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LncRNA TP73-AS1 promotes malignant progression of hepatoma by regulating microRNA-103

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Abstract. – OBJECTIVE: The aim of this study was to investigate long non-coding RNA (lncRNA) TP73-AS1 expression in hepatocellular carcinoma (HCC) tissues and cells, and to further investigate whether it can accelerate the progression of HCC by regulating microRNA-103.

PATIENTS AND METHODS: Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was performed to examine TP73-AS1 expression in 60 pairs of HCC tissues and adjacent ones, and the association between lncRNATP73-AS1 level and clinical indicators of HCC as well as patients' prognosis was analyzed. Meanwhile, qRT-PCR was used to further verify TP73-AS1 expression in HCC cell lines. The lncRNA TP73-AS1 knockdown model was constructed using lentivirus in the HCC cell lines, including Bel-7402 and HepG2. Cell counting kit-8 (CCK-8), 5-Ethynyl-2'-deoxyuridine (EdU), and flow cytometry assays were performed to figure out the influence of TP73-AS1 on the basic biological function of the HCC cells. Finally, the involved potential regulatory mechanism was explored using cell recovery experiments, and the relationship between TP73-AS1 and microRNA-103 was further studied.

RESULTS: QRT-PCR results indicated that TP73-AS1 expression in HCC samples was conspicuously enhanced compared with paracancerous tissues, and patients with a relatively high level of TP73-AS1 had a higher tumor stage and a lower overall survival rate. Meanwhile, the proliferation ability of cells in the sh-TP73-AS1 group was strikingly lower than that in the control group, while cell apoptosis showed the opposite trend. Besides, qRT-PCR results indicated a negative correlation between microRNA-103 and TP73-AS1 in HCC tissue specimens. The results of the luciferase reporting assay revealed that TP73-AS1 could be targeted by microRNA-103 through binding site. In addition, the cell recovery experiment demonstrated that TP73-AS1 and microRNA-103 might have a mutual regulation, and the two of which could together affect the malignant progression of HCC.

CONCLUSIONS: TP73-AS1 expression was conspicuously enhanced both in HCC tissues and cell lines, which were associated with advanced tumor stage and poor prognosis. In addition, TP73-AS1 could accelerate the proliferation of HCC cells by regulating microRNA-103.

Key Words:
TP73-AS1, MiRNA-103, HCC, Proliferation.

Introduction

Hepatocellular carcinoma (HCC) is one of the common malignant tumors in China. Its incidence rate ranks third in China, while the mortality rate ranks the second[1-3]. Currently, surgery is the main method for treatment of liver cancer, but most patients with liver cancer are already in the advanced stage, and thus, they lost the opportunity of surgical treatment. However, liver cancer lacks other effective treatment methods, resulting in a low 5-year survival[4-6]. Therefore, it is of great significance to improve the prognosis of patients with HCC by systematically revealing the pathogenesis of this disease, clarifying the regulatory relationship between various molecules in cancer cells, finding the pathogenic factors that play a vital role in the pathogenesis of this cancer, developing new drugs for effective treatment, and enriching therapeutic methods[7,8].

Long non-coding RNA (lncRNA) is a non-coding RNA greater than 200 nucleotides in length. In the RNA contained in the cells, the proportion of lncRNA far exceeds the well-known mRNA, which is more than 90%, and mRNA only accounts

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for about 2%[9,10]. Many studies[11-14] have shown that lncRNA exert a vital influence on the development of tumors, which undoubtedly brings new hopes to reveal the mechanism of tumor development. Therefore, the systematic and in-depth research and clarification of the molecular mechanism of lncRNA in the progression of liver cancer have far-reaching significance for the development of molecularly targeted drugs, the improvement of liver cancer treatment methods, and the survival rate of patients[15,16]. LncRNA TP73-AS1 is located at 1p36.32, and there are very few reports on the role of lncRNA TP73-AS1 in the development of tumors at home and abroad[17-19]. Therefore, this experiment comprehensively analyzed the level and biological effects of lncRNATP73-AS1 in HCC, and initially explored its molecular mechanism of tumor regulation.

MicroRNAs are a class of endogenous short-chain non-coding small-molecule ribonucleotides that bind to the 3'-UTR of mRNA to degrade the targeted mRNA, thereby negatively regulating the level of transcription of the target gene[17-23]. In recent years, microRNA-103 has been studied in a variety of tumors; however, its roles in HCC remains elusive. Therefore, this study investigated whether lncRNA TP73-AS1 provides an experimental basis for its clinical application by regulating miRNA-103.

Patients and Methods

Patients and Liver Cancer Samples

In this study, 60 pairs of liver tissue were selected from surgically treated cases of HCC, and invasive tumor specimens and their paracancerous tissues were stored at -80°C. This study was approved by the Ethics Committee of Xi'an XD Group Hospital. Patients and their families have been fully informed that their specimens would be used for scientific research, and all participating patients signed a written informed consent.

Cell Lines and Reagents

Six human HCC cells (Bel-7402, HepG2, MHCC88H, SMMC-7221, Huh7, Hep3B) and one human normal liver cell line (LO2) were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA), and Dulbecco's Modified Eagle's Medium (DMEM) and the fetal bovine serum (FBS) were purchased from Life Technologies (Gaithersburg, MD, USA). The hepatocellular carcinoma cell line was cultured in a DMEM medium containing 10% FBS, penicillin (100 U/mL), and streptomycin (100 μg/mL). All cells were cultured at 37°C, in a 5% CO_2 incubator, and digested with 1% trypsin + EDTA (Ethylene Diamine Tetraacetic Acid) for passage when they reached 80%-90% of confluence.

Transfection

The negative control group (shRNA) and the lentivirus containing the TP73-AS1 knockdown sequence (sh-TP73-AS1) were purchased from Shanghai Jima Company (Shanghai, China). The cells were seeded in 6-well plates and grown to a cell density of 40%, then, the transfection was performed according to the manufacturer's instructions, and the cells were collected 48 h later for quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) analysis and cell function experiments.

Cell Proliferation Assay

The cells after 48 h of transfection were collected and re-seeded into 96-well plates at 2000 cells per well. After being cultured for 24 h, 48 h, 72 h, and 96 h, cell counting kit-8 (CCK-8) reagent (Dojindo Laboratories, Kumamoto, Japan) was added in the cells. After incubation for 2 h, the optical density (OD) value of each well was measured in the microplate reader at 490 nm of absorption wavelength.

Colony Formation Assay

After 48 h of transfection, the cells were collected, and 200 cells were seeded in each well of a 6-well plate and cultured in complete medium for 2 weeks. The medium was replaced after one week and then changed twice a week. After 2 weeks, the cells were cloned and washed twice with Phosphate-Buffered Saline (PBS). The cells were fixed in 2 ml of methanol for 20 min. After the methanol was aspirated, the cells were stained with 0.1% crystal violet for 20 minutes, washed 3 times with PBS, photographed and counted.

5-Ethynyl-2'-Deoxyuridine (EdU) Assay

To demonstrate the effect of TP73-AS1 on cell proliferation, the EDU assay was performed according to the manufacturer's requirements. After transfection for 24 h, the cells were incubated with 50 μm EDU for 2 h and then stained with AdoLo and 4',6-diamidino-2-phenylindole (DAPI). The number of EDU-positive cells was detected by fluorescence microscopy. The display rate of EDU positive was shown as the ratio of the

number of EDU positive cells to the total DAPI chromogenic cells.

QRT-PCR Assay

1 mL of TRIzol (Invitrogen, Carlsbad, CA, USA) was used to lyse the cells, and the total RNA was extracted. The initially extracted RNA was treated with DNase I to remove the genomic DNA and purify the RNA. RNA reverse transcription was performed according to the Prime Script Reverse Transcription Kit instructions, the real time-PCR was performed according to the SYBR® Premix Ex Taq™ kit instructions (TaKaRa, Otsu, Shiga, Japan), and the PCR reaction was performed using the StepOne Plus Real-time PCR System (Applied Biosystems, Foster City, CA, USA). The following primers were used for qRT-PCR reaction: TP73-AS1: F: 5'-CCGGTTTTCCAGTTCTTGCAC-3', R: 5'-GCCTCACAGGGAAACTTCATGC-3'; glyceraldehyde 3-phosphate dehydrogenase (GAPDH): F: 5'-CGCTCTCTGCTCCTCCTGTTC-3', R: 5'-ATCCGTTGACTCCGACCTTCAC-3'; miRNA-103: F: 5'-CGCTAGAAGCTTTTGGGTTAATACTCCATTGAG-3', R: 5'-GCCCTAGACCATGGATTTGTCATTTTGTAAAACT-3'; U6: F: 5'-CGCTTCGGCAGCACATATAC-3', R: 5'-TTCACGAATTTGCGTGTCAT-3'.

The Bio-Rad PCR instrument was used to analyze and process the data with the software iQ5 2.0 (Bio-Rad, Hercules, CA, USA). The GAPDH and U6 genes were used as internal parameters.

Dual Luciferase Reporting Assay

A specific fragment of the target promoter was inserted in front of the luciferase level sequence to construct a report plasmid. The transcription factor plasmid to be detected was co-transfected with the reporter plasmid into the Bel-7402 and HepG2 cells. A specific luciferase substrate was added, and luciferase reacted with the substrate to generate fluorescence. By measuring the intensity of the fluorescence, the activity of the luciferase was analyzed to verify whether the transcription factor could interact with the target promoter fragment.

Statistical Analysis

The statistical analysis was performed using GraphPad Prism 5 V5.01 software (La Jolla, CA, USA). The statistical differences between the two groups or groups were analyzed using the Student's *t*-test. The comparison between the groups was done using One-way ANOVA test followed by post-hoc test (Least Significant Difference). Independent experiments were repeated at least three times for each experiment, and the data were expressed as mean ± standard deviation ($\bar{x}\pm s$). $p<0.05$ was considered statistically significant.

Results

TP73-AS1 was Highly Expressed in HCC Tissues and Cell Lines

In order to determine the role of TP73-AS1 in HCC, we collected 60 pairs of HCC samples

Table I. Association of lncRNA TP73-AS1 and miR-103 expression with clinicopathologic characteristics of hepatocelluar carcinoma.

Parameters	Number of cases	TP73-AS1 expression		*p*-value	miR-103 expression		*p*-value
		Low (%)	High(%)		Low (%)	High (%)	
Age (years)			0.190				0.452
<60	25	15	10		9	16	
≥60	35	15	20		16	19	
Gender				0.302			0.432
Male	30	17	13		11	19	
Female	30	13	17		14	16	
T stage				0.018			0.015
T1-T2	35	22	13		10	25	
T3-T4	25	8	17		15	10	
Lymph node metastasis				0.108			0.124
No	38	22	16		13	25	
Yes	22	8	14		12	10	
Distance metastasis				0.067			0.170
No	35	21	14		12	23	
Yes	25	9	16		13	12	

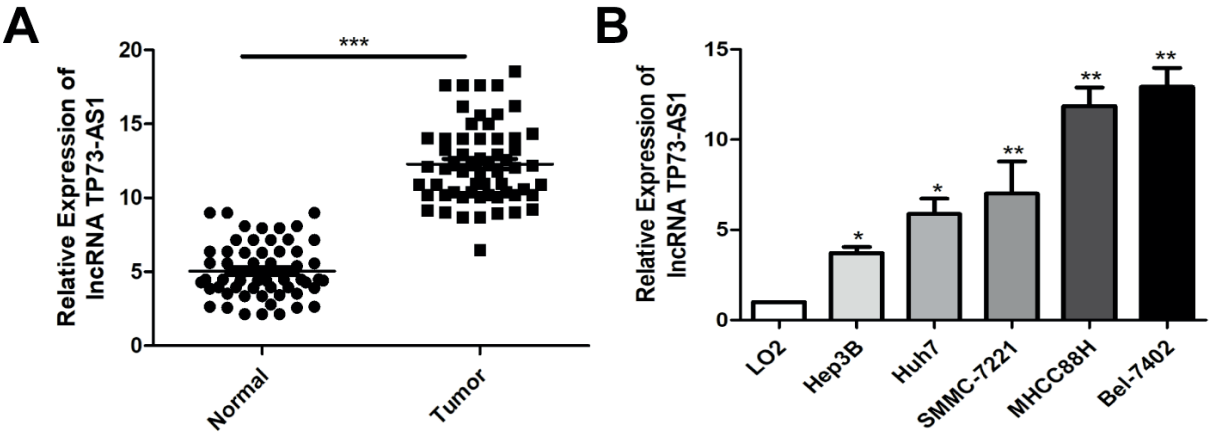

Figure 1. TP73-AS1 is highly expressed in hepatocellular carcinoma tissues and cell lines. ***A,*** qRT-PCR was used to detect the differential expression of TP73-AS1 in hepatocellular carcinoma tumor tissues and adjacent tissues; ***B,*** qRT-PCR was used to detect the expression level of TP73-AS1 in osteosarcoma cell lines. Data are mean ± SD, *p<0.05, **p<0.01, ***p<0.001.

and adjacent ones and detected TP73-AS1 level in these samples. The results revealed that TP73-AS1 was elevated in the HCC tissue samples (Figure 1A), suggesting that TP73-AS1 might act as an oncogene in liver cancer. In addition, TP73-AS1 was found conspicuously higher in the OS cells than in human normal liver cell lines (LO2), and the difference was statistically significant (Figure 1B).

TP73-AS1 Level was Correlated with Pathological Staging and Overall Survival in HCC Paatients

Based on the mRNA results of TP73-AS1 in 60 pairs of HCC specimens and paracancerous ones, we divided these tissue samples into the high-level group and low-level group and analyzed the relationship between TP73-AS1 level and age, sex, tumor stage, lymph node, or distant metastasis of HCC patients. As shown in Table I, the highly expressed lncRNATP73-AS1 was positively correlated with the pathological stage of HCC, but not with age, gender, distant, or lymph node metastasis.

Knockdown of TP73-AS1 Inhibited Cell Proliferation, and Promoted Cell Apoptosis

To investigate the function of TP73-AS1 in HCC, we constructed a knockdown TP73-AS1 lentiviral vector. After transfecting the TP73-AS1 lentiviral vector in the Bel-7402 and HepG2 cell lines, the qRT-PCR assay was performed to verify the interference efficiency (Figure 2A). In the Bel-7402 and HepG2 cell lines, after TP73-AS1 down-regulation, the CCK-8, EDU, and flow cytometry assays were performed to detect cell proliferation and apoptosis ability. The results showed that, compared with the control group, the cell proliferation ability of the cells in the TP73-AS1 silencing group was conspicuously reduced (Figure 2B, 2C, 2D), while cell apoptosis was conversely increased (Figure 2E).

Direct Binding of TP73-AS1 and MicroRNA-103

As shown in Figure 3A, in order to further verify the binding of microRNA-103 to TP73-AS1, we cloned the TP73-AS1 sequence into the luciferase reporter plasmid pmirGLO, and also constructed the mutation vector pmirGLO-TP73-AS1-mut. Then, pmirGLO-TP73-AS1-wt, pmirGLO-TP73-AS1-mut or pmirGLO, and microRNA-103 were co-transfected into Bel-7402 and HepG2 cells, respectively, for luciferase reporter gene experiments. The results showed that the overexpression of microRNA-103 conspicuously attenuated the luciferase activity of the wild type TP73-AS1 vector (p<0.05) without attenuating the luciferase activity containing the mutant vector (p>0.05) or the empty vector (p>0.05), further demonstrating that TP73-AS1 can be targeted by microRNA-103 through this binding site.

MicroRNA-103 was Lowly Expressed in Hepatocellular Carcinoma Tissues or Cell Samples

QRT-PCR experiments confirmed that the microRNA-103 level was conspicuously decreased in HCC tissues when comparing with paracancerous ones (Figure 3B). Subsequently, microR-

NA-103 was also found lowly expressed in HCC cell lines (Figure 3C). Therefore, we selected 16 pairs of samples in HCC patients and detected the mRNA expression of TP73-AS1 and microRNA-103. The results indicated that mRNA levels of TP73-AS1 and microRNA-103 showed a negative correlation in HCC tissues (Figure 3D). Subsequently, after transfecting the TP73-AS1 knockdown lentiviral vector in the Bel-7402 and HepG2 cell lines, the expression of microRNA-103 in the cells of TP73-AS1 silencing group was found conspicuously enhanced (Figure 3E). At the same time, we analyzed the association between microRNA-103 expression and pathology as well as patients' prognosis in HCC. As shown in Table I, the low level of microRNA-103 was positively correlated with the pathological stage of HCC, but not with age, gender, lymph node, or distant metastasis.

Overexpression of MicroRNA-103 Inhibited Cell Growth and Accelerated Cell Apoptosis

To investigate the function of microRNA-103 in HCC, we constructed a microRNA-103 overexpressing lentiviral vector. After transfection of the lentiviral vector in the Bel-7402 and HepG2 cell lines, the interference efficiency was subsequently confirmed (Figure 4A), and the CCK-8, EDU, and flow cytometry were used to detect cell growth and apoptosis. The results showed that compared with miR-NC, the cell proliferation ability of microRNA-103 overexpression group was conspicuously decreased (Figure 4B, 4C, 4D) while cell apoptosis was oppositely enhanced (Figure 4E).

TP73-AS1 Modulated MicroRNA-103 Expression in Hepatocellular Carcinoma Tissues and Cell Lines

To further explore the ways in which lncRNATP73-AS1 promotes the malignant progression of HCC, we found a possible relationship between TP73-AS1 and microRNA-103 through relevant bioinformatics analysis (Figure 5A). In addition, to further explore the interaction between TP73-AS1 and microRNA-103 in HCC cells, we down-regulated microRNA-103 in an HCC cell line that had been transfected with sh-TP73-AS1 to further explore the mutual regulation between the two, and the transfected effi-

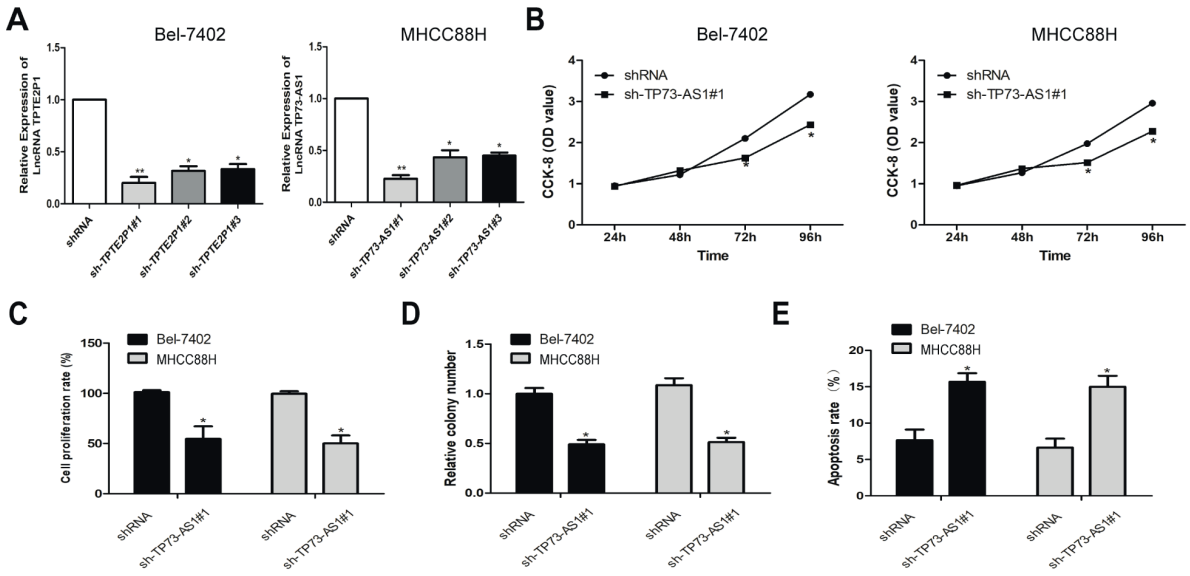

Figure 2. Silencing TP73-AS1 inhibits the proliferation of hepatocellular carcinoma cells. ***A,*** qRT-PCR verified the interference efficiency of TP73-AS1 after transfection of TP73-AS1 knockout vector in Bel-7402 and HepG2 cell lines. ***B,*** CCK-8 assay was used to analyzed the effect of silenced-TP73-AS1 on proliferation of hepatocellular carcinoma cells including Bel-7402 and HepG2 cell lines. ***C,*** EDU assay was used to detect the effect of silencing TP73-AS1 on proliferation of hepatocellular carcinoma cells including Bel-7402 and HepG2 cell lines. ***D,*** Cell cloning assay was used to detect the effect of silencing TP73-AS1 on proliferation of hepatocellular carcinoma cells including Bel-7402 and HepG2 cell lines. ***E,*** Flow cytometry assay was used to detect the effect of silencing TP73-AS1 on apoptosis of hepatocellular carcinoma cells including Bel-7402 and HepG2 cell lines. Data are mean ± SD, *$p<0.05$.

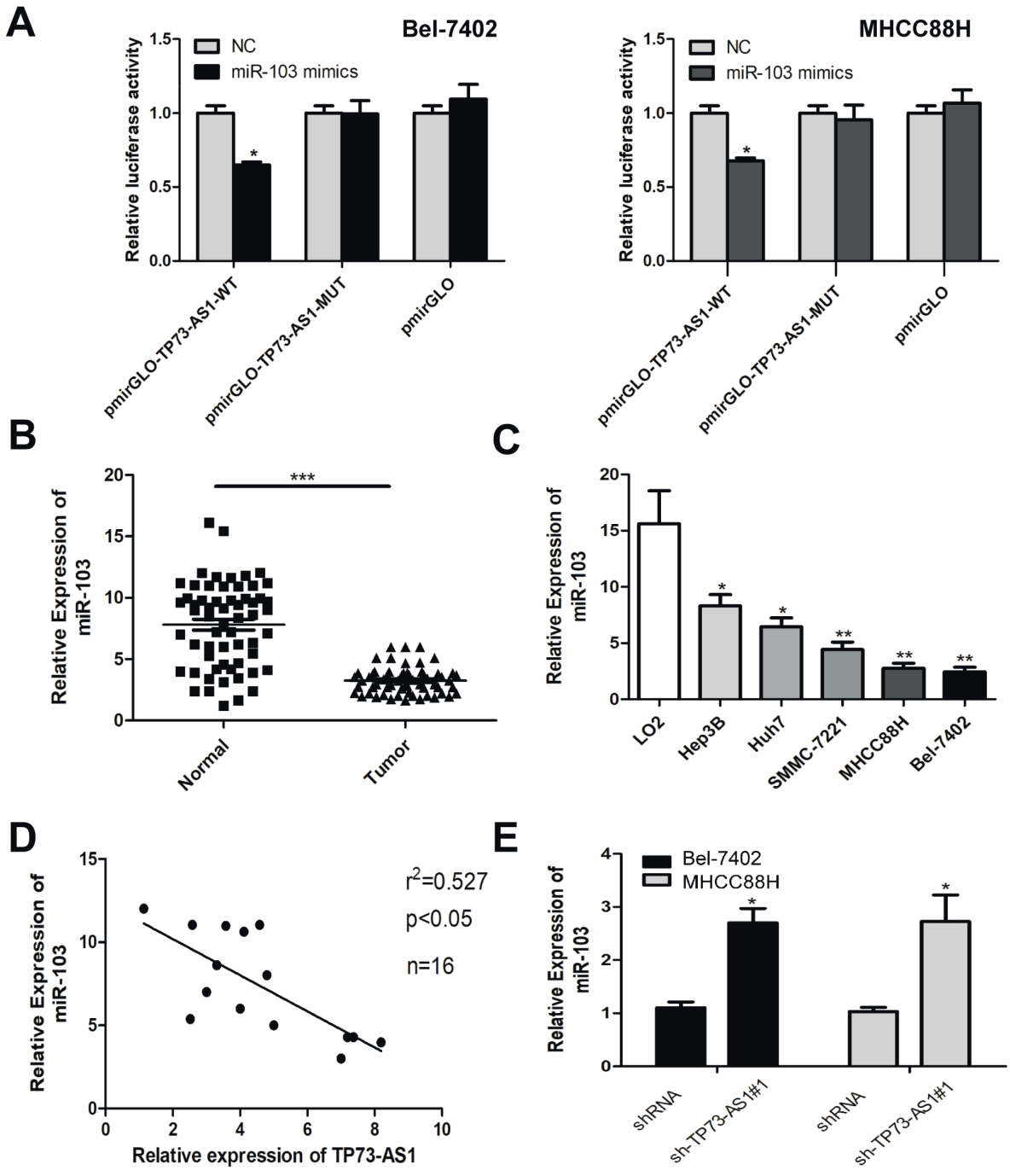

Figure 3. Direct targeting of miR-103 by TP73-AS1. ***A,*** Dual luciferase reporter assay was performed to demonstrate direct targeting of TP73-AS1 and miR-103. The dual luciferase reporter gene assay in the Bel-7402 and HepG2 cell lines showed that overexpression of miR-103 significantly attenuated the luciferase activity of the wild-type TP73-AS1 vector ($p<0.001$) without attenuating that of mutant vector ($p>0.05$) or empty vector ($p>0.05$). ***B,*** qRT-PCR was used to detect differential expression of miR-103 in hepatocellular carcinoma and adjacent tissues. ***C,*** qRT-PCR verified the mRNA expression level of miR-103 after transfection of TP73-AS1 in hepatocellular carcinoma cell lines. ***D,*** There was a negative association between TP73-AS1 and miR-103 expression in hepatocellular carcinoma tissues. ***E,*** Silencing TP73-AS1 significantly increased miR-103 levels. Data are mean ± SD, *$p<0.05$, **$p<0.01$, ***$p<0.001$.

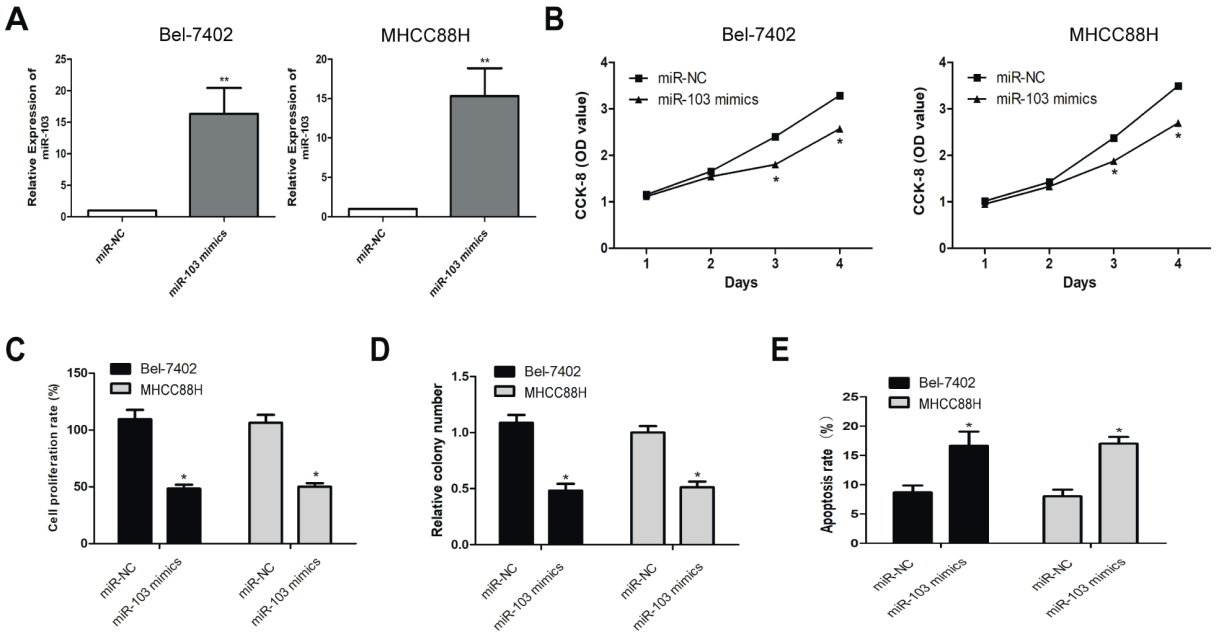

Figure 4. Overexpression of miR-103 inhibits hepatocyte cancer cell proliferation. ***A,*** qRT-PCR verified the interference efficiency of miR-103 after transfection of miR-103 overexpression vector in Bel-7402 and HepG2 cell lines. ***B,*** CCK-8 assay was used to analyzed the effect of overexpression of miR-103 on proliferation of hepatocellular carcinoma cells including Bel-7402 and HepG2 cell lines. ***C,*** EDU assay was used to analyzed the effect of overexpression of miR-103 on proliferation of hepatocellular carcinoma cells. ***D,*** Cell cloning assay was used to analyzed the effect of overexpression of miR-103 on proliferation of hepatocellular carcinoma cells. ***E,*** Flow cytometry assay was used to analyzed the effect of overexpression of miR-103 on apoptosis of hepatocellular carcinoma cells. Data are mean ± SD, **p*<0.05.

ciency was verified using qPCR (Figure 5B). Subsequently, we conducted the EDU experiments and flow cytometry, and found that microRNA-103 could counteract the effect of TP73-AS1 on HCC cells (Figure 5C).

Discussion

HCC (HCC) is a malignant tumor with strong invasive ability, high metastasis, and poor prognosis. The mortality caused by patients accounts for third place in the world for tumor-related diseases[1-3]. Therefore, finding new biological indicators for early diagnosis and prognosis of liver cancer is of great significance for the treatment and improvement of prognosis of liver cancer patients[5-8]. LncRNAs are a class of endogenous RNA a transcript length of more than 200 nt that cannot encode proteins. They often have a polyA tail structure and account for about 80% of non-coding RNA[9-11]. With the deepening of later research, we have found that some tumor-related lncRNAs have also been gradually reported, such as lncRNA H19, which is a non-coding RNA expressed by maternal characteristics and has a biphasic effect, such as promoting cancer and suppressing cancer[24,25].

TP73-AS1 is a recently discovered long-chain non-coding RNA that is mapped to chromosome 1[17-19]. Scholars[17-19] have shown that TP73-AS1 is highly expressed in lung cancer, and its high level is associated with poor prognosis in patients with osteosarcoma. In addition, it has been found that the abnormal level of TP73-AS1 is associated with poor prognosis in patients with renal clear cell carcinoma. In the present study, we have found through tissue verification that the level of TP73-AS1 in HCC tissues is conspicuously higher than that in paracancerous specimens, and is positively correlated with the pathological stage and poor prognosis of HCC. Therefore, we believe that TP73-AS1 may be involved in the progression of liver cancer. TP73-AS1 is proved as a disease-related gene, and experiments were performed *in vitro*. Subsequently, CCK-8 and EDU experiments were performed, and silencing TP73-AS1 was found to inhibit the proliferation of HCC cells.

At present, lncRNA exerts its biological functions such as regulation and modification mainly by competitively binding miRNA and thereby

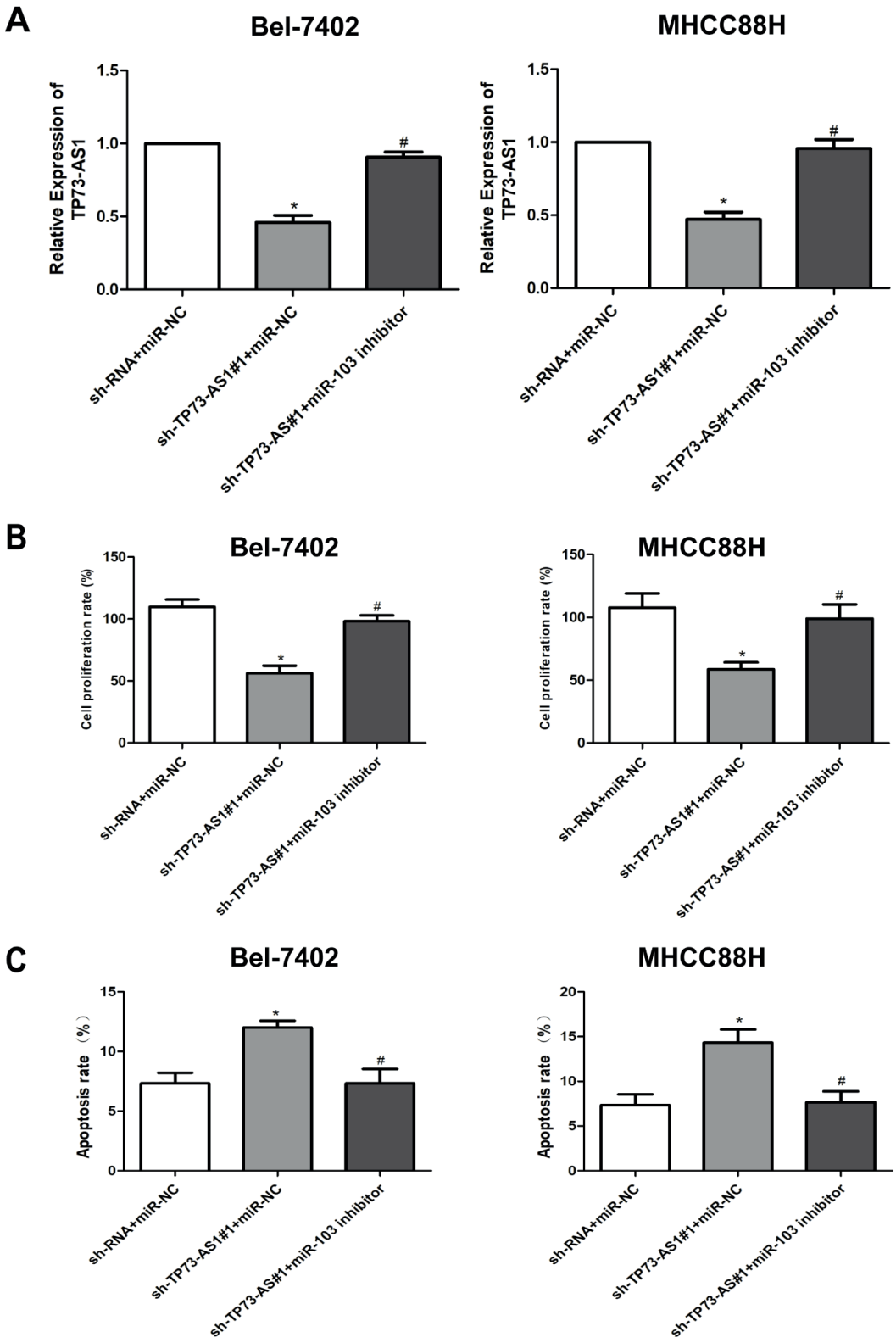

Figure 5. TP73-AS1 regulates the expression of miR-103 in hepatocellular carcinoma tissues and cell lines. ***A,*** TP73-AS1 expression levels in TP73-AS1 and miR-103 co-transfected cell lines were detected by qRT-PCR. ***B,*** EDU assay was used to detect the effect of co-transfection of TP73-AS1 and miR-103 on cell proliferation of HCC cell lines. ***C,*** Flow cytometry assay was used to detect the effect of co-transfection of TP73-AS1 and miR-103 on cell apoptosis of HCC cell lines. Data are mean ± SD, *$p<0.05$.

affecting the regulation of its target mRNA[26-28]. Previous studies have predicted through bioinformatics analysis that miRNA-103 may interact with TP73-AS1. MiRNA-103 is a key molecule in the miRNA family. The results of this work showed that miRNA-103 was lower expressed in HCC specimens than in normal tissues and could inhibit the proliferation of HCC cells[29,30]. In the present research, we used bioinformatics methods to analyze the TP73-AS1 sequence containing a miRNA-103 binding site, and verified the combination of TP73-AS1 and downstream miRNA-103 by molecular biology experiments such as the bioinformatics and the dual luciferase reporter gene assay. Meanwhile, the levels of TP73-AS1 and microRNA-103 were just negatively correlated in HCC cell lines. In addition, we performed a recovery study in HCC cells and found that miRNA-103 could counteract the role of lncRNA TP73-AS1 in HCC cell lines. It was suggested that lnRNA TP73-AS1 can inhibit miRNA-103 expression so as to promote the proliferation of HCC cells.

Conclusions

We found that LncRNA TP73-AS1 had a high expression in liver cancer tissues and cells, which might result in an advanced tumor stage and a poor prognosis of patients. In addition, TP73-AS1 may prompt the malignant process of this disease via regulating miRNA-103.

Conflict of Interests

The Authors declare that they have no conflict of interests.

References

1) Kale P, Khillan V, Sarin SK. Novel association of Streptococcus gallolyticus subspecies pasteurianus and hepatocellular carcinoma: opening new frontiers. Scand J Gastroenterol 2018; 53: 1354-1357.
2) Wu J, Jiang ZM, Xie Y, He XQ, Lin XH, Hu JL, Peng YZ. MiR-218 suppresses the growth of hepatocellular carcinoma by inhibiting the expression of proto-oncogene Bmi-1. J BUON 2018; 23: 604-610.
3) Liu ZH, Zhang YF, Xu ZD, Z HL, Y FZ, Z DX. UNC119 promoted cell growth and migration by Wnt/beta-catenin signal and TGF-beta/EMT signal pathway in hepatocellular carcinoma. J BUON 2018; 23: 1717-1724.
4) Agarwal R, Cao Y, Hoffmeier K, Krezdorn N, Jost L, Meisel AR, Jungling R, Dituri F, Mancarella S, Rotter B, Winter P, Giannelli G. Precision medicine for hepatocellular carcinoma using molecular pattern diagnostics: results from a preclinical pilot study. Cell Death Dis 2017; 8: e2867.
5) Xiao Y, Yan W, Guo L, Meng C, Li B, Neves H, Chen PC, Li L, Huang Y, Kwok HF, Lin Y. Digitoxin synergizes with sorafenib to inhibit hepatocellular carcinoma cell growth without inhibiting cell migration. Mol Med Rep 2017; 15: 941-947.
6) Shen J, He L, Li C, Wen T, Chen W, Lu C, Yan L, Li B, Yang J. Prognostic nomograms for patients with resectable hepatocellular carcinoma incorporating systemic inflammation and tumor characteristics. Oncotarget 2016; 7: 80783-80793.
7) Li W, Wang Y, Kellner DB, Zhao L, Xu L, Gao Q. Efficacy of RetroNectin-activated cytokine-induced killer cell therapy in the treatment of advanced hepatocellular carcinoma. Oncol Lett 2016; 12: 707-714.
8) Liu Z, Zhang Y, Xu Z. UNC119 promotes the growth and migration of hepatocellular carcinoma via Wnt/β/catenin and TGF/β-EMT signaling pathways. J BUON 2018; 23: 188-192.
9) Niu J, Lin Y, Liu P, Yu Y, Su C, Wang X. Microarray analysis on the lncRNA expression profile in male hepatocellular carcinoma patients with chronic hepatitis B virus infection. Oncotarget 2016; 7: 76169-76180.
10) He Y, Meng XM, Huang C, Wu BM, Zhang L, Lv XW, Li J. Long noncoding RNAs: Novel insights into hepatocellular carcinoma. Cancer Lett 2014; 344: 20-27.
11) Liu JY, Zhu Q, Li J, Zhao S, Li L. The retrovirus-mediated antisense human telomerase RNA (hTR) gene limits the growth of hepatocellular carcinoma growth in cell culture and animals. Dig Dis Sci 2008; 53: 1122-1130.
12) Yang M, Wang M, Li X, Xie Y, Xia X, Tian J, Zhang K, Tang A. The role of lncRNAs in signaling pathway implicated in CC. J Cell Biochem 2019; 120: 2703-2712.
13) Abbastabar M, Sarfi M, Golestani A, Khalili E. LncRNA involvement in hepatocellular carcinoma metastasis and prognosis. EXCLI J 2018; 17: 900-913.
14) Chen Z, Hu X, Wu Y, Cong L, He X, Lu J, Feng J, Liu D. Long non-coding RNA XIST promotes the development of esophageal cancer by sponging miR-494 to regulate CDK6 expression. Biomed Pharmacother 2019; 109: 2228-2236.
15) de Oliveira JC, Oliveira LC, Mathias C, Pedroso GA, Lemos DS, Salviano-Silva A, Jucoski TS, Lobo-Alves SC, Zambalde EP, Cipolla GA, Gradia DF. Long non-coding RNAs in cancer: another layer of complexity. J Gene Med 2019; 21: e3065.
16) Zhang L, Wang L, Wang Y, Chen T, Liu R, Yang W, Liu Q, Tu K. LncRNA KTN1-AS1 promotes tumor growth of hepatocellular carcinoma by targeting

miR-23c/ERBB2IP axis. Biomed Pharmacother 2019; 109: 1140-1147.

17) Liu C, Ren L, Deng J, Wang S. LncRNA TP73-AS1 promoted the progression of lung adenocarcinoma via PI3K/AKT pathway. Biosci Rep 2019; 39: BSR20180999.

18) Xiong DD, Dang YW, Lin P, Wen DY, He RQ, Luo DZ, Feng ZB, Chen G. A circRNA-miRNA-mRNA network identification for exploring underlying pathogenesis and therapy strategy of hepatocellular carcinoma. J Transl Med 2018; 16: 220.

19) Liu G, Zhao X, Zhou J, Cheng X, Ye Z, Ji Z. LncRNA TP73-AS1 promotes cell proliferation and inhibits cell apoptosis in clear cell renal cell carcinoma through repressing KISS1 expression and inactivation of PI3K/Akt/mTOR signaling pathway. Cell Physiol Biochem 2018; 48: 371-384.

20) Mohr AM, Mott JL. Overview of microRNA biology. Semin Liver Dis 2015; 35: 3-11.

21) Pinzon N, Li B, Martinez L, Sergeeva A, Presumey J, Apparailly F, Seitz H. MicroRNA target prediction programs predict many false positives. Genome Res 2017; 27: 234-245.

22) Kunz M, Gottlich C, Walles T, Nietzer S, Dandekar G, Dandekar T. MicroRNA-21 versus microRNA-34: Lung cancer promoting and inhibitory microRNAs analysed in silico and in vitro and their clinical impact. Tumour Biol 2017; 39: 1393383234.

23) Li Y, Ren W, Wang X, Yu X, Cui L, Li X, Zhang X, Shi B. MicroRNA-150 relieves vascular remodeling and fibrosis in hypoxia-induced pulmonary hypertension. Biomed Pharmacother 2019; 109: 1740-1749.

24) Sun W, Lv J, Duan L, Lin R, Li Y, Li S, Fu C, Zhao L, Xin S. Long noncoding RNA H19 promotes vascular remodeling by sponging let-7a to upregulate the expression of cyclin D1. Biochem Biophys Res Commun 2019; 508: 1038-1042.

25) Zhang Y, Mi L, Xuan Y, Gao C, Wang YH, Ming HX, Liu J. LncRNA HOTAIRM1 inhibits the progression of hepatocellular carcinoma by inhibiting the Wnt signaling pathway. Eur Rev Med Pharmacol Sci 2018; 22: 4861-4868.

26) Xiang Y, Huang Y, Sun H, Pan Y, Wu M, Zhang J. Deregulation of miR-520d-3p promotes hepatocellular carcinoma development via lncRNA MIAT regulation and EPHA2 signaling activation. Biomed Pharmacother 2019; 109: 1630-1639.

27) Chen J, Huang X, Wang W, Xie H, Li J, Hu Z, Zheng Z, Li H, Teng L. LncRNA CDKN2BAS predicts poor prognosis in patients with hepatocellular carcinoma and promotes metastasis via the miR-153-5p/ARHGAP18 signaling axis. Aging (Albany NY) 2018; 10: 3371-3381.

28) Gong J, Wang J, Liu T, Hu J, Zheng J. LncRNA FEZF1AS1 contributes to cell proliferation, migration and invasion by sponging miR4443 in hepatocellular carcinoma. Mol Med Rep 2018; 18: 5614-5620.

29) Xu Q, Li Y, Shang YF, Wang HL, Yao MX. MiRNA-103: molecular link between insulin resistance and nonalcoholic fatty liver disease. World J Gastroenterol 2015; 21: 511-516.

30) Weber DG, Johnen G, Bryk O, Jockel KH, Bruning T. Identification of miRNA-103 in the cellular fraction of human peripheral blood as a potential biomarker for malignant mesothelioma--a pilot study. PLoS One 2012; 7: e30221.