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LncRNA MT1JP inhibits the malignant progression of hepatocellular carcinoma through regulating AKT

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Abstract. – OBJECTIVE: The purpose of this study was to investigate the expression profile of long non-coding RNA (lncRNA) MT1JP in hepatocellular carcinoma (HCC), and to explore the relationship between its expression level and the clinical indicators, as well as the prognosis of HCC patients.

PATIENTS AND METHODS: In this study, the expression level of MT1JP in 45 pairs of tumor tissue specimens and paracancerous ones collected from HCC patients were examined through quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) method, and the interplay between MT1JP expression and clinical indicators, as well as the prognosis of HCC patients, was also analyzed. Meanwhile, the expression of MT1JP in HCC cell lines was further verified by qRT-PCR. In addition, MT1JP overexpression model was constructed using lentivirus in HCC cell lines (Hub7 and HepG2), and then, Cell Counting Kit-8 (CCK-8), cell colony formation assay, and flow cytometry were performed to examine the impact of MT1JP on the HCC cell functions. Additionally, whether MT1JP exerts its biological characteristics through protein kinase B (AKT) was finally explored.

RESULTS: In this experiment, qRT-PCR results showed that the expression level of lncRNA MT1JP in tumor tissues of HCC patients was remarkably lower than that in adjacent tissues, and the difference was statistically significant. Meanwhile, compared with patients with high expression of MT1JP, patients with low expression of MT1JP had a higher pathological staging and a lower overall survival rate. In addition, overexpression of MT1JP remarkably attenuated the proliferation ability of HCC cells but enhanced cell apoptosis rate at the same time. Finally, Western blot results revealed that the overexpression of MT1JP may markedly reduce the AKT expression, thereby suppressing the malignant progression of HCC.

CONCLUSIONS: LncRNA MT1JP expression is remarkably decreased in HCC tumor tissue samples, which is associated with pathological stage and poor prognosis of HCC patients. In addition, MT1JP may inhibit the malignant progression of HCC by downregulating AKT.

Key Words:
LncRNA MT1JP, AKT, HCC, Malignant progression.

Introduction

Hepatocellular carcinoma (HCC) is the most common cancer in the liver and ranks third in the world among cancer-related deaths. About 750,000 people die of liver cancer every year globally[1,2]. Currently, surgical resection and liver transplantation are the most effective treatments for HCC[3,4]. Due to the fast speed, high invasiveness of malignancy, and the obscure symptoms of liver cancer, most patients have developed to

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the middle and late stage, thus missing the best time for surgical treatment[5,6]. Even after surgical resection, the risk of postoperative recurrence is still relatively high[7]. Therefore, it is of great significance to explore early diagnostic markers for HCC diagnosis to improve the prognosis of patients with HCC[8,9].

Non-coding RNAs are also involved in regulating a variety of physiological functions, including tumor development, as well as cell proliferation, migration, and apoptosis[10-12]. Among them, long non-coding RNA (lncRNA), mostly generated by RNA transcriptase II transcription, is located in the nucleus or cytoplasm with a length of more than 200 nt, and it has no ability to encode proteins[13,14]. LncRNA can be divided into 5 types according to the relative positions and protein-coding genes in the genome, each of them has different biological functions and different mechanisms of gene regulation[15,16]. LncRNAs are competing endogenous RNAs (ceRNAs) containing miRNA response elements (MERs), which can inhibit the regulation of miRNAs on downstream genes by competitively binding miRNA sites. The study on the role of lncRNA in HCC may contribute to a further understand of the pathogenesis of HCC.

In this study, lncRNA MTIJP was selected from the results of lncRNA expression profile microarray that had been done in the previous stage due to its significantly different expression between tumor tissues and adjacent tissues of HCC patients. Besides, no relevant reports about this molecule have ever been found in the research of liver cancer. LncRNA MT1JP is a non-coding RNA with a length of 977 nt located on chromosome 16[17]. In our study, quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was performed to verify the expression of the lncRNA in HCC tissue samples, and its relationship with clinical characteristics was also analyzed. Subsequently, the effect of MTlJP on the function of liver cancer cells was detected through relevant cell experiments, providing new possibilities for the diagnosis and clinical targeted therapy of liver cancer. As a result, in this study, the recombinant adenovirus of lncRNA MT1JP was constructed, and the *in vitro* cell model of MT1JP overexpression and knockdown was used to study the induction and activation of protein kinase B (AKT) by MT1JP, as well as its effect on HCC cell biological behavior. Furthermore, the molecular pathogenic mechanism of the abnormal activation of AKT induced by lncRNA MT1JP in the occurrence of HCC was explored.

Patients and Methods

Patients and HCC Samples

A total of 45 cases of HCC tumor tissue specimens and adjacent tissue specimens were obtained from fresh specimens through biopsy or surgical resection in the department of general surgery and oncology in our hospital, and stored in a refrigerator at -80°C. All the 45 patients with HCC were aged from 30 to 78 years old. All cases were diagnosed by two senior directors of pathologists and the results were confirmed to be accurate. This study was approved by the Ethics Committee of our Hospital. Signed written informed consents were obtained from all participants before the study.

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 the hospital, and Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were purchased from American Life Technologies (Gaithersburg, MD, USA). The cells were cultured with DMEM high glucose medium containing 10% FBS, penicillin (100 U/mL), and streptomycin (100 μg/mL) in a 37°C, 5% CO_2 incubator. In addition, cells were digested with 1% trypsin + EDTA (ethylenediaminetetraacetic acid) for passage when the fusion degree reached 80-90%.

Transfection

The lentiviral vectors containing the NC sequences or MT1JP sequences were purchased from Shanghai Jima Company (Shanghai, China). The cells were plated in 6-well plates and grew to a cell density of 70%, followed by lentiviral transfection. After 48 h, the cells were collected for quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) analysis and cell function experiments.

Cell Proliferation Assay

The proliferation of the three cell lines was examined using the Cell Counting Kit-8 (CCK-8) kit (Dojindo Molecular Technologies, Kumamoto, Japan). The transfected cells were collected and plated into 96-well plates at 2000 cells per well. After cultured for 24 h, 48 h, 72 h, and 96 h respectively, 10 μL of CCK-8 solution was added per well for incubation for 1 h, and then, the optical density (OD) at the absorption wavelength of 445 nm of each well was measured in the microplate reader.

Colony Formation Assay

After 48 h of transfection, 200 collected cells were seeded in each well of a 6-well plate and cultured with complete medium for 2 weeks. The medium was changed after one week, and then, twice a week. The medium should not be replaced as much as possible in the previous week to avoid cell adhesion. After 2 weeks, the cells were cloned and then fixed in 2 mL of methanol for 20 minutes. After the methanol was aspirated, the cells were stained with 0.1% crystal violet staining solution for 20 minutes, washed 3 times with phosphate-buffered saline (PBS), photographed, and counted under a light-selective environment.

Flow Cytometry

The cell density was adjusted to about 1×10^6 cells/mL. After the medium was discarded, the cells were washed twice with PBS and gently resuspended with 0.5 mL of pre-cooled 1× binding buffer. Next, 1.25 μL of Annexin V-fluorescein isothiocyanate (FITC) was added for incubation at room temperature and light-proof reaction for 15 min. Later, the cells were centrifuged at 1000 × g for 5 min at room temperature, and the supernatant was removed. After gently resuspending the cells with 0.5 mL of pre-cooled l×binding buffer, 10 μL of propidium iodide (PI) was added, and the samples were placed on ice and stored in the dark, followed by flow cytometry analysis (FACSCalibur, BD Biosciences, Detroit, MI, USA).

QRT-PCR

Total RNA was extracted from HCC cell lines and tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and RNA was reversely transcribed into complementary deoxyribose nucleic acid (cDNA) using PrimeScript RT Reagent. qRT-PCR was performed using SYBR® Premix Ex Taq™ and StepOne Plus Real-time PCR System (TaKaRa, Otsu, Shiga, Japan). Data analysis was performed using ABI Step One software (Applied Biosystems, Foster City, CA, USA) and the relative expression levels of mRNA were calculated using the $2^{-\Delta\Delta Ct}$ method. The following primers used for the qRT-PCR are listed below: MT1JP F: 5'-TACCGAGCTCGGATCCTTGCGGTCTCTC-CATTTATCG-3', R: 5'-TACCGAGCTCG-GATCCTTGCGGTCTCTCCATTTATCG-3; AKT F: 5'-TGGGGAAGATTGGGAAAGGC-3', R: 5'-TCGCCCATTGGCTACATCTC-3'; glyceraldehyde 3-phosphate dehydrogenase (GAPDH) F: 5'-GCAGGGGGGAGCCAAAAGGGT-3', R: 5'-TGGGTGGCAGTGATGGCATGG-3'.

Western Blot

The transfected cells were lysed using cell lysis buffer, shaken on ice for 30 minutes, and centrifuged at 14,000 × g for 15 minutes at 4°C. The total protein concentration was calculated by bicinchoninic acid (BCA) Protein Assay Kit (Pierce, Rockford, IL, USA). Next, the extracted proteins were separated using a 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene difluoride membrane (PVDF; Roche, Basel, Switzerland). Finally, Western blot analysis was performed according to standard procedures. The primary antibodies, anti-AKT and anti-GAPDH, and the secondary antibodies were both purchased from Cell Signaling Technology (Danvers, MA, USA).

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 5 V5.01 software (La Jolla, CA, USA). Differences between the two groups were analyzed using the Student's *t*-test. The comparisons among multiple groups were 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 data were expressed as mean ± standard deviation. $p<0.05$ was considered statistically significant.

Results

Expression Pattern of MT1JP in HCC and Its Association with Clinicopathological Factors

To determine the difference in the expression levels of MT1JP in tumor tissue samples and paracancerous ones of patients with HCC, qRT-PCR was conducted to reveal that the expression of MT1JP in the former was lower than that in the latter (Figure 1A), suggesting that MT1JP may act as a tumor suppressor gene in HCC. At the same time, the expression level of MT1JP in common HCC cell lines was detected by qRT-PCR, and it was found that MT1JP showed the lowest expression in Hub7 and HepG2 cell lines, which were therefore selected for subsequent study (Figure 1B).

According to the mRNA levels of MT1JP, the above tissue samples were divided into high and low expression group, and the number of cells in each group was counted. Subsequently, the Chi-square test was applied to analyze the interplay between MT1JP mRNA expression and clinical indicators of HCC patients including age, patho-

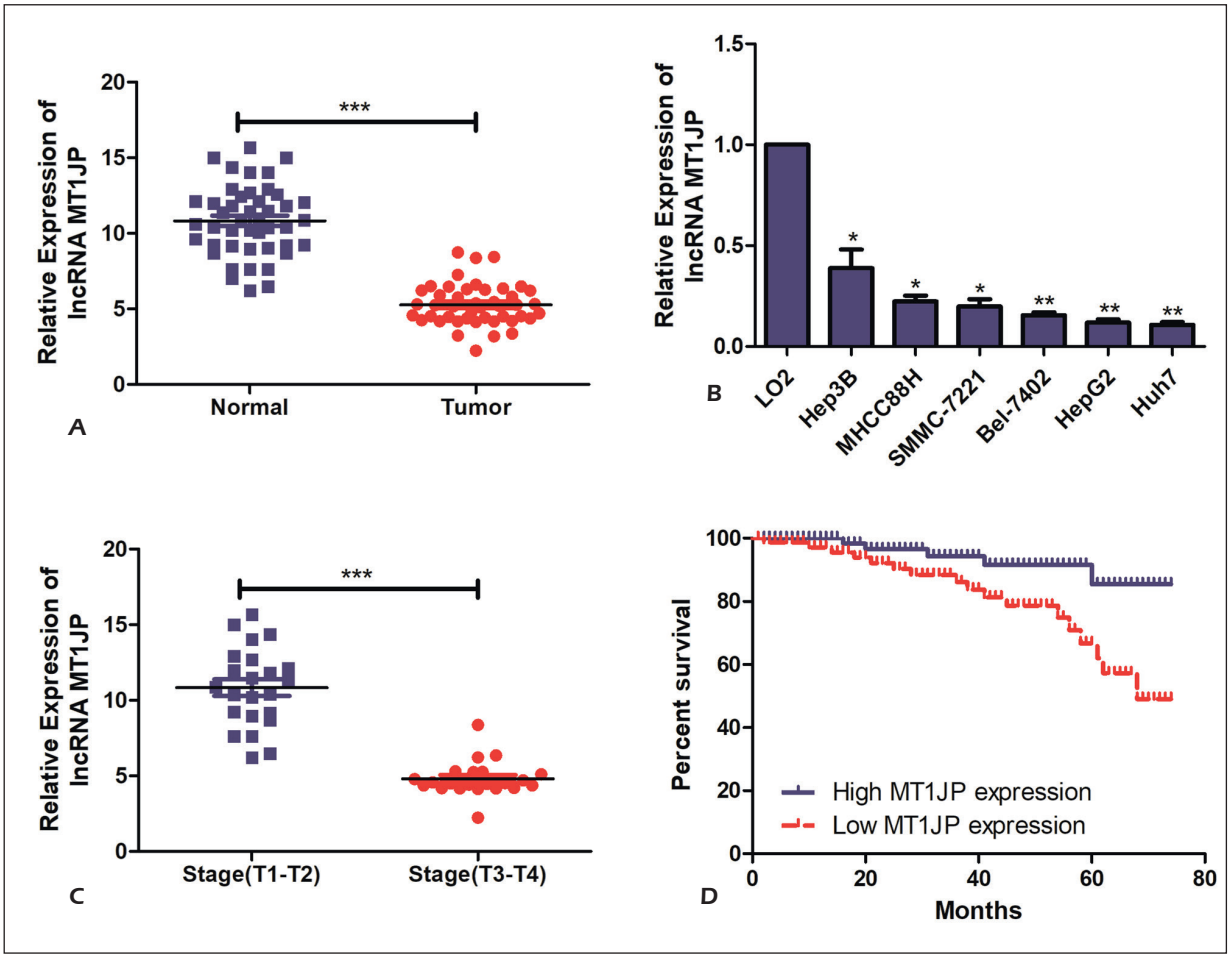

Figure 1. MT1JP is underexpressed in hepatocellular carcinoma tissues and cell lines. **A**, QRT-PCR is used to detect the difference in expression of MT1JP in hepatocellular carcinoma tumor tissues and adjacent tissues. **B**, QRT-PCR is used to detect the expression level of MT1JP in hepatocellular carcinoma cell lines. **C**, QRT-PCR is applied to observe the difference in expression of MT1JP in different pathological stages of patients with HCC. **D**, Kaplan Meier survival curve of patients with HCC based on MT1JP expression is shown. The prognosis of patients with low expression is significantly poor than that of high expression group. Data are mean ± SD, *p<0.05, **p<0.01, ***p<0.001.

logical stage, lymph node or distant metastasis of HCC patients. As shown in Table I, the low expression of MT1JP was positively correlated with the pathological stage of HCC patients (Figure 1C). In addition, the Kaplan-Meier survival curve showed that the low expression of MT1JP was remarkably associated with poor prognosis of HCC by collecting relevant follow-up data. In other words, the lower the expression level of MT1JP, the worse the prognosis of HCC patients (p<0.05; Figure 1D).

Downregulation of MT1JP Contributed to Cell Proliferation, and Inhibited Cell Apoptosis in HCC

To explore the effects of MT1JP on HCC cell function, the MT1JP overexpression model was successfully constructed by lentivirus, and its transfection efficiency was verified by qRT-PCR (Figure 2A). The proliferation rate of HCC cells was found markedly attenuated after overexpression of MT1JP (Figure 2B and 2C). Subsequently, in order to further study the impact of MT1JP on the apoptosis of HCC cells, flow cytometry, and Annexin V-FITC/PI double staining results revealed that the apoptosis ability of HCC cells was remarkably enhanced after overexpression of MT1JP when compared with the NC group (Figure 2D).

Downregulation of MT1JP Decreased the Expression Level of AKT

To further explore the ways in which MT1JP inhibits the malignant progression of HCC, after

Table I. Association of LncRNA MT1JP expression with clinicopathologic characteristics of hepatocellular carcinoma.

Parameters	No. of cases	MT1JP expression		*p*-value
		High (%)	Low (%)	
Age (years)				0.592
<60	20	12	8	
≥60	25	13	12	
Gender				0.423
Male	22	12	10	
Female	23	13	10	
T stage				0.023
T1-T2	22	16	6	
T3-T4	23	9	14	
Lymph node metastasis				0.540
No	27	16	11	
Yes	18	9	9	
Distance metastasis				0.577
No	29	17	12	
Yes	16	8	8	

overexpression of MT1JP, Western blot, and qRT-PCR results revealed that the expression of AKT in HCC cell lines was markedly reduced (Figure 3A). Furthermore, Western blot and qRT-PCR assays confirmed the overexpression efficiency of AKT overexpression vector (Figure 3B). In addition, it was found by qRT-PCR that AKT expression was increased in HCC tumor specimens compared with the paracancerous normal ones (Figure 3C), which revealed a negative correlation between AKT and MT1JP expression in HCC tissues (Figure 3D).

MT1JP Modulated AKT Expression in Human HCC Cells

To further explore the interaction between MT1JP and AKT, HCC cell lines were co-transfectedMT1JP and AKT, and the transfection efficiency was confirmed through Western blot (Figure 4A). Subsequently, CCK-8, plate cloning experiment, and flow cytometry assay demonstrated that the overexpression of AKT remarkably enhanced cell proliferation capacity while attenuated the apoptosis ability of HCC cells in the MT1JP upregulation group (Figure 4B, 4C, 4D), indicating that AKT could counteract the impact of MT1JP overexpression on the malignant progression of HCC cells.

Discussion

Hepatocellular carcinoma (HCC), as one of the major malignancies in the world, ranks sixth in morbidity and third in mortality. About 700,000 people die of liver cancer every year, with a mortality rate of 93%[1-3]. At present, surgical resection is still the preferred treatment method[4,5]. Studies[6,7] have shown that the 5-year overall survival rate of patients with HCC after treatment is less than 12%. Due to insidious onset, high malignancy, rapid progress, complex treatment, and short survival time, there is no effective and reliable method for early screening and prognosis judgment[7]. Therefore, it is of great theoretical significance and application value to elucidate the relationship between the clinicopathological characteristics of HCC and relevant molecules[6,7].

Advances in high-throughput screening, sequencing, micro-matrix, and other technologies make lncRNAs highly valued for people. LncRNAs do not encode protein products by themselves but exert their biological functions in organisms with multiple mechanisms[8-11]. They can regulate gene expression mainly through epigenetic regulation, transcriptional regulation, and post-transcriptional regulation[12]. In addition, lncRNA is likely to be engaged in all aspects of the occurrence, development, and metastasis of malignant tumors, which makes it become one of the hotspots in oncology research[17,18]. At present, abnormal expression of lncRNA MT1JP has been found[19,20] in various tumors of different organs, which may be associated with malignant progression of tumors. In this study, the role of lncRNA MT1JP in the occurrence and development of HCC was first investigated in 45 pairs of clinical specimens from HCC patients.

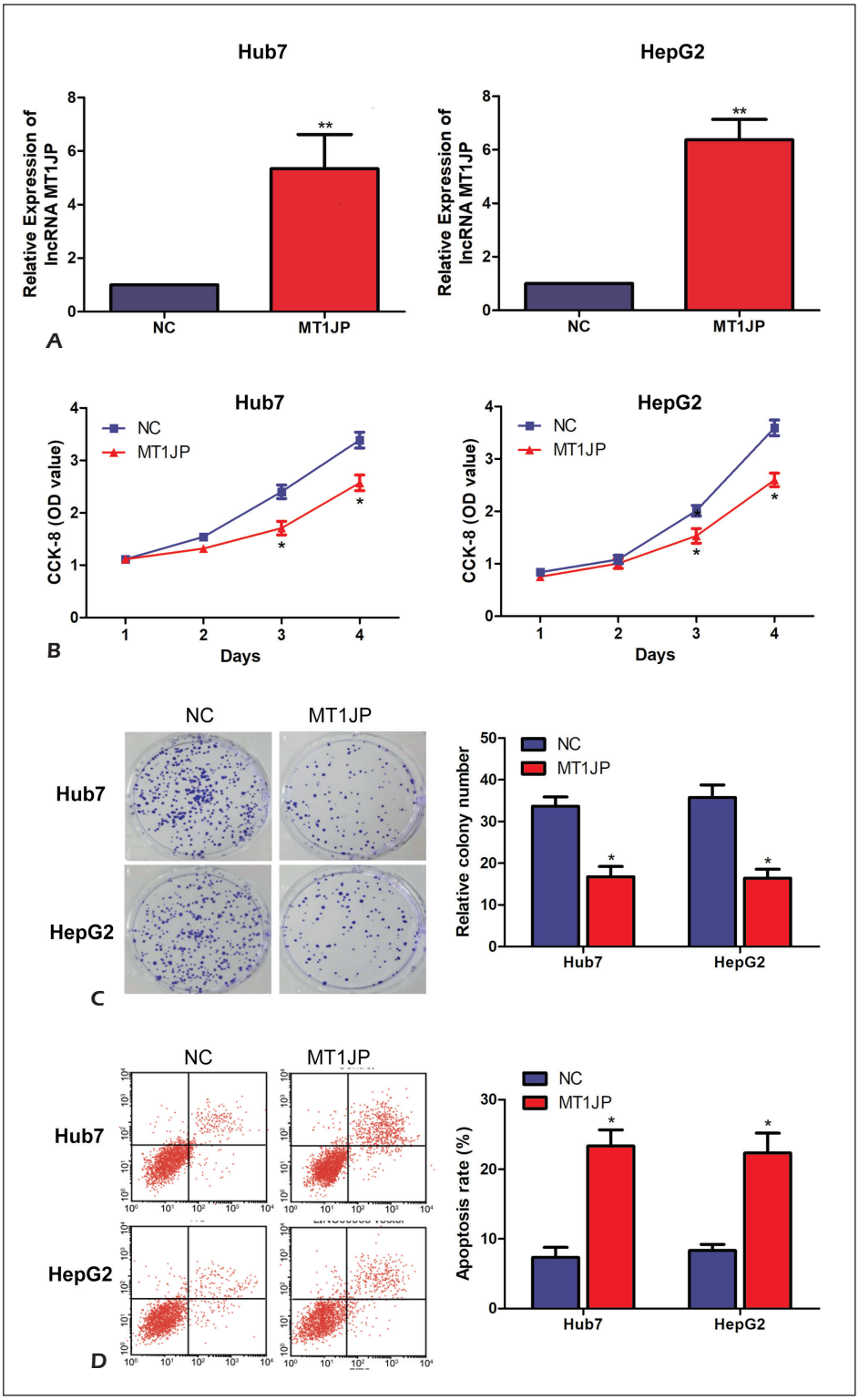

Figure 2. Overexpression of MT1JP inhibits HCC cell proliferation and promotes cell apoptosis. **A**, Verification of transfection efficiency after lentiviral transfection of MT1JP overexpression vector in Hub7 and HepG2 cell lines via qRT-PCR. **B**, Detection of the effect of transfection of the MT1JP overexpression vector on proliferation of Hub7 and HepG2 cell lines via Western blotting and qRT-PCR assays. **C**, Plate cloning experiments for the number of HCC positive cells after transfection of the MT1JP overexpression vector in the Hub7 and HepG2 cell lines (magnification: 10×). **D**, Detection of the apoptosis ability of HCC cells after transfection of MT1JP overexpression vector in Hub7 and HepG2 cell lines via cell flow cytometry. Data are mean ± SD, *p<0.05, **p<0.01.

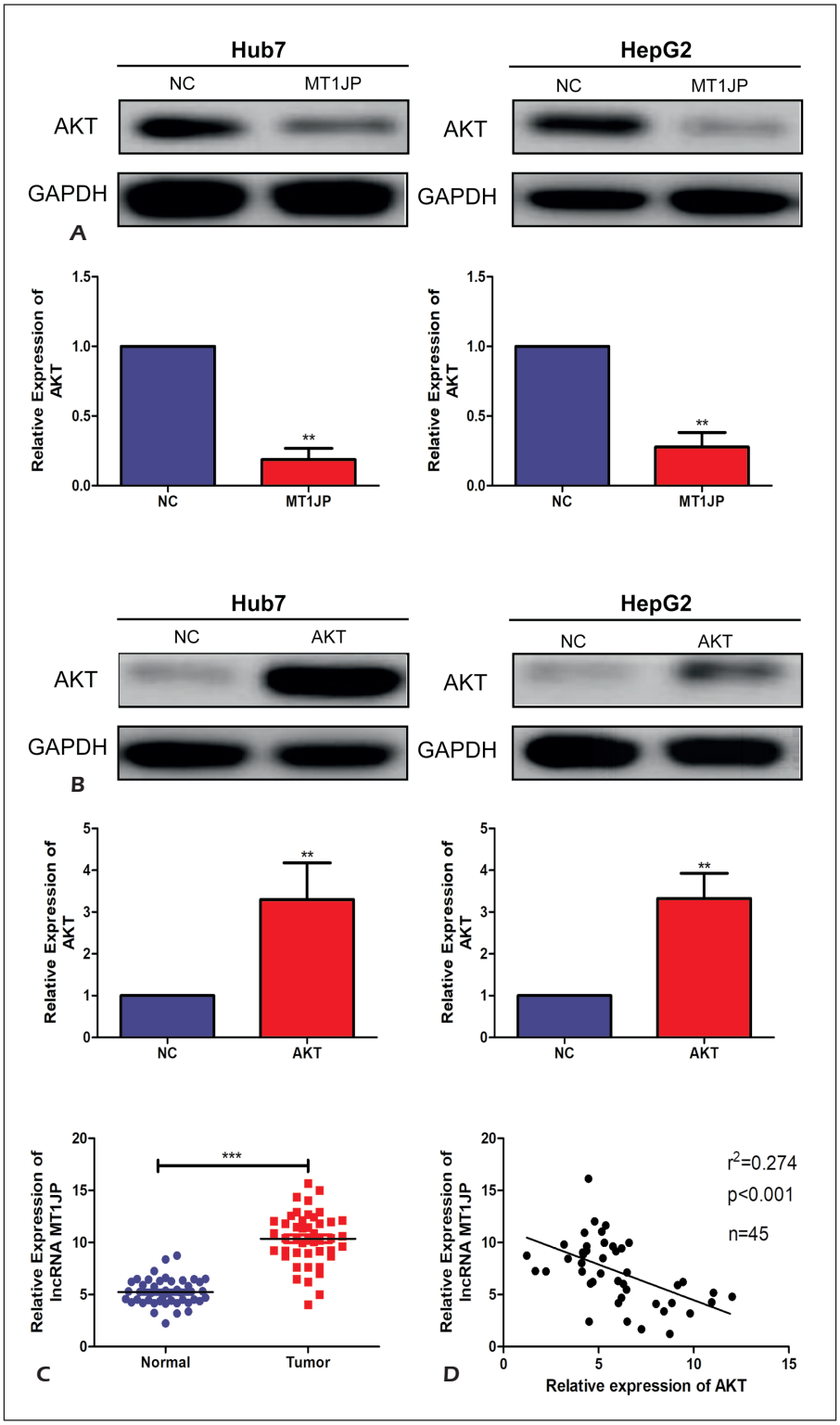

Figure 3. Overexpression of MT1JP reduces the expression of AKT. **A**, Examination of the effect of overexpression of MT1JP on AKT expression in Hub7 and HepG2 cell lines via Western blotting and qRT-PCR assays. **B**, Detection of AKT transfection efficiency after overexpression of AKT in Hub7 and HepG2 cell lines via Western blotting and qRT-PCR assays. **C**, QRT-PCR is used to detect the difference in expression of AKT in hepatocellular carcinoma tumor tissues and adjacent tissues. **D**, QRT-PCR is used to detect the difference in expression of MT1JP and AKT in hepatocellular carcinoma tissues. Data are mean ± SD, $^{**}p<0.01$, $^{***}p<0.001$.

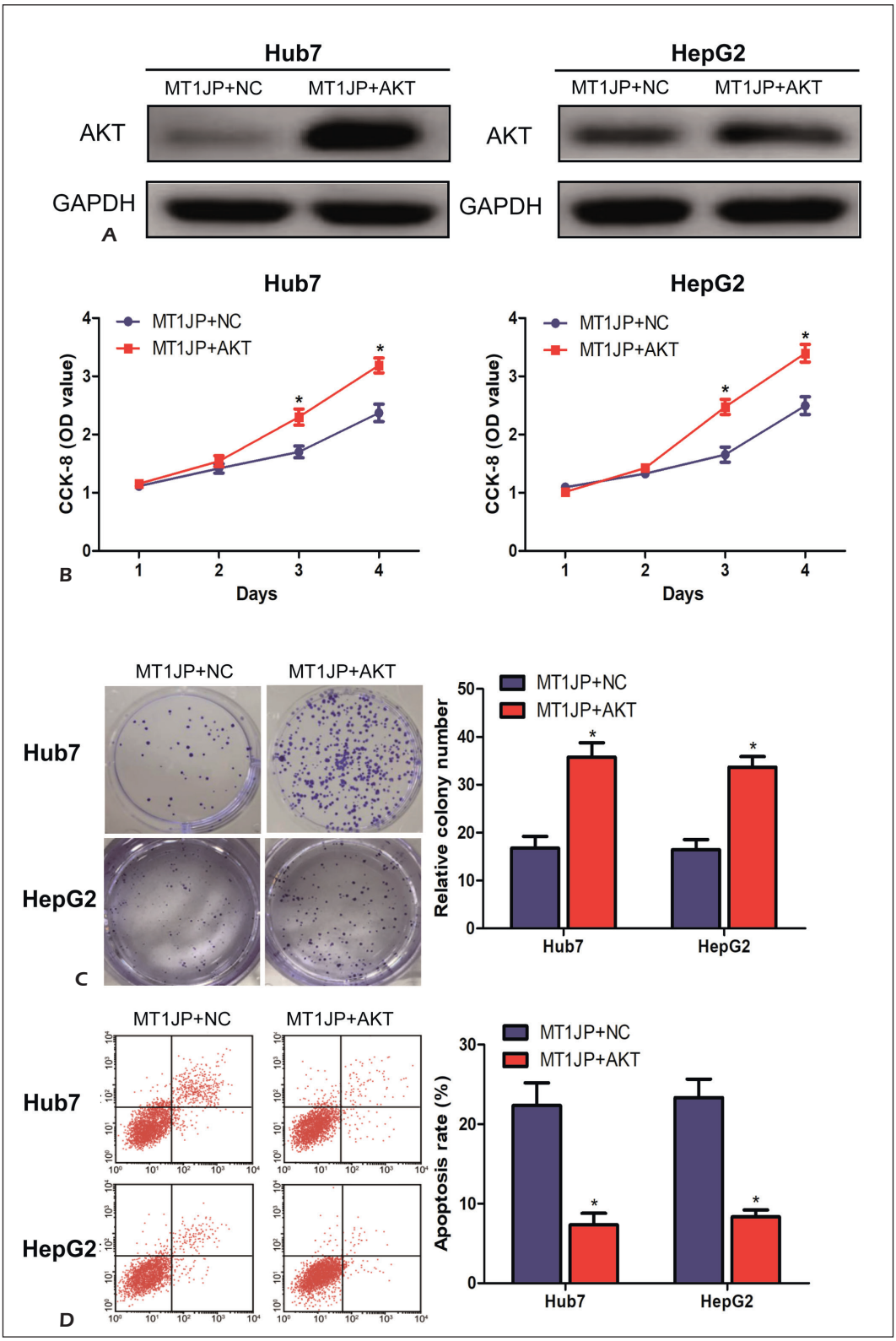

Figure 4. MT1JP regulates AKT in HCC cells. **A**, AKT expression levels in HCC cell lines co-transfected with MT1JP and AKT were detected by Western blotting. **B**, Detection of the proliferation of HCC cells after overexpression of MT1JP and AKT in Hub7 and HepG2 cell lines via CCK-8 assay. **C**, Plate cloning experiments of the number of hepatocellular carcinoma positive cells after overexpression of MT1JP and AKT in the Hub7 and HepG2 cell lines (magnification: 10×). **D**, Detection of the cell apoptosis ability after overexpression of MT1JP and AKT in the Hub7 and HepG2 cell lines via cell flow cytometry.

Detection of the expression of lncRNA MT1JP at the transcriptional level and protein level in fresh HCC surgical specimens and hepatocellular cancer cell lines demonstrated that MT1JP expression in HCC tumor tissues or cell lines was reduced to different degrees compared to that in paracancerous tissues or normal liver cell line LO2, indicating that MT1JP may play a pivotal role in the development of HCC. Meanwhile, the relationship between HCC patients with high and low MT1JP expression and some indicators, including age, gender, pathological stage, lymph node metastasis, and distant metastasis were investigated, and it was found that MT1JP expression was positively correlated with the pathological stage of HCC patients and Kaplan-Meier survival curve also revealed that low MT1JP expression was remarkably correlated with poor prognosis of HCC patients. In other words, the lower the MT1JP expression was, the worse the prognosis of HCC patients would be. Subsequently, CCK-8, plate cloning and flow cytometry experiments demonstrated that MT1JP could inhibit the proliferation while promoting the apoptosis of HCC cells and suggesting an important role of MT-1JP in the progression of HCC. However, the exact molecular mechanism still remains elusive.

Increased AKT expression is relevant to the progression of various human malignant tumors[21]. Current *in vivo* and *in vitro* experimental studies[22,23] suggest that AKT pathway plays a pivotal role in primary invasion and secondary metastasis of colorectal cancer, lung cancer, and other cancers. Therefore, the study on the occurrence and regulatory mechanism of AKT is of great significance for finding the target targets for the treatment of malignant tumors, especially tumor cell metastasis. In this study, Western blot results showed that AKT protein expression was remarkably reduced after MT1JP was overexpressed, and the two showed a negative correlation in HCC tissues, as well as in cell lines. These results suggest that lncRNA MT1JP may exert a significant function in inhibiting cell proliferation and promoting apoptosis of HCC cells through modulating AKT.

Conclusions

In summary, the expression of lncRNA MT-1JP in HCC tissues was remarkably decreased, which was significantly correlated with the pathological stage and poor prognosis of HCC patients. Additionally, MT1JP may inhibit the malignant progression of HCC *via* downregulating AKT.

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Conflict of Interests

The authors declare that they have no conflict of interests.

References

1) Hartke J, Johnson M, Ghabril M. The diagnosis and treatment of hepatocellular carcinoma. Semin Diagn Pathol 2017; 34: 153-159.
2) Wang W, Huang Z, Guo B, Liu S, Xiao W, Liang J. Short- and long-term outcomes of laparoscopic hepatectomy in elderly patients with hepatocellular carcinoma. J BUON 2018; 23: 971-978.
3) Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A, Roberts LR. A global view of hepatocellular carcinoma: trends, risk, prevention and management. Nat Rev Gastroenterol Hepatol 2019; 16: 589-604.
4) Hsu CY, Wang CW, Cheng AL, Kuo SH. Hypofractionated particle beam therapy for hepatocellular carcinoma-a brief review of clinical effectiveness. World J Gastrointest Oncol 2019; 11: 579-588.
5) De Mattia E, Cecchin E, Guardascione M, Foltran L, Di Raimo T, Angelini F, D'Andrea M, Toffoli G. Pharmacogenetics of the systemic treatment in advanced hepatocellular carcinoma. World J Gastroenterol 2019; 25: 3870-3896.
6) Neureiter D, Stintzing S, Kiesslich T, Ocker M. Hepatocellular carcinoma: therapeutic advances in signaling, epigenetic and immune targets. World J Gastroenterol 2019; 25: 3136-3150.
7) Raoul JL, Frenel JS, Raimbourg J, Gilabert M. Current options and future possibilities for the systemic treatment of hepatocellular carcinoma. Hepat Oncol 2019; 6. HEP11.
8) Bhan A, Soleimani M, Mandal SS. Long noncoding RNA and cancer: a new paradigm. Cancer Res 2017; 77: 3965-3981.
9) Pardini B, Sabo AA, Birolo G, Calin GA. Noncoding RNAs in extracellular fluids as cancer biomarkers: the new frontier of liquid biopsies. Cancers (Basel) 2019; 11. pii: E1170.
10) Jiang N, Pan J, Fang S, Zhou C, Han Y, Chen J, Meng X, Jin X, Gong Z. Liquid biopsy: circulating exosomal long noncoding RNAs in cancer. Clin Chim Acta 2019; 495: 331-337.
11) Sarfi M, Abbastabar M, Khalili E. Long noncoding RNAs biomarker-based cancer assessment. J Cell Physiol 2019; 234: 16971-16986.
12) Zhang G, Pian C, Chen Z, Zhang J, Xu M, Zhang L, Chen Y. Identification of cancer-related miRNA-lncRNA biomarkers using a basic miRNA-lncRNA network. PLoS One 2018; 13: e0196681.

13) Wang M, Jiang S, Yu F, Zhou L, Wang K. Noncoding RNAs as molecular targets of resveratrol underlying its anticancer effects. J Agric Food Chem 2019; 67: 4709-4719.

14) Zhao J, Li L, Han ZY, Wang ZX, Qin LX. Long noncoding RNAs, emerging and versatile regulators of tumor-induced angiogenesis. Am J Cancer Res 2019; 9: 1367-1381.

15) Miranda-Castro R, De-Los-Santos-Alvarez N, Lobo-Castanon MJ. Long noncoding RNAs: from genomic junk to rising stars in the early detection of cancer. Anal Bioanal Chem 2019; 411: 4265-4275.

16) Shi H, Xu Y, Yi X, Fang D, Hou X. Current research progress on long noncoding RNAs associated with hepatocellular carcinoma. Anal Cell Pathol (Amst) 2019; 2019: 1534607.

17) Zhu C, Ma J, Li Y, Zhang Y, Da M. Low expression of long noncoding RNA MT1JP is associated with poor overall survival in gastric cancer patients: protocol for meta-analysis. Medicine (Baltimore) 2018; 97: e10394.

18) Odell ID, Flavell RA. HER2 joins AKT to inhibit STING immunity. Nat Cell Biol 2019; 21: 917-918.

19) Jaiswal N, Gavin MG, Quinn WR, Luongo TS, Gelfer RG, Baur JA, Titchenell PM. The role of skeletal muscle Akt in the regulation of muscle mass and glucose homeostasis. Mol Metab 2019; 28: 1-13.

20) Hegazy RR, Mansour DF, Salama AA, Abdel-Rahman RF, Hassan AM. Regulation of PKB/Akt-pathway in the chemopreventive effect of lactoferrin against diethylnitrosamine-induced hepatocarcinogenesis in rats. Pharmacol Rep 2019; 71: 879-891.

21) Kahraman DC, Kahraman T, Cetin-Atalay R. Targeting PI3K/Akt/mTOR pathway identifies differential expression and functional role of IL8 in liver cancer stem cell enrichment. Mol Cancer Ther 2019; 18: 2146-2157.

22) Ren M, Xu W, Xu T. Salidroside represses proliferation, migration and invasion of human lung cancer cells through AKT and MEK/ERK signal pathway. Artif Cells Nanomed Biotechnol 2019; 47: 1014-1021.

23) Zhu Y, Zhong Y, Long X, Zhu Z, Zhou Y, Ye H, Zeng X, Zheng X. Deoxyshikonin isolated from Arnebia euchroma inhibits colorectal cancer by down-regulating the PI3K/Akt/mTOR pathway. Pharm Biol 2019; 57: 412-423.