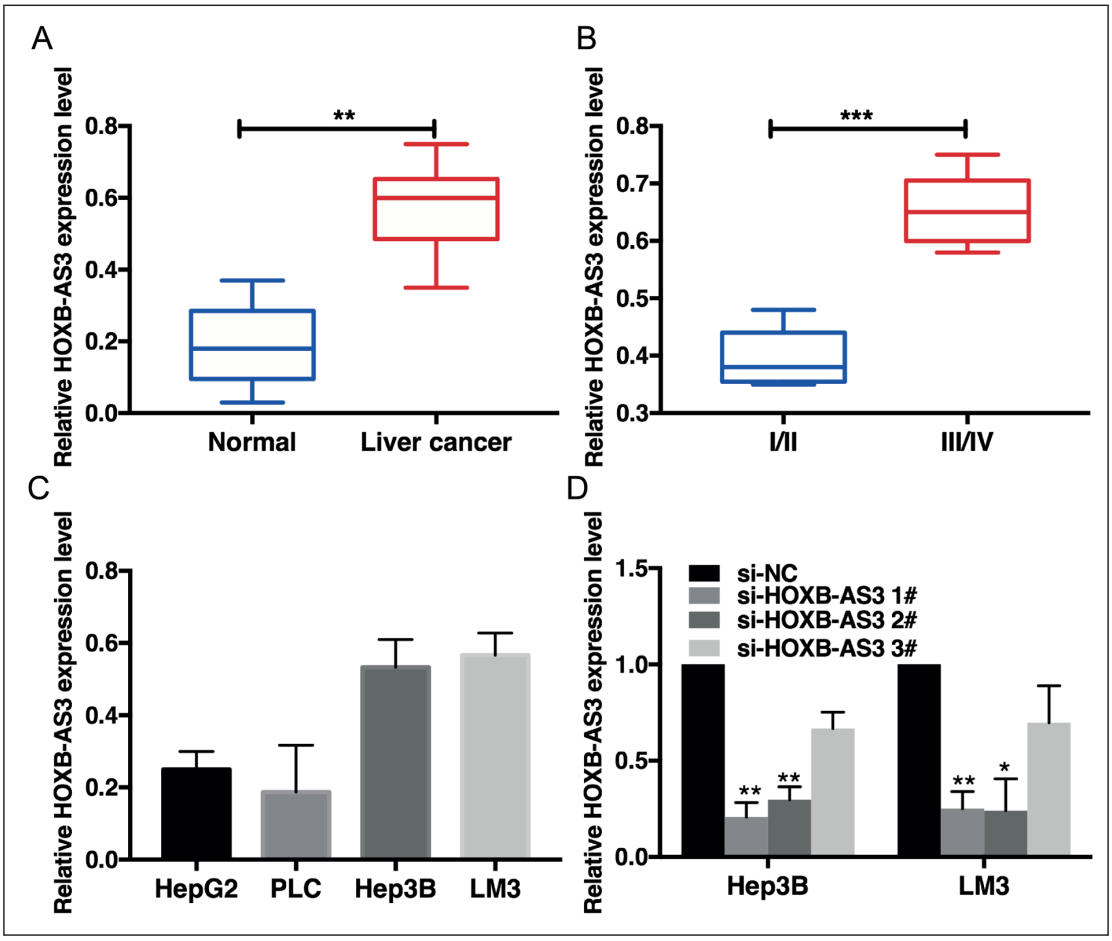

Figure 1. HOXB-AS3 was highly expressed in HCC tissues. ***A,*** HOXB-AS3 is highly expressed in hepatocellular carcinoma tissues. ***B,*** The expression of HOXB-AS3 was significantly higher in advanced hepatocellular carcinoma tissues than that of early stage. ***C,*** Expression of HOXB-AS3 in hepatoma cells, with higher expression in Hep3B and LM3. ***D,*** si-HOXB-AS3 1# and si-HOXB-AS3 2# were most efficient in down-regulating HOXB-AS3 expression.

3#) were transfected into the Hep3B and LM3 cell lines, respectively, and we found that both Hep3B and LM3 transfected with si-DUXAP10 1# and si-HOXB-AS3 2# could significantly inhibit the expression of HOXB-AS3 (Figure 1D). So si-DUXAP10 1# and si-HOXB-AS3 2 were selected for following study.

Interference with HOXB-AS3 Expression Inhibited the Hepatoma Cell Proliferation and Induced Apoptosis as well as Cell Cycle Arrest

We detected cell proliferation after knocking down HOXB-AS3 in Hep3B and LM3 cell lines. Plate cloning experiments demonstrated that knockdown of HOXB-AS3 markedly inhibited cell proliferation (Figure 2A). Subsequently, we examined the effect of HOXB-AS3 on apoptosis and cell cycle by flow cytometry. It was found that knockdown of HOXB-AS3 strikingly induced apoptosis of hepatoma cells (Figure 2B), leading to cell cycle arrest in the G0/G1 phase (Figure 2C).

HOXB-AS3 can Inhibit p53 Expression by Binding to DNMT1

We knocked down HOXB-AS3 in Hep3B and LM3 cell lines and detected the expression of tumor suppressor genes including p15, p21, p27, and p53 by qRT-PCR. The results showed that after HOXB-AS3 level decreased, p53 expression was strikingly up-regulated in both cell lines (Figure 3A, 3B). Western Blot results indicated that p53 protein levels reduced after knockdown of HOXB-AS3 in Hep3B and LM3 cell lines (Figure 3C). To further explore the regulatory mechanism of HOXB-AS3 on p53, we first detected the subcellular localization of HOXB-AS3 using a nucleoplasm separation assay. It was found that HOXB-AS3 mainly distributed in the nucleus

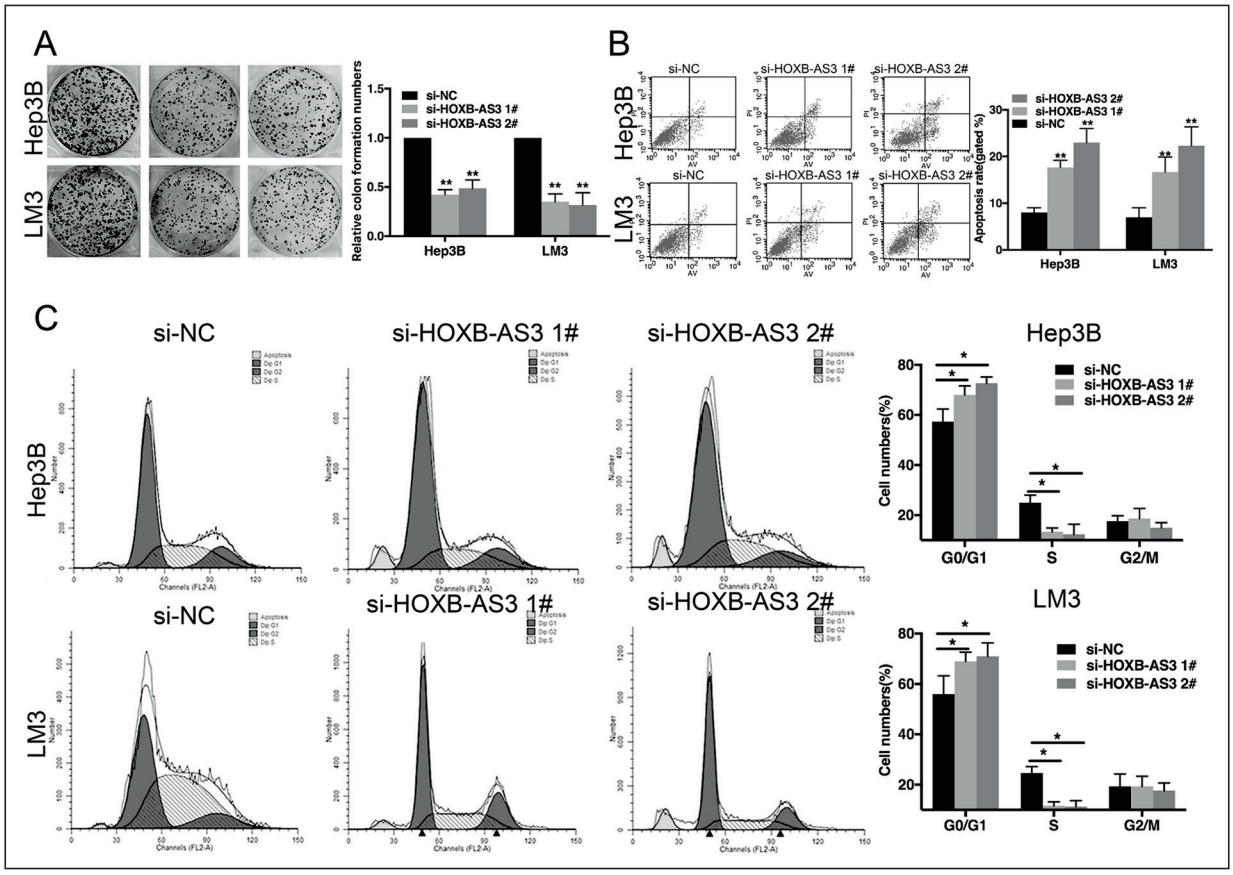

Figure 2. Interference with HOXB-AS3 inhibits hepatoma cells proliferation, induces apoptosis and cell arrests. ***A,*** After interference with HOXB-AS3, the proliferation of hepatoma cells were significantly downregulated. ***B,*** After interference with HOXB-AS3, the apoptosis of hepatoma cells were significantly increased. ***C,*** After cell interference with HOXB-AS3, the cell cycle arrested at G0/G1 phase.

(Figure 3D). This implied that HOXB-AS3 might play its regulatory role at transcriptional level. Previous studies have illustrated that DNMT1 can inhibit p53 expression by binding to the p53 promoter region[16,17]. Therefore, we wondered whether HOXB-AS3 could be stably expressed by binding to DNMT1 so as to decrease p53 expression. RIP and CHIP experiments were subsequently performed in the Hep3 cell line. The result of RIP experiment demonstrated that HOXB-AS3 could bind to DNMT1 (Figure 3E), and the CHIP experiment confirmed that DNMT1 could bind to the promoter region of p53 (Figure 3F). In addition, CHIP assay further confirmed that knocking down HOXB-AS3 in Hep3 cell line decreased the binding ability of DNMT1 to p53 promoter (Figure 3G). The interference of DNMT1 in the Hep3 cell line also markedly down-regulated the p53 protein expression (Figure 3H). These results indicated that HOXB-AS3 could inhibit p53 expression by binding to DNMT1.

P53 Reversed the Carcinogenesis of HOXB-AS3

P53 mRNA and protein expression were found significantly up-regulated after transfection with pcDNA-p53 in Hep3 cell lines (Figure 4A, 4B). We then examined the effect of p53 on the cell cycle by flow cytometry. The results demonstrated that overexpression of p53 induced cell cycle arrest in the G0/G1 phase (Figure 4C). After HOXB-AS3 was upregulated, we used CCK8 plate cloning experiment to test the cell proliferation ability; as a result, the cell proliferation ability was found significantly enhanced. When simultaneously overexpressing p53 and HOXB-AS3, the cell proliferation was partially reduced but was still higher than that in control group (Figure 4D). These results indicated that HOXB-AS3 could promote tumor cell proliferation by inhibiting the expression of p53.

Discussion

In recent years, long-chain non-coding RNA has become a hot topic as a new factor that affects tumors development. Many studies have shown that lncRNAs play an important role in the development of liver cancer. For example, lncRNA H19, which is a maternal specific expressed non-coding RNA[18], has a biphasic effect of promoting and inhibiting tumor. It was found that H19 can mediate the metastasis and malignant transformation of hepato-

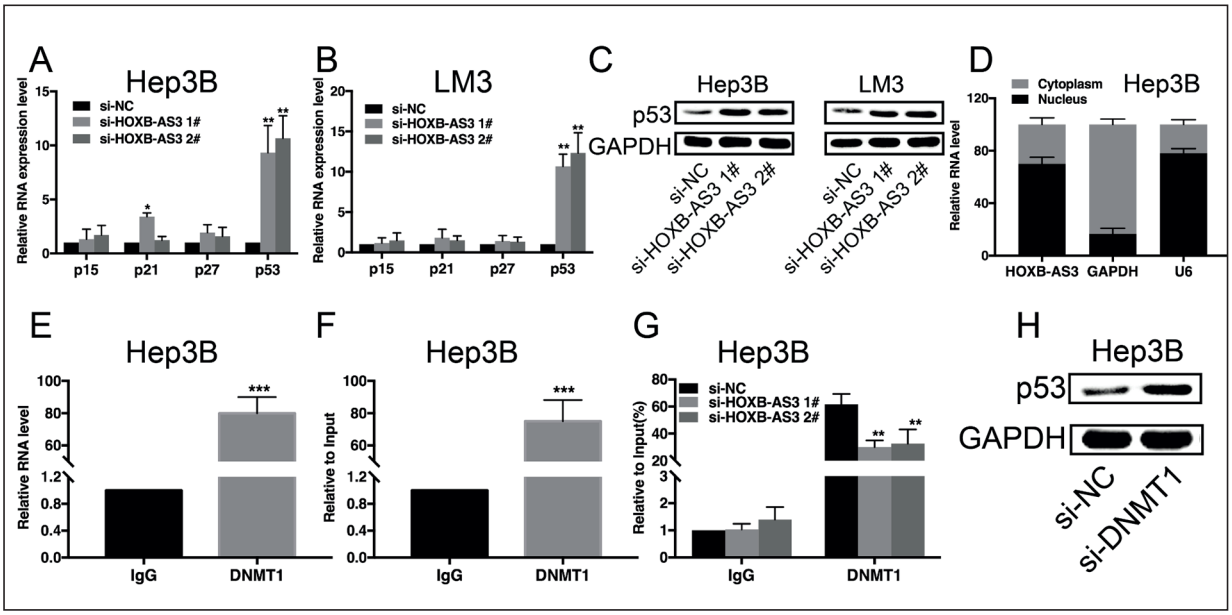

Figure 3. HOXB-AS3 inhibited p53 expression by binding to DNMT1. ***A, & B,*** After interference with HOXB-AS3, the p53 expression of Hep3B and LM3 cells were significantly up-regulated. ***C,*** After interference with HOXB-AS3, the expression of p53 protein of Hep3B and LM3 cells were significantly up-regulated. ***D,*** HOXB-AS3 was mainly distributed in the nucleus. ***E,*** HOXB-AS3 can bind to DNMT1 promotor. ***F,*** DNMT1 can bind to the p53 promoter region. ***G,*** After the interference with HOXB-AS3, the binding of DNMT1 to p53 promoter was downregulated. ***H,*** After the interference with HOXB-AS3, p53 expression was down-regulated.

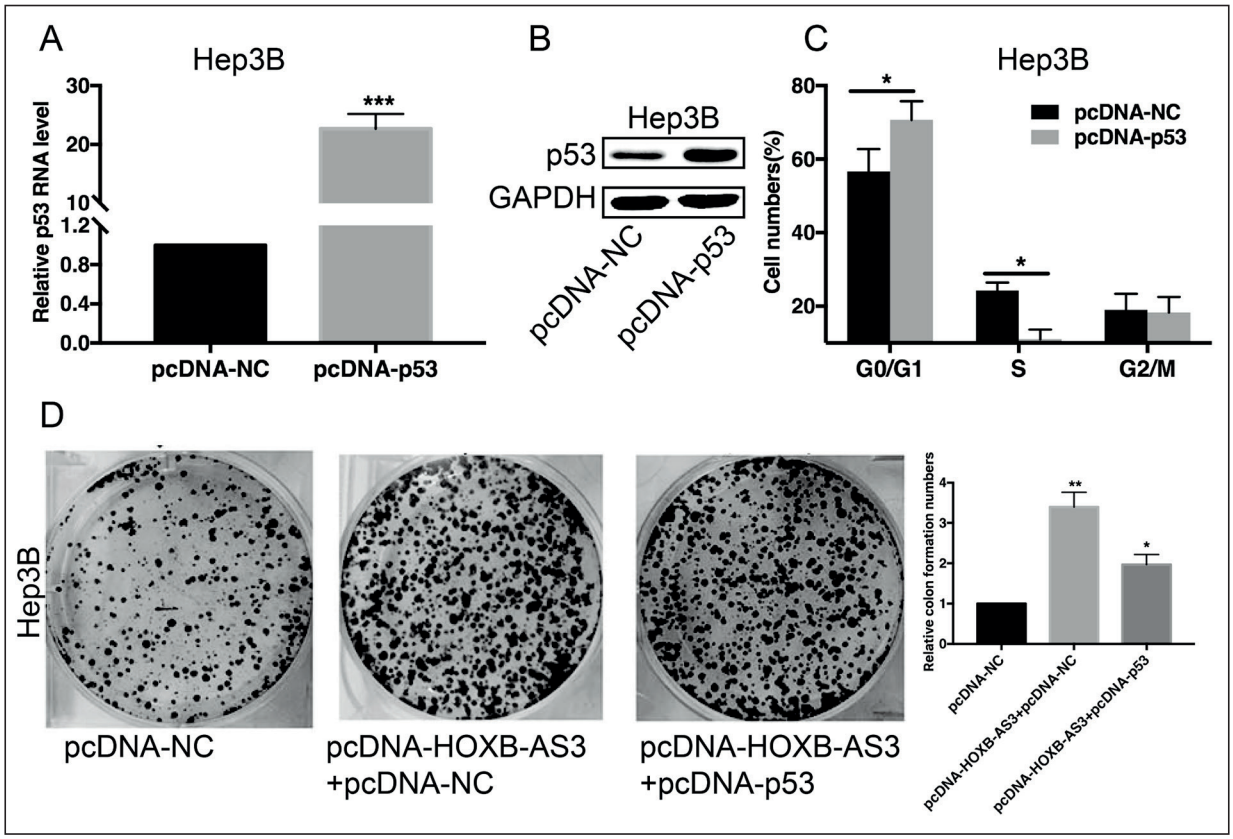

Figure 4. p53 can reverse the carcinogenic effects of HOXB-AS3. ***A,*** & ***B,*** After transfection of pcDNA-p53, p53 RNA (A) and protein (B) levels were significantly up-regulated. ***C,*** After overexpression of p53, the cell cycle arrested at G0/G1 phase. ***D,*** Overexpression of p53 can partially reverse the hepatoma cell proliferation promoted by HOXB-AS3.

ma cells through activating microRNA-200 family. H19 can also be used as a precursor molecule of miRNA-675 to downregulate the expression of the gene RB. And H19 can also act as a tumor suppressor in the mouse model[19-22]. In addition, high expression of lncRNA-HEIH in liver cancer is closely related to tumor recurrence and prognosis in hepatocellular carcinoma caused by hepatitis B virus (HBV). It acts as a proto-oncogene to promote the development of hepatocellular carcinoma[23]. The high expression transcript of liver cancer (HULC) can be combined with microRNA-372 to increase the expression of miR-372's target gene, PRKACB, thereby promoting the proliferation of hepatoma cells[24]. Previous studies have found that HOXB-AS3 was involved in the occurrence and development of colon cancer, and was abnormally expressed in many tumor cells, but its regulation mechanism in liver cancer has not been reported yet.

In this study, qRT-PCR was used to detect the expression of HOXB-AS3 in hepatocarcinoma tissues, corresponding paracancerous tissues and hepatoma cell lines. The results demonstrated that the expression of HOXB-AS3 was strikingly high in HCC tissues and hepatoma cells. We next explored the function of HOXB-AS3 in hepatoma cells and found that HOXB-AS3 could promote the proliferation of hepatoma cells and inhibit apoptosis.

Previous studies have illustrated that lncRNAs are able to exert regulation effect by inhibiting the expression of target genes. Based on this theory, we further found that HOXB-AS3 can exert its biological functions by inhibiting p53 expression. Additionally, the result of qRT-PCR and Western blot showed that HOXB-AS3 influenced the mRNA and protein expression of p53. Hence, we speculated that P53 might be a target gene of HOXB-AS3.

lncRNAs can participate in regulatory networks by binding to transcription factors. We, therefore, considered whether HOXB-AS3 can bind to a certain transcription factor and cause changes in

the expression of p53, thus leading to biological changes in HCC. RIP experiment was then performed to verifying our speculation. The results demonstrated that HOXB-AS3 did bind to DNMT1 and stabilize DNMT1 expression. DNMT1 is the most significant methyltransferase in the human body that can support the methylation of newly synthesized DNA strands[26]. In tumor cells, hypermethylation of tumor suppressor genes and abnormal proliferation along with differentiation of cells are all associated with increased DNMT1 activity. Studies have demonstrated that high expression of DNMT1 has a close relationship with the occurrence, drug resistance and prognosis of many diseases such as non-small cell lung cancer, leukemia and endometrial cancer[27-29]. We used CHIP experiments to verify that HOXB-AS3 could down-regulate the expression of target gene p53 by binding to DNMT1. This provided a reasonable explanation of how HOXB-AS3 led to biological changes in HCC.

In this study, we explored the relationship between HOXB-AS3, DNMT1 and p53, and clearly analyzed their functions and mechanisms. But this is only a small part in the study of lncRNA and cancer. We expect that such research on lncRNAs may become new breakthrough in cancer treatment in the future, thus providing new research ideas for the treatment and improvement of prognosis of HCC patients.

Conclusions

We demonstrated that HOXB-AS3 was strikingly down-regulated in HCC. Highly expressed HOXB-AS3 can promote the proliferation of HCC cells and inhibit apoptosis. The mechanism may be that HOXB-AS3 can inhibit p53 expression by binding to DNMT1.

Acknowledgements

This study was supported by Natural Science Fundation of Zhejiang Project, China (LQ18H290003).

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Stacy S, Hyder O, Cosgrove D, Herman JM, Kamel I, Geschwind JF, Gurakar A, Anders R, Cameron A, Pawlik TM. Patterns of consultation and treatment of patients with hepatocellular carcinoma presenting to a large academic medical center in the US. J Gastrointest Surg 2013; 17: 1600-1608.
2) Shariff MI, Cox IJ, Gomaa AI, Khan SA, Gedroyc W, Taylor-Robinson SD. Hepatocellular carcinoma: current trends in worldwide epidemiology, risk factors, diagnosis and therapeutics. Expert Rev Gastroenterol Hepatol 2009; 3: 353-367.
3) Ataide EC, Boin IF, Almeida JR, Seva-Pereira T, Stucchi RS, Cardoso AR, Caruy CA, Escanhoela CA. Prognostic factors for hepatocellular carcinoma recurrence: experience with 83 liver transplantation patients. Transplant Proc 2011; 43: 1362-1364.
4) Lee SC, Tan HT, Chung MC. Prognostic biomarkers for prediction of recurrence of hepatocellular carcinoma: current status and future prospects. World J Gastroenterol 2014; 20: 3112-3124.
5) Dinger ME, Pang KC, Mercer TR, Mattick JS. Differentiating protein-coding and noncoding rna: challenges and ambiguities. PLoS Comput Biol 2008; 4: e1000176.
6) Kapranov P, Cheng J, Dike S, Nix DA, Duttagupta R, Willingham AT, Stadler PF, Hertel J, Hackermuller J, Hofacker IL, Bell I, Cheung E, Drenkow J, Dumais E, Patel S, Helt G, Ganesh M, Ghosh S, Piccolboni A, Sementchenko V, Tammana H, Gingeras TR. Rna maps reveal new rna classes and a possible function for pervasive transcription. Science 2007; 316: 1484-1488.
7) Guttman M, Amit I, Garber M, French C, Lin MF, Feldser D, Huarte M, Zuk O, Carey BW, Cassady JP, Cabili MN, Jaenisch R, Mikkelsen TS, Jacks T, Hacohen N, Bernstein BE, Kellis M, Regev A, Rinn JL, Lander ES. Chromatin signature reveals over a thousand highly conserved large non-coding rnas in mammals. Nature 2009; 458: 223-227.
8) Su YJ, Yu J, Huang YQ, Yang J. Circulating long noncoding rna as a potential target for prostate cancer. Int J Mol Sci 2015; 16: 13322-13338.
9) Jiao ZY, Tian Q, Li N, Wang HB, Li KZ. Plasma long non-coding rnas (lncrnas) serve as potential biomarkers for predicting breast cancer. Eur Rev Med Pharmacol Sci 2018; 22: 1994-1999.
10) Patil VS, Zhou R, Rana TM. Gene regulation by non-coding rnas. Crit Rev Biochem Mol Biol 2014; 49: 16-32.
11) Green DR, Kroemer G. Cytoplasmic functions of the tumour suppressor p53. Nature 2009; 458: 1127-1130.
12) Munoz-Fontela C, Mandinova A, Aaronson SA, Lee SW. Emerging roles of p53 and other tumour-suppressor genes in immune regulation. Nat Rev Immunol 2016; 16: 741-750.
13) Bykov V, Eriksson SE, Bianchi J, Wiman KG. Targeting mutant p53 for efficient cancer therapy. Nat Rev Cancer 2018; 18: 89-102.
14) Cheng X, Blumenthal RM. Mammalian dna methyltransferases: a structural perspective. Structure 2008; 16: 341-350.

15) Hermann A, Gowher H, Jeltsch A. Biochemistry and biology of mammalian dna methyltransferases. Cell Mol Life Sci 2004; 61: 2571-2587.

16) Yang J, Platt LT, Maity B, Ahlers KE, Luo Z, Lin Z, Chakravarti B, Ibeawuchi SR, Askeland RW, Bondaruk J, Czerniak BA, Fisher RA. Rgs6 is an essential tumor suppressor that prevents bladder carcinogenesis by promoting p53 activation and dnmt1 downregulation. Oncotarget 2016; 7: 69159-69172.

17) Georgia S, Kanji M, Bhushan A. Dnmt1 represses p53 to maintain progenitor cell survival during pancreatic organogenesis. Genes Dev 2013; 27: 372-377.

18) Chandra A, Lan S, Zhu J, Siclari VA, Qin L. Epidermal growth factor receptor (egfr) signaling promotes proliferation and survival in osteoprogenitors by increasing early growth response 2 (egr2) expression. J Biol Chem 2013; 288: 20488-20498.

19) Yin P, Navarro A, Fang F, Xie A, Coon JS, Richardson C, Bulun SE. Early growth response-2 expression in uterine leiomyoma cells: regulation and function. Fertil Steril 2011; 96: 439-444.

20) To SQ, Simpson ER, Knower KC, Clyne CD. Involvement of early growth response factors in tnfalpha-induced aromatase expression in breast adipose. Breast Cancer Res Treat 2013; 138: 193-203.

21) Liu X, Shi H, Liu B, Li J, Liu Y, Yu B. Mir-330-3p controls cell proliferation by targeting early growth response 2 in non-small-cell lung cancer. Acta Biochim Biophys Sin (Shanghai) 2015; 47: 431-440.

22) Nafez S, Oikawa K, Odero GL, Sproule M, Ge N, Schapansky J, Abrenica B, Hatherell A, Cadonic C, Zhang S, Song X, Kauppinen T, Glazner GW, Grilli M, Czubryt MP, Eisenstat DD, Albensi BC. Early growth response 2 (egr-2) expression is triggered by nf-kappab activation. Mol Cell Neurosci 2015; 64: 95-103.

23) Zhang L, Yang F, Yuan JH, Yuan SX, Zhou WP, Huo XS, Xu D, Bi HS, Wang F, Sun SH. Epigenetic activation of the mir-200 family contributes to H19-mediated metastasis suppression in hepatocellular carcinoma. Carcinogenesis 2013; 34: 577-586.

24) Zhuo W, Ge W, Meng G, Jia S, Zhou X, Liu J. Microrna20a promotes the proliferation and cell cycle of human osteosarcoma cells by suppressing early growth response 2 expression. Mol Med Rep 2015; 12: 4989-4994.

25) Burns FR, Lanham KA, Xiong KM, Gooding AJ, Peterson RE, Heideman W. Analysis of the zebrafish sox9b promoter: identification of elements that recapitulate organ-specific expression of sox9b. Gene 2016; 578: 281-289.

26) Boland MJ, Christman JK. Characterization of dnmt3b:thymine-dna glycosylase interaction and stimulation of thymine glycosylase-mediated repair by dna methyltransferase(s) and rna. J Mol Biol 2008; 379: 492-504.

27) Xing J, Stewart DJ, Gu J, Lu C, Spitz MR, Wu X. Expression of methylation-related genes is associated with overall survival in patients with non-small cell lung cancer. Br J Cancer 2008; 98: 1716-1722.

28) Klisovic RB, Stock W, Cataland S, Klisovic MI, Liu S, Blum W, Green M, Odenike O, Godley L, Burgt JV, Van Laar E, Cullen M, Macleod AR, Besterman JM, Reid GK, Byrd JC, Marcucci G. A phase i biological study of mg98, an oligodeoxynucleotide antisense to dna methyltransferase 1, in patients with high-risk myelodysplasia and acute myeloid leukemia. Clin Cancer Res 2008; 14: 2444-2449.

29) Liao X, Siu MK, Chan KY, Wong ES, Ngan HY, Chan QK, Li AS, Khoo US, Cheung AN. Hypermethylation of ras effector related genes and dna methyltransferase 1 expression in endometrial carcinogenesis. Int J Cancer 2008; 123: 296-302.