European **Rev**iew for **Med**ical and **Pharmacol**ogical **Sci**ences 2021; 25: 1853-1860

MiR-335-5p inhibits proliferation of Huh-7 liver cancer cells *via* targeting the Oct4/Akt pathway

Y.-Y. JI[1], Y. SONG[2], A.-N. WANG[3]

[1]Department of Infectious Diseases, Jiaozhou Central Hospital of Qingdao, Qingdao, China
[2]Department of Gastroenterology and Hepatology, Zoucheng People's Hospital, Zoucheng, China
[3]Department of Internal Medicine, Jiaozhou People's Hospital, Jiaozhou, China

Yuanyuan Ji and Yan Song contributed equally to this work

Abstract. – OBJECTIVE: The aim of this study was to detect the expression of micro ribonucleic acid (miR)-335-5p in the liver tissues of patients with liver cancer, and to explore its effect on liver cancer and mechanism using Huh7 human liver cancer cells.

PATIENTS AND METHODS: Liver tissues were collected from patients with liver cancer. The expression of miR-335-5p in tissues was detected via quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Subsequently, Huh7 cells were transfected with miR-335-5p in vitro. After overexpressing miR-335-5p, changes in the expression of octamer-binding transcription factor 4 (Oct4) gene were observed via qRT-PCR. Furthermore, the proliferation of Huh7 cells and the protein expressions of protein kinase B (Akt) and phosphorylated Akt (p-Akt) were detected using cell counting kit (CCK)-8 assay and Western blotting (WB), respectively.

RESULTS: Compared with Control group, the expression of miR-335-5p increased significantly in the liver tissues of liver cancer patients ($p<0.01$). In comparison with those in negative group, the messenger RNA (mRNA) expression of Oct4 and the proliferation rate of Huh7 cells were both significantly inhibited in miR-335-5p group ($p<0.01$, $p<0.05$). After overexpression of miR-335-5p, the protein expression level of p-Akt decreased remarkably ($p<0.01$).

CONCLUSIONS: MiR-335-5p directly binds to the 3' untranslated region (3'UTR) of Oct4 mRNA to restrain the phosphorylation of Akt, thereby inhibiting Huh7 cell proliferation.

Key Words:
Liver cancer, Huh7 cells, Oct4, MiR-335-5p, Proliferation.

Introduction

Liver cancer, an invasive cancer, is the sixth most common cancer worldwide. Meanwhile, it is also the third leading cause of cancer deaths. Liver cancer can be classified into two types, including hepatocellular carcinoma (HCC) and hepatoblastoma (HB)[1-3]. Both HCC and HB possess distinct cytological features[4]. For instance, HepG2 was initially recognized as an HCC cell line, but was misidentified and proved to originate from HB[5]. Currently, the global morbidity rate of liver cancer varies greatly among sexes and regions[3]. Moreover, the morbidity rate of liver cancer in different regions reflects the regional differences in exposure to hepatitis viruses and environmental pathogens. In developing countries, hepatitis B virus (HBV) infection and hepatitis C virus (HCV) infection represent 60% and 33% of total liver cancer cases, respectively[6]. In recent years, liver resection and transplantation are efficacious in patients with early liver cancer. However, due to the delayed detection, the prognosis of most patients is still poor, with a low survival rate[7-9]. Hence, it is desperately needed to explore the potential mechanism of liver cancer to elucidate its pathogenesis, and to develop a novel target for the treatment of liver cancer.

Micro ribonucleic acids (MiRNAs) are a main class of short non-coding RNAs extensively researched[10]. They are structurally highly conservative and can affect the inhibition on translation or messenger RNA (mRNA) cleavage. MiRNAs have also been confirmed to abolish the biological functions of target genes, thereby playing an important role in malignant tumors[11-13]. To date, more than 2,000 kinds of miRNAs have been identified in humans, playing pivotal roles in various biological processes[14-16]. Increasingly more studies have demonstrated that miRNAs exert a critical role in the development and progression of human tumors, especially liver cancer. Dysregulation of miRNAs has also been recognized as the potential molecular marker of tumors[17-19]. Pre-

Corresponding Author: Anna Wang, BM; e-mail: wanganna1976@163.com

vious reports[20,21] have indicated that some miRNAs, including miR-24a, miR-30a-3p, miR-138, miR-203 and miR-451, are associated with tumor progression. Moreover, numerous miRNAs have been verified to be involved in the development of liver cancer. In particular, miR-28-5p modulates the proliferation of liver cancer stem cells *via* the IGF-1 pathway[22]. High expression of miR-196a is associated with the progression of HCC in young patients[23]. Additionally, miR-101 regulates the chemoresistance of liver cancer cells to cis-platinum through the DNA-PKCs signaling pathway[24]. MiR-225-5p, widely explored in cancers, represses the metastasis of gastric cancer by targeting B-cell lymphoma-W (Bcl-W) and specific protein 1[25]. Furthermore, its overexpression can inhibit the expression of EphA4 in breast cancer, thereby inhibiting breast cancer cell proliferation[26]. According to researches into colorectal cancer, the overall survival is significantly shorter in patients with down-regulation of miR-335-5p. However, its overexpression can restrain the migration and invasion of tumor cells. Therefore, miR-335-5p serves as a potential target for the treatment of colorectal cancer[27]. In addition, miR-335-5p up-regulates the expression of CRKL, eventually suppressing the proliferation and migration of bladder cancer cells[28]. However, the functions of miR-335-5p in mediating the proliferation and metastasis of liver cancer cells have not been fully elucidated. Therefore, the molecular mechanism of miR-335-5p in liver cancer needs to be further explored.

It is worth noting that the phosphatidylinositol 3-hydroxy kinase/protein kinase B (PI3K/Akt) signaling pathway is a canonical regulator in the development of liver cancer[29]. Dysregulation of this pathway induces cell growth disorder. As the cores of this pathway, both PI3K and Akt can mediate downstream biological effects *via* various molecules, such as NF-κB, VEGF and FOXO[30]. Sun et al[31] found that the PI3K/Akt pathway plays a pivotal role in a variety of solid tumors. It has also been discovered that octamer-binding transcription factor 4 (Oct4), with the carcinogenic potential, induces stem-like properties and resists apoptosis in cancer[32,33]. The present study, therefore, aimed to examine whether miR-335-5p and Oct4 were correlated with the PI3K/Akt signaling pathway.

Patients and Methods

Cells and Reagents

Huh7 human liver cancer cells were purchased from the Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China); RNA extraction kit from QIAGEN (Hilden, Germany); Lipofectamine 2000 transfection reagent from Invitrogen (Carlsbad, CA, USA); miR-335-5p mimics from Thermo Fisher Scientific, Inc. (Waltham, MA, USA); Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) from Gibco (Rockville, MD, USA); Luciferase plasmids from Promega Corporation (Madison, WI, USA); radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China), polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA, USA), enhanced chemiluminescence (ECL) Plus Western blotting detection reagent and horseradish peroxidase (HRP)-coupled secondary antibodies from ImmunoWay (Plano, TX, USA).

Collection of Clinical Samples

Paired HCC tissue samples (n=20) and adjacent non-tumor tissue samples (n=20) were collected from HCC patients who received radical surgery from May 2015 to December 2017 in Jiaozhou Central Hospital of Qingdao. All the patients did not undergo chemotherapy or radiotherapy before surgery. Collected tissue samples were immediately cryopreserved in liquid nitrogen at -80°C for use. This investigation was approved by the Ethics Committee of Jiaozhou Central Hospital of Qingdao. Signed written informed consents were obtained from all participants before the study.

Target Gene Prediction and Analysis

Target genes of miR-335-5p were predicted using TargetScan, and the resulting target genes were uploaded to Metascape for enrichment analysis. Based on species, TargetScan[34], an online database dedicated to analyzing the miRNA target genes of mammalians, is divided into the following sub-databases: TargetScanHuman, TargetScanMouse, TargetScanFly, TargetScanWorm and TargetScanFish. Metascape[35] integrates multiple authoritative database resources from GO, KEGG, Uniprot and DrugBank. Therefore, it supports pathway enrichment analysis, biological process annotation, gene-related protein-protein interaction network analysis and drug analysis concerned.

Cell Culture and Transfection

Huh7 human liver cancer cells were first evenly distributed into a cell culture flask. The cells were cultured in DMEM containing 10% FBS in

an incubator with 5% CO_2 at 37°C. For cell transfection, Huh7 cells were first seeded into 24-well plates. Subsequently, the cells were transfected with 100 nM miR-335-5p mimics (miR-335-5p group) or negative controls (Negative group) according to the instructions of Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The Oct4 overexpression system plasmid [complementary deoxyribonucleic acid 3 (pcDNA)-Oct4] was constructed by inserting intact Oct4 sequences into the pcDNA3.1 plasmid. On the second day, the pcDNA-Oct4 plasmid was transfected into Huh7 cells *via* Lipofectamine 2000. At 48 h after transfection, the cells were harvested for subsequent experiments.

Cell Proliferation via Cell Counting Kit (CCK)-8 Assay

First, Huh7 cells were inoculated into 96-well plates (Thermo Fisher Scientific, Inc., Waltham, MA, USA) at a density of 1×10^4 cells/well. Then, the cells were transfected with 1 pM miR-335-5p mimics or negative controls, followed by culture for 24, 48 and 72 h. At each time point, 10 μL of CCK-8 reagent (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) was added to each well, followed by culture at 37°C for another 2 h in the dark. Optical density at 450 nm (OD_{450}) was finally measured using a spectrophotometer (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Each experimental condition was determined in triplicate, and all the experiments were repeated for at least 3 times.

Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR)

Total RNAs in tissues and cells were extracted using TRIzol reagent and quantified by NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, extracted RNAs were reversely transcribed into cDNA using reverse transcriptase. Mature miR-335-5p relative to U6 and Oct4 relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were quantified *via* qRT-PCR using 7900HT fast real-time PCR system (Applied Biosystems, Foster City, CA, USA). Primer sequences used in this study were listed in Table I.

Luciferase Reporter Gene Assay

Huh7 cells cultured in 24-well plates were transfected with PGL3 Luciferase reporter plasmids containing mutant-type or wild-type Oct4 3' untranslated region (3'UTR) and miR-335-5p using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA). After culture for 24 h, the Luciferase activity was measured according to the manufacturer's protocols of Dual-Luciferase reporter analysis system (Promega, Madison, WI, USA).

Western Blotting

Total proteins were extracted from Huh7 cells transfected with miR-335-5p using RIPA lysis buffer. Subsequently, protein samples were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes. After being sealed by 5% skim milk powder, the membranes were incubated with primary antibodies of Akt and phosphorylated Akt (p-Akt) overnight. On the next day, the membranes were incubated with HRP-coupled secondary antibodies. Immunoreactive bands were finally detected using the enhanced chemiluminescence (ECL) exposure system.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 software (IBM Corp., Armonk, NY, USA) was utilized for statistical analysis. Measurement data were presented as mean ± standard deviation. Independent-samples *t*-test was used to compare the difference between two groups. $p<0.05$ suggested that the differences were statistically significant.

Table I. QRT-PCR primer sequences.

Gene	Forward (5′-3′)	Reverse (5′-3′)
GAPDH	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
Oct4	CTTGAATCCCGAATGGAAAGGG	CCTTCCCAAATAGAACCCCCA
MiR-335-5p	TCAAGAGCAATAACGAAAAATGT	TGGTGTCGTGGAGTCG

Results

Up-regulation of MiR-335-5p Expression in Liver Cancer Patients

First, the information of liver cancer patients was statistically explained (Table II). QRT-PCR analysis showed that the expression of miR-335-5p increased significantly in the liver tissues of liver cancer patients when compared with Control group (p<0.01) (Figure 1).

Predicted Target Genes of MiR-335-5p

TargetScan predicted that miR-335-5p had 289 target genes, including Oct4 (POU5F1). Subsequently, the target gene set was up-loaded to the online tool Metascape for enrichment analysis. A series of biological processes and pathways in which the target genes were involved were finally acquired. Besides, the biological processes covered abundant categories (Figure 2).

Transfection with MiR-335-5p Induced MiR-335-5p Expression

To verify whether transfection with miR-335-5p induced overexpression of miR-335-5p in Huh7 cells, qRT-PCR was conducted. The results confirmed that the expression of miR-335-5p in miR-335-5p group was significantly higher than that in Negative group (p<0.01; Figure 3).

MiR-335-5p Overexpression Inhibited Oct4 Gene Expression

The changes in Oct4 expression were detected *via* qRT-PCR to observe whether miR-335-5p overexpression could alter the expression of Oct4. According to the results, the expression of Oct4 was evidently down-regulated after miR-335-5p overexpression compared with that in Negative group (p<0.01; Figure 4).

Table II. Statistics of information of 20 liver cancer patients.

Parameter	n
Age	
>50	10
≤50	10
Sex	
Male	10
Female	10
TMN stage	
I/II	10
III/IV	10

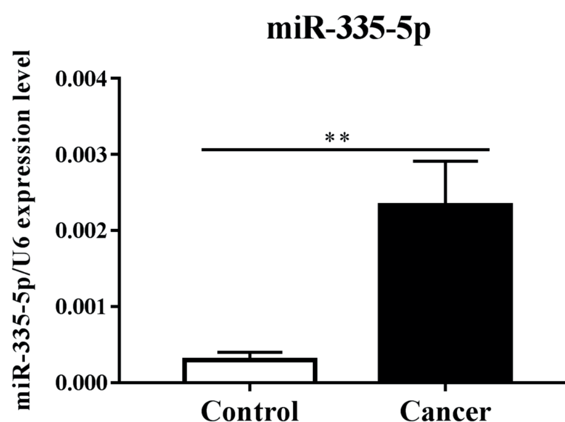

Figure 1. QRT-PCR results reveal that the expression of miR-335-5p is up-regulated in the liver tissues of liver cancer patients. Note: compared with that in control group, the expression of miR-335-5p increases in patients with liver cancer (**p<0.01).

MiR-335-5p Overexpression Restrained Huh7 Cell Proliferation

The changes in the proliferation of Huh7 human liver cancer cells after miR-335-5p overexpression was detected by CCK-8 assay. It was discovered that the OD_{450} of Huh7 cells remarkably declined at 48 and 72 h in miR-335-5p group (p<0.05). These results implied that the proliferation rate of Huh7 cells was distinctly repressed (Figure 5).

MiR-335-5p Directly Bound to Oct4 to Inhibit the Activity of the Akt Pathway

To clarify the mechanism of action of miR-335-5p in liver cancer, the changes in the protein expressions of Akt and p-Akt after miR-335-5p overexpression were detected by Western blotting. The results indicated that miR-335-5p overexpression significantly down-regulated the protein expression of p-Akt (p<0.01; Figure 6). The interaction between miR-335-5p and Oct4 was determined *via* Luciferase reporter gene assay. It was found that the activity of wild-type Oct4 reporter plasmid increased remarkably in Huh7 cells with miR-335-5p overexpression (p<0.01). However, no evident changes were observed in the mutant-type Oct4 reporter system (Figure 7).

Discussion

MiRNAs are the most extensively researched non-coding RNAs currently. Previous studies have established that increasing miRNA molecules are identified as markers. Many miRNAs

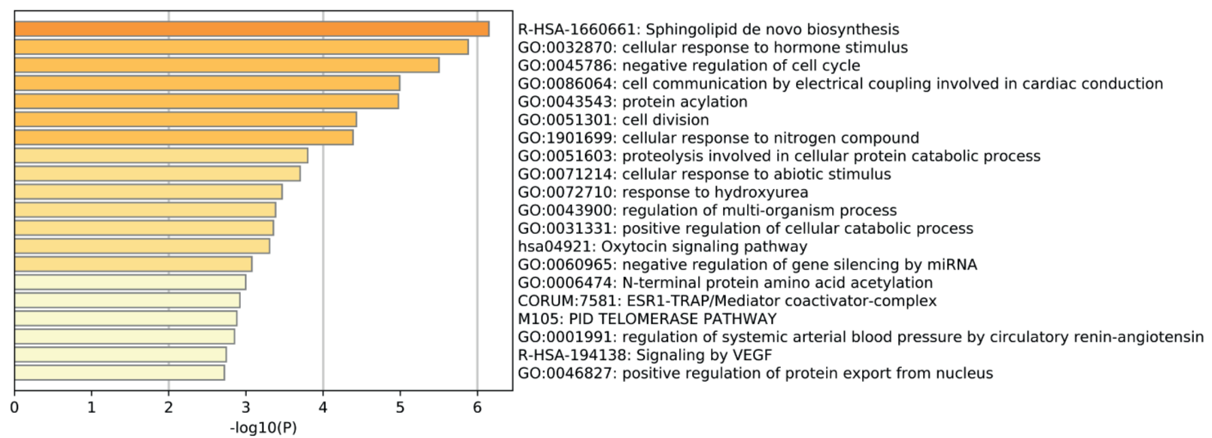

Figure 2. Statistics of enriched terms of miR-335-5p target genes. Note: $p<0.05$ for all the terms.

have been found associated with the occurrence of liver cancer[36]. Of note, miR-370 down-regulates the expression of PIM1 in HCC to resist cancer development[37]. MiR-548b directly binds to the mRNA of high mobility group box 1 to repress the invasion of HCC[38]. MiR-335-5p, widely explored in cancers, targets intercellular adhesion molecule 1 (ICAM-1) to suppress the invasion and metastasis of thyroid cancer cells[39]. Wang et al[40] proposed that miR-335-5p overexpression can be taken as a new treatment strategy for HCC since it suppresses the proliferation and migration of liver cancer cells. However, its mechanism of action has not been fully elucidated. Our results showed that miR-335-5p, as a novel liver cancer-related miRNA molecule, significantly inhibited the growth of Huh7 human liver cancer cells. All these findings suggested that miR-335-5p functioned as a tumor suppressor miRNA.

Besides, our results revealed that miR-335-5p served as a therapeutic target for liver cancer. Compared with other studies, the present research not only verified the expression of miR-335-5p in liver cancer, but also partly corroborated the expression and mechanism of miR-335-5p *in vitro*. Moreover, the differential expression of miR-335-5p predicted its important role in disease progression. Online website predicted that Oct4 acted as the target gene of miR-335-5p in HCC. Meanwhile, miR-335-5p could bind to Oct4 to inhibit the proliferation of Huh7 liver cancer cells. Oct4 (POU5F1), a stem-related gene, is expressed in embryonic stem cells, germ cells and various cancer cells[41-43]. Zeineddine et al[44] have

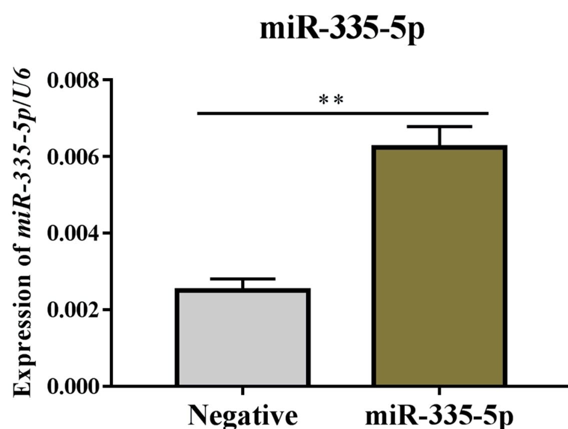

Figure 3. Transfection with miR-335-5p induces the expression of miR-335-5p. Note: in comparison with that in negative group, the expression level of miR-335-5p is substantially up-regulated in miR-335-5p group (**$p<0.01$).

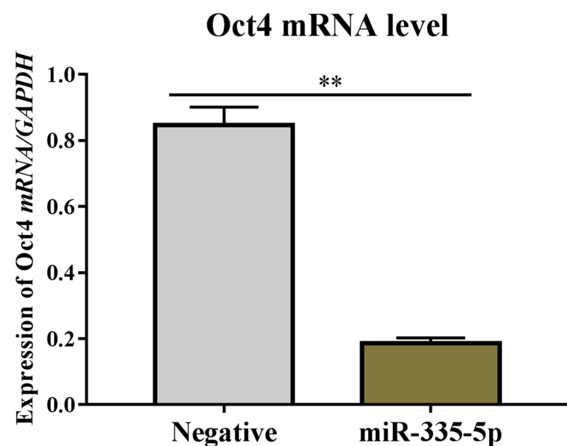

Figure 4. Transfection with miR-335-5p mimics inhibits the expression of Oct4 in Huh7 cells. Note: the expression of Oct4 in miR-335-5p group is notably lower than that in negative group (**$p<0.01$).

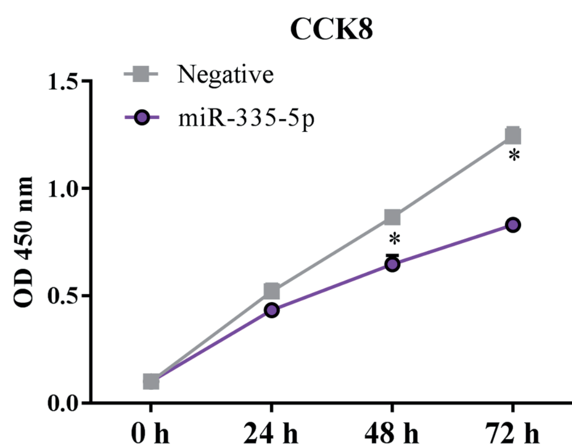

Figure 5. MiR-335-5p overexpression represses the proliferation of Huh7 cells. Note: the OD_{450} of Huh7 cells distinctly declines at 48 and 72 h in miR-335-5p group compared with that in negative group (*$p<0.05$).

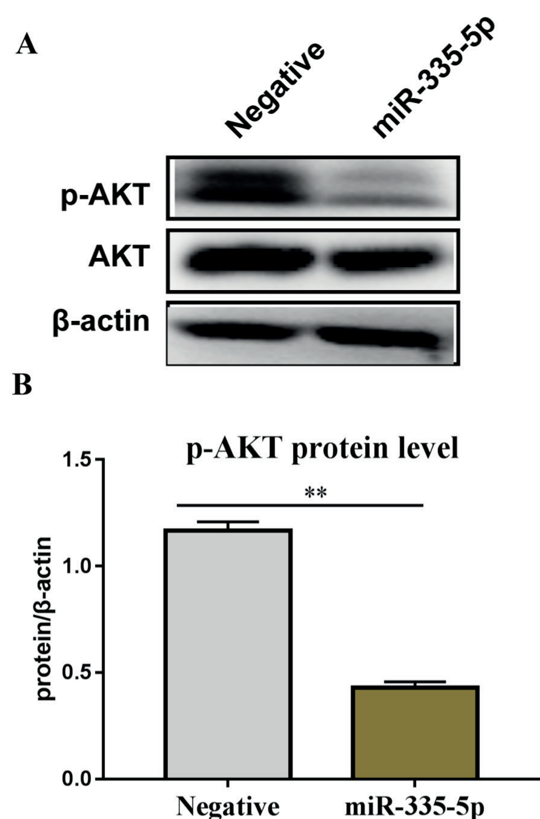

Figure 6. MiR-335-5p overexpression inhibits p-Akt expression. **A,** Akt and p-Akt protein electrophoresis bands after transfection with miR-335-5p in Huh7 cells. **B,** Quantitative results of Akt and p-Akt proteins after transfection with miR-335-5p in Huh7 cells. Note: **$p<0.01$: there is a statistically significant difference between negative group and miR-335-5p group.

considered Oct4 as a major regulator of stem cells. According to previous researches, the expression of Oct4 is correlated with the recurrence of liver cancer by depending on STAT3[45]. It has been reported that Oct4 has several targets, including ICAM-1, CPNE1 and Bcl-W[39,46,47]. However, there has not yet been a report that miR-335-5p functions by modulating Oct4 in liver cancer. In this study, bioinformatics analysis showed that Oct4 was likely to be a target of miR-335-5p. Their interaction in HCC has also been verified. The Akt signaling pathway plays a crucial role in the growth of liver cancer cells[48,49]. At present, the potential mechanism of miR-335-5p *via* the Akt signaling pathway has not been fully reported. Therefore, it was assumed that miR-335-5p inhibited Oct4 expression to regulate the activity of the Akt signaling pathway. The present study laid a solid foundation for the development and verification of miRNAs that could serve as diagnostic markers or therapeutic targets for liver cancer. Despite relatively full prediction and verification, there are still some deficiencies. For example, the assumption was not verified *in vivo*, so the mechanism of miR-335-5p in liver cancer patients remains to be explored in depth. Additionally, whether miR-335-5p could truly serve as a marker in liver cancer it needs to be further confirmed for better clinical application.

Conclusions

MiR-335-5p is differentially expressed in liver tissues of patients with liver cancer. Oct4 serves

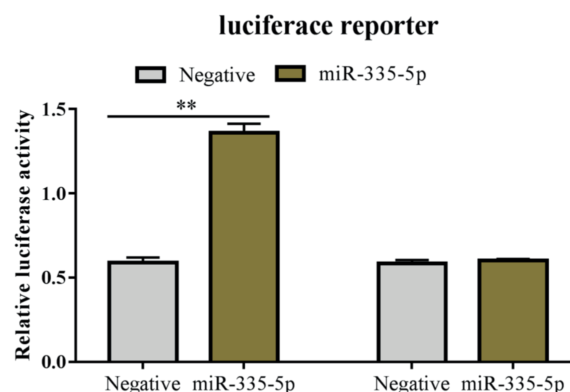

Figure 7. Luciferase activity of wild-type and mutant-type Oct4 reporters in Huh7 cells after transfection with negative or miR-335-5p. Note: **$p<0.01$: there is a statistically significant difference between negative group and miR-335-5p group.

as the target gene of miR-335-5p. These results lay a foundation for further investigation of the mechanism of miR-335-5p in liver cancer. Moreover, miR-335-5p inhibits Oct4 gene expression to restrain the activation of the downstream Akt signaling pathway. The novelty of this study was that our findings could provide scientific bases for the treatment of liver cancer with miR-335-5p and put forward the possibility of miR-335-5p as the potential treatment target for liver cancer.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Liu Y, Wang Y, Sun X, Mei C, Wang L, Li Z, Zha X. MiR-449a promotes liver cancer cell apoptosis by downregulation of Calpain 6 and POU2F1. Oncotarget 2016; 7: 13491-13501.
2) Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010; 127: 2893-2917.
3) Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.
4) Azlin AH, Looi LM, Cheah PL. Tissue microarray immunohistochemical profiles of p53 and pRB in hepatocellular carcinoma and hepatoblastoma. Asian Pac J Cancer Prev 2014; 15: 3959-3963.
5) Lopez-Terrada D, Cheung SW, Finegold MJ, Knowles BB. Hep G2 is a hepatoblastoma-derived cell line. Hum Pathol 2009; 40: 1512-1515.
6) Parkin DM. The global health burden of infection-associated cancers in the year 2002. Int J Cancer 2006; 118: 3030-3044.
7) Liu CY, Chen KF, Chen PJ. Treatment of liver cancer. Cold Spring Harb Perspect Med 2015; 5: a21535.
8) Maluccio M, Covey A. Recent progress in understanding, diagnosing, and treating hepatocellular carcinoma. CA Cancer J Clin 2012; 62: 394-399.
9) Huang KW, Jayant K, Lee P, Yang P, Hsiao C, Habib N, Sodergren MH. Positive immuno-modulation following radiofrequency assisted liver resection in hepatocellular carcinoma. J Clin Med 2019; 8: 385.
10) Kloosterman WP, Plasterk RH. The diverse functions of microRNAs in animal development and disease. Dev Cell 2006; 11: 441-450.
11) Zhou L, Liu F, Wang X, Ouyang G. The roles of microRNAs in the regulation of tumor metastasis. Cell Biosci 2015; 5: 32.
12) Ambros V. The functions of animal microRNAs. Nature 2004; 431: 350-355.
13) Ding T, Xu J, Sun M, Zhu S, Gao J. Predicting microRNA biological functions based on genes discriminant analysis. Comput Biol Chem 2017; 71: 230-235.
14) Xu N, Zhang J, Shen C, Luo Y, Xia L, Xue F, Xia Q. Cisplatin-induced downregulation of miR-199a-5p increases drug resistance by activating autophagy in HCC cell. Biochem Biophys Res Commun 2012; 423: 826-831.
15) Carthew RW, Sontheimer EJ. Origins and mechanisms of miRNAs and siRNAs. Cell 2009; 136: 642-655.
16) Jia XQ, Cheng HQ, Qian X, Bian CX, Shi ZM, Zhang JP, Jiang BH, Feng ZQ. Lentivirus-mediated overexpression of microRNA-199a inhibits cell proliferation of human hepatocellular carcinoma. Cell Biochem Biophys 2012; 62: 237-244.
17) Wang Y, Chen F, Zhao M, Yang Z, Li J, Zhang S, Zhang W, Ye L, Zhang X. The long noncoding RNA HULC promotes liver cancer by increasing the expression of the HMGA2 oncogene via sequestration of the microRNA-186. J Biol Chem 2017; 292: 15395-15407.
18) Chen R, Liao JY, Huang J, Chen WL, Ma XJ, Luo XD. Downregulation of SRC kinase signaling inhibitor 1 (SRCIN1) expression by microRNA-32 promotes proliferation and epithelial-mesenchymal transition in human liver cancer cells. Oncol Res 2018; 26: 573-579.
19) Yin S, Fan Y, Zhang H, Zhao Z, Hao Y, Li J, Sun C, Yang J, Yang Z, Yang X, Lu J, Xi JJ. Differential TGFβ pathway targeting by miR-122 in humans and mice affects liver cancer metastasis. Nat Commun 2016; 7: 11012.
20) Chen KJ, Hou Y, Wang K, Li J, Xia Y, Yang XY, Lv G, Xing XL, Shen F. Reexpression of Let-7g microRNA inhibits the proliferation and migration via K-Ras/HMGA2/snail axis in hepatocellular carcinoma. Biomed Res Int 2014; 2014: 742417.
21) Ma Y, She XG, Ming YZ, Wan QQ. MiR-24 promotes the proliferation and invasion of HCC cells by targeting SOX7. Tumour Biol 2014; 35: 10731-10736.
22) Xia Q, Han T, Yang P, Wang R, Li H, Zhang J, Zhou X. MicroRNA-28-5p regulates liver cancer stem cell expansion via IGF-1 pathway. Stem Cells Int 2019; 2019: 8734362.
23) Wang SY, Chen CL, Hu YC, Chi Y, Huang YH, Su CW, Jeng WJ, Liang YJ, Wu JC. High expression of microRNA-196a is associated with progression of hepatocellular carcinoma in younger patients. Cancers (Basel) 2019; 11: 1549.
24) Chai Z, Yin X, Chen J, Shi J, Sun J, Liu C, Liu F, Cheng S. MicroRNA-101 modulates cisplatin chemoresistance in liver cancer cells via the DNA-PKcs signaling pathway. Oncol Lett 2019; 18: 3655-3663.
25) Xu Y, Zhao F, Wang Z, Song Y, Luo Y, Zhang X, Jiang L, Sun Z, Miao Z, Xu H. MicroRNA-335 acts as a metastasis suppressor in gastric cancer by targeting Bcl-w and specificity protein 1. Oncogene 2012; 31: 1398-1407.
26) Dong Y, Liu Y, Jiang A, Li R, Yin M, Wang Y. MicroRNA-335 suppresses the proliferation, migration, and invasion of breast cancer cells by targeting EphA4. Mol Cell Biochem 2018; 439: 95-104.
27) Sun Z, Zhang Z, Liu Z, Qiu B, Liu K, Dong G. MicroRNA-335 inhibits invasion and metastasis of colorectal cancer by targeting ZEB2. Med Oncol 2014; 31: 982.

28) Liu XK, Chen D, Li X. MiR-335 suppresses cell proliferation and migration by upregulating CRKL in bladder cancer. Eur Rev Med Pharmacol Sci 2019; 23: 2399-2408.
29) Fruman DA, Chiu H, Hopkins BD, Bagrodia S, Cantley LC, Abraham RT. The PI3K pathway in human disease. Cell 2017; 170: 605-635.
30) Eijkelenboom A, Burgering BM. FOXOs: signalling integrators for homeostasis maintenance. Nat Rev Mol Cell Biol 2013; 14: 83-97.
31) Sun Y, Tian H, Wang L. Effects of PTEN on the proliferation and apoptosis of colorectal cancer cells via the phosphoinositol-3-kinase/Akt pathway. Oncol Rep 2015; 33: 1828-1836.
32) Hochedlinger K, Yamada Y, Beard C, Jaenisch R. Ectopic expression of Oct-4 blocks progenitor-cell differentiation and causes dysplasia in epithelial tissues. Cell 2005; 121: 465-477.
33) Chen YC, Hsu HS, Chen YW, Tsai TH, How CK, Wang CY, Hung SC, Chang YL, Tsai ML, Lee YY, Ku HH, Chiou SH. Oct-4 expression maintained cancer stem-like properties in lung cancer-derived CD133-positive cells. PLoS One 2008; 3: e2637.
34) Agarwal V, Bell GW, Nam JW, Bartel DP. Predicting effective microRNA target sites in mammalian mRNAs. Elife 2015; 4: e05005.
35) Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi AH, Tanaseichuk O, Benner C, Chanda SK. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. Nat Commun 2019; 10: 1523.
36) Callegari E, Gramantieri L, Domenicali M, D'Abundo L, Sabbioni S, Negrini M. MicroRNAs in liver cancer: a model for investigating pathogenesis and novel therapeutic approaches. Cell Death Differ 2015; 22: 46-57.
37) Pan XP, Wang HX, Tong DM, Li Y, Huang LH, Wang C. MiRNA-370 acts as a tumor suppressor via the downregulation of PIM1 in hepatocellular carcinoma. Eur Rev Med Pharmacol Sci 2017; 21: 1254-1263.
38) Yun Z, Meng F, Jiang P, Yue M, Li S. MicroRNA-548b suppresses aggressive phenotypes of hepatocellular carcinoma by directly targeting high-mobility group box 1 mRNA. Cancer Manag Res 2019; 11: 5821-5834.
39) Luo L, Xia L, Zha B, Zuo C, Deng D, Chen M, Hu L, He Y, Dai F, Wu J, Wang C, Wang Y, Zhang Q. MiR-335-5p targeting ICAM-1 inhibits invasion and metastasis of thyroid cancer cells. Biomed Pharmacother 2018; 106: 983-990.
40) Wang F, Li L, Piontek K, Sakaguchi M, Selaru FM. Exosome miR-335 as a novel therapeutic strategy in hepatocellular carcinoma. Hepatology 2018; 67: 940-954.
41) Babaie Y, Herwig R, Greber B, Brink TC, Wruck W, Groth D, Lehrach H, Burdon T, Adjaye J. Analysis of Oct4-dependent transcriptional networks regulating self-renewal and pluripotency in human embryonic stem cells. Stem Cells 2007; 25: 500-510.
42) Hattori N, Nishino K, Ko YG, Hattori N, Ohgane J, Tanaka S, Shiota K. Epigenetic control of mouse Oct-4 gene expression in embryonic stem cells and trophoblast stem cells. J Biol Chem 2004; 279: 17063-17069.
43) Wang L, Guo ZY, Zhang R, Xin B, Chen R, Zhao J, Wang T, Wen WH, Jia LT, Yao LB, Yang AG. Pseudogene OCT4-pg4 functions as a natural micro RNA sponge to regulate OCT4 expression by competing for miR-145 in hepatocellular carcinoma. Carcinogenesis 2013; 34: 1773-1781.
44) Zeineddine D, Hammoud AA, Mortada M, Boeuf H. The Oct4 protein: more than a magic stemness marker. Am J Stem Cells 2014; 3: 74-82.
45) Chang TS, Wu YC, Chi CC, Su WC, Chang PJ, Lee KF, Tung TH, Wang J, Liu JJ, Tung SY, Kuo LM, Ho HN, Ling TY, Huang YH. Activation of IL6/IGFIR confers poor prognosis of HBV-related hepatocellular carcinoma through induction of OCT4/NANOG expression. Clin Cancer Res 2015; 21: 201-210.
46) Wang K, Jin W, Song Y, Fei X. LncRNA RP11-436H11.5, functioning as a competitive endogenous RNA, upregulates BCL-W expression by sponging miR-335-5p and promotes proliferation and invasion in renal cell carcinoma. Mol Cancer 2017; 16: 166.
47) Tang H, Zhu J, Du W, Liu S, Zeng Y, Ding Z, Zhang Y, Wang X, Liu Z, Huang J. CPNE1 is a target of miR-335-5p and plays an important role in the pathogenesis of non-small cell lung cancer. J Exp Clin Cancer Res 2018; 37: 131.
48) Zhu MX, Wei CY, Zhang PF, Gao DM, Chen J, Zhao Y, Dong SS, Liu BB. Elevated TRIP13 drives the AKT/mTOR pathway to induce the progression of hepatocellular carcinoma via interacting with ACTN4. J Exp Clin Cancer Res 2019; 38: 409.
49) Toh TB, Lim JJ, Hooi L, Rashid M, Chow EK. Targeting Jak/Stat pathway as a therapeutic strategy against SP/CD44+ tumorigenic cells in Akt/beta-catenin-driven hepatocellular carcinoma. J Hepatol 2020; 72: 104-118..