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## MicroRNA-98-5p Inhibits Cell Proliferation and Induces Cell Apoptosis in Hepatocellular Carcinoma via Targeting IGF2BP1

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Some microRNAs (miRs) have been demonstrated to play promoting or tumor-suppressing roles in the development and progression of hepatocellular carcinoma (HCC). However, the regulatory mechanism of miR-98-5p in HCC still remains largely unclear. In the present study, our data showed that miR-98-5p was significantly downregulated in 84 cases of HCC tissues compared to the matched adjacent nontumor tissues. In addition, downregulation of miR-98-5p was associated with tumor size, portal vein tumor embolus, node metastasis, and clinical stage in HCC. HCC patients with low expression of miR-98-5p showed a shorter survival time compared with those with high miR-98-5p levels. Moreover, the expression of miR-98-5p was also reduced in HCC cell lines (HepG2, Hep3B, LM3, and SMCC7721) compared to the normal liver cell line THLE-3. Overexpression of miR-98-5p significantly decreased LM3 cell growth by inducing cell cycle arrest at the G<sub>1</sub> stage and cell apoptosis. Insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1) was then identified as a novel target gene of miR-98-5p, and its protein expression was negatively regulated by miR-98-5p in LM3 cells. Overexpression of IGF2BP1 eliminated the effects of miR-98-5p overexpression on the proliferation, cell cycle, and apoptosis of LM3 cells. Finally, we found that IGF2BP1 was upregulated in HCC, and its expression was negatively correlated to miR-98-5p levels. In summary, we demonstrate that miR-98-5p could inhibit HCC cell proliferation while inducing cell apoptosis, partly at least, via inhibition of its target gene IGF2BP1, and we suggest that miR-98-5p may become a promising therapeutic candidate for HCC treatment.

**Key words:** Cell proliferation; Cell cycle; Cell apoptosis; Hepatocellular carcinoma (HCC); MicroRNAs (miRs); Insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1)

### INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common human malignancies worldwide and causes a large number of deaths<sup>1,2</sup>. In recent years, the incidence of HCC has increased rapidly<sup>1,2</sup>. Although great efforts have been made to improve surgical resection, radiotherapy, and chemotherapy, the outcome of HCC treatments remains unsatisfactory<sup>2</sup>. Therefore, it is urgently needed to explore the molecular mechanism underlying HCC growth, which may help develop effective strategies for HCC treatment.

MicroRNAs (miRs), a class of noncoding RNAs that are 18–25 nucleotides in length, have been found to act

as key regulators for gene expression via directly binding to 3'-untranslated regions (3'-UTRs) of their target mRNAs, causing mRNA degradation or protein translation inhibition<sup>3–5</sup>. By inhibiting the protein expression of their target genes, miRs participate in the regulation of a variety of biological processes, such as cell proliferation, apoptosis, cell cycle progression, angiogenesis, and so forth<sup>6–8</sup>. Moreover, many miRs have been found to be involved in the development and malignant progression of human cancers, including HCC<sup>9–11</sup>. Among these miRs, miR-98 could inhibit tumor angiogenesis and invasion by targeting activin receptor-like kinase-4 and matrix metalloproteinase-11<sup>12</sup>. Moreover, miR-98 has

been reported to play a suppressive role in several different types of cancer<sup>13-15</sup>. For instance, Yang et al. reported that miR-98 inhibited cell proliferation and invasion of non-small cell carcinoma lung cancer (NSCLC) by targeting PAK1<sup>13</sup>. Recently, miR-98-5p has been demonstrated to play a suppressive role in HCC, partially by targeting CTHRC1 and SALL4<sup>6,16</sup>. However, the molecular mechanism of miR-98-5p underlying HCC growth has not been fully uncovered.

Insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1), a member of the IGF2BP family, contains four K homology domains and two RNA recognition motifs<sup>17</sup>. It functions by binding to the mRNAs of certain genes, including insulin-like growth factor 2,  $\beta$ -actin, and  $\beta$ -transducin repeat-containing protein, and thus regulates their translation, thereby becoming involved in the regulation of cell proliferation and survival<sup>17,18</sup>. Recently, IGF2BP1 was reported to function as an oncogene in different cancer types, including HCC, by promoting tumor cell proliferation, migration, and invasion<sup>18-20</sup>. However, the detailed regulatory mechanism underlying IGF2BP1 expression in HCC still remains largely unclear.

In this study, we aimed to explore the molecular mechanism of miR-98-5p underlying HCC growth.

## MATERIALS AND METHODS

### Clinical Tissue Samples

This study was approved by the ethics committee of Xiangya Hospital, Central South University, Changsha, P.R. China. Primary HCC tissues ( $n=84$ ) and their matched adjacent nontumor tissues were collected from Xiangya Hospital between March 2009 and September 2010. The clinical characteristics of HCC patients involved in this study are summarized in Table 1, and all written informed consents were obtained. Tissues were immediately snap frozen in liquid nitrogen after surgical removal and stored in liquid nitrogen before use.

### Real-Time qPCR

Total RNA was extracted from tissues or cell lines using TRIzol Reagent (Thermo Fisher, Waltham, MA, USA) and then converted into cDNA using the Reverse Transcription Kit (Thermo Fisher) according to the manufacturer's instructions. The miR expression detection was performed using PrimeScript<sup>®</sup> miRNA RT-PCR Kit (Takara, Tokyo, Japan) on an ABI 7300 plus thermocycler (Thermo Fisher). U6 was used as an internal reference. The mRNA expression was examined using the standard

**Table 1.** Association Between miR-98-5p Expression and Clinicopathological Characteristics in Hepatocellular Carcinoma

Variables	Cases ( $n=84$ )	Low miR-98-5p Level ( $n=54$ )	High miR-98-5p Level ( $n=30$ )	<i>p</i> Value
Age (years)				0.349
$\leq 55$	32	23	9	
$> 55$	52	31	21	
Sex				0.487
Male	51	31	20	
Female	33	23	10	
Tumor size (cm)				0.016*
$\leq 5$ cm	55	30	25	
$> 5$ cm	29	24	5	
Differentiation				0.158
Well and moderately	56	39	17	
Poor	28	15	13	
Nodal metastasis				0.011*
Present	39	31	8	
Absent	45	23	22	
Portal vein tumor embolus				0.017*
Present	28	23	5	
Absent	56	31	25	
Clinical stage				0.001*
I-II	43	20	23	
III-IV	41	34	7	
HBV infection				0.770
Present	69	45	24	
Absent	15	9	6	

\*The difference has statistical significance.

SYBR-Green RT-PCR Kit (Takara). GAPDH was used as an internal reference. The primers used are shown as follows: IGF2BP1, 5'-GCGGCCAGTTCCTGGTCAA-3' (forward) and 5'-TTGGGCACCGAATGTTCAATC-3' (reverse); GAPDH, 5'-CTGGGCTACACTGAGCACC-3' (forward) and 5'-AAGTGGTCGTTGAGGGCAATG-3' (reverse). The reaction condition was 95°C for 3 min, followed by 40 cycles of 95°C for 30 s and 60°C for 30 s. The relative expression was analyzed by the  $2^{-\Delta\Delta C_t}$  method.

#### Cell Culture

Human HCC cell lines (HepG2, Hep3B, LM3, and SMCC7721) and normal liver THLE-3 cells were obtained from the Cell Center of Xiangya Medical School, Central South University, Changsha, P.R. China. All cell lines were cultured in DMEM (Thermo Fisher, Carlsbad, CA, USA) with 10% fetal bovine serum (FBS; Thermo Fisher) at 37°C in a humidified incubator containing 5% CO<sub>2</sub>.

#### Western Blot

Cells were lysed in RIPA buffer containing protease and phosphatase inhibitors (Thermo Fisher) for 30 min. Cell lysates were centrifuged at 13,000×g for 30 min at 4°C. The supernatant was collected. The protein concentration was determined with a BCA protein assay kit (Beyotime Biotechnology, Shanghai, P.R. China). Protein (50 µg) was separated in 12% SDS-PAGE gel and transferred onto a polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA, USA). The PVDF membrane was blocked in 5% nonfat milk (Yili, Beijing, P.R. China) overnight at 4°C, and then incubated with primary antibodies against IGF2BP1 and GAPDH (Abcam, Cambridge, MA, USA) at room temperature for 3 h. After washing with TBST three times, the PVDF membrane was incubated with the secondary antibody (Abcam) at room temperature for 40 min. The immunoblots on the PVDF membrane were visualized with an enhanced chemiluminescence (ECL) kit (Thermo Fisher).

#### Cell Transfection

Lipofectamine 2000 (Thermo Fisher) was used to perform cell transfection according to the manufacturer's instructions. LM3 cells were transfected with scramble miR (miR-NC), miR-98-5p mimics, negative control (NC) inhibitor, miR-98-5p inhibitor, or cotransfected with miR-98-5p mimics and pc-DNA3.1-IGF2BP1 plasmid, or miR-98-5p mimics and blank pc-DNA3.1 vector, respectively. Experiments were carried out 48 h after transfection.

#### MTT Assay

LM3 cells ( $5 \times 10^4$ ) were seeded into a 96-well plate, and 100 µl of fresh serum-free medium containing 0.5 g/L MTT (Sigma-Aldrich, St. Louis, MO, USA) was added to each well. After incubation at 37°C for 0, 24, 48, and 72 h,

the medium was removed. Fifty microliters of DMSO (Sigma-Aldrich) was then added to each well and incubated at 37°C for 10 min. The A570 of each sample was measured using a plate reader (TECAN Infinite M200, Switzerland).

#### Cell Cycle Analysis

LM3 cells ( $1 \times 10^6$ ) were washed twice with DPBS, resuspended in 70% ethanol, and fixed overnight at -20°C. Cells were then washed twice in PBS with 3% BSA and incubated for 30 min at room temperature in propidium iodide (PI) staining buffer containing 3% BSA, 40 µg/ml PI, and 0.2 mg/ml RNase in PBS. DNA content analyses were carried out using a flow cytometer (C6; BD Biosciences, San Jose, CA, USA).

#### Cell Apoptosis Assay

Annexin-V-FITC/PI Apoptosis Detection Kit (Roche, Basel, Switzerland) was used to detect cell apoptosis according to the manufacturer's instructions. In brief, LM3 cells were collected by trypsinization, washed twice in PBS, and resuspended in 500 µl of 1× binding buffer, which was then added with 5 µl of annexin V-FITC and 5 µl of PI. After incubation at room temperature in the dark for 10 min, fluorescence was analyzed with a flow cytometer (C6; BD Biosciences).

#### Bioinformatic Prediction

TargetScan ([www.targetscan.org/](http://www.targetscan.org/)) was used to predict the putative target genes of miR-98-5p according to the manufacturer's instructions.

#### Luciferase Reporter Assay

The wild type (WT) or mutant type (MT) of IGF2BP1 3'-UTR was constructed by PCR and QuickChange Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA) according to the manufacturer's instructions and then inserted into the MCS in the psiCHECK<sup>TM2</sup> vector (Promega, Madison, WI, USA). LM3 cells were cultured to 70%–80% confluence in a 24-well plate and cotransfected with 100 ng of WT-IGF2BP1-3'-UTR or MT-IGF2BP1-3'-UTR plasmid, and 50 nM of miR-98-5p mimics or miR-NC using Lipofectamine 2000 according to the manufacturer's instructions. The activity of *Renilla* luciferase and firefly luciferase was determined using the Dual-luciferase Reporter Assay System (Promega) 48 h after transfection. *Renilla* luciferase activity was normalized to firefly luciferase activity.

#### Statistical Analysis

All experiments were performed at least in triplicate. The data were expressed as the mean ± standard deviation (SD). Statistical analysis was performed using SPSS 19.0 (SPSS, Armonk, NY, USA). The statistical correlation of

data between groups was analyzed by Student's *t*-test. The association between miR-98-5p expression and clinical characteristics in HCC was analyzed using chi-square test. A value of  $p < 0.05$  was considered as statistically significant.

## RESULTS

### *miR-98-5p Is Downregulated in HCC*

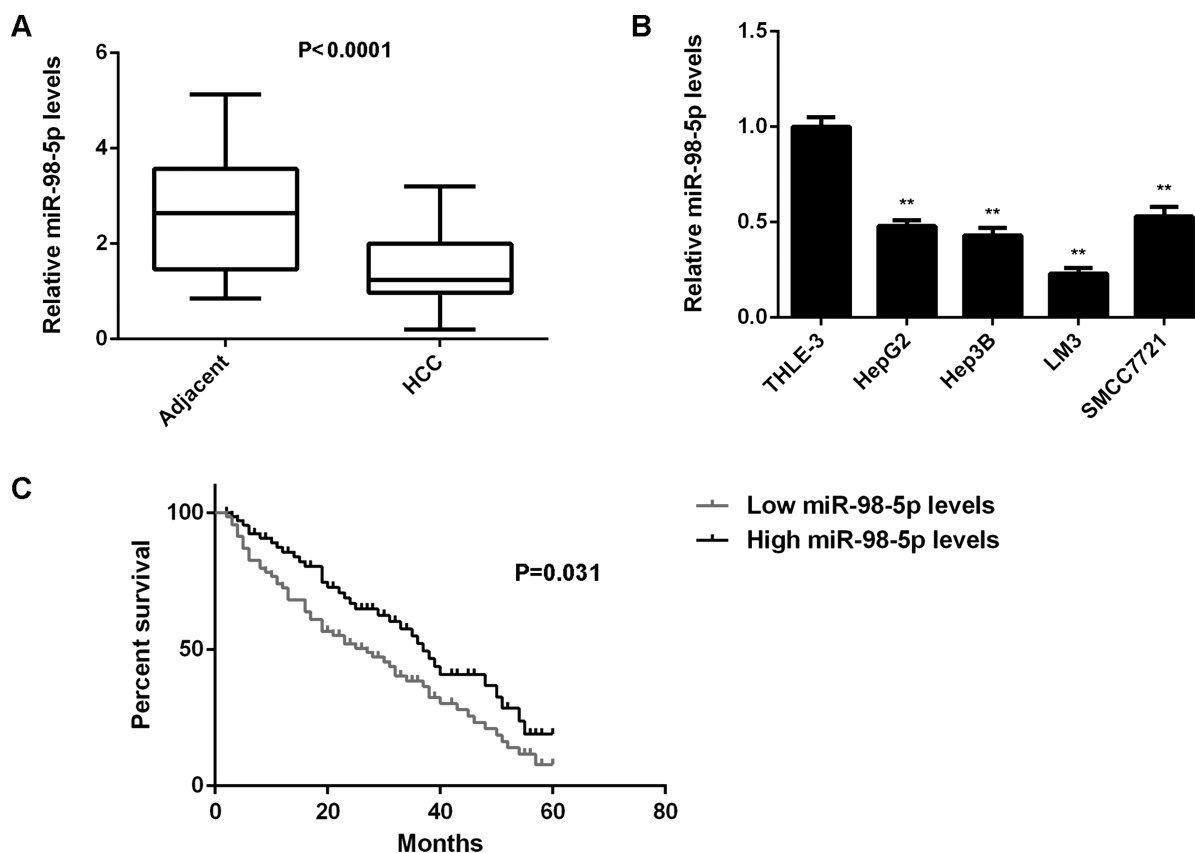
In this study, we first examined the miR-98-5p expression in HCC tissues and matched adjacent nontumor tissues using real-time qPCR. The miR-98-5p levels were significantly reduced in HCC tissues compared to matched adjacent nontumor tissues (Fig. 1A). We then examined the expression of miR-98-5p in HCC cell lines (HepG2, Hep3B, LM3, and SMCC7721) and normal liver THLE-3 cells. We found that miR-98-5p was also downregulated in HCC cell lines compared with THLE-3 cells (Fig. 1B).

We further studied the clinical significance of miR-98-5p in HCC. According to the mean value of miR-98-5p

expression, we divided HCC patients into two groups: low miR-98-5p expression and high miR-98-5p expression. We found that low expression of miR-98-5p was significantly associated with tumor size, differentiation, portal vein tumor embolus, node metastasis, and clinical stage in HCC (Table 1), suggesting that downregulation of miR-98-5p may be involved in the malignant progression of HCC. Moreover, we found that HCC patients with low expression of miR-98-5p showed a shorter survival time compared with those having high miR-98-5p levels, suggesting that the reduced expression of miR-98-5p may be used for predicting poor prognosis of HCC patients (Fig. 1C).

### *miR-98-5p Inhibits LM3 Cell Proliferation and Induces Cell Cycle Arrest and Cell Apoptosis*

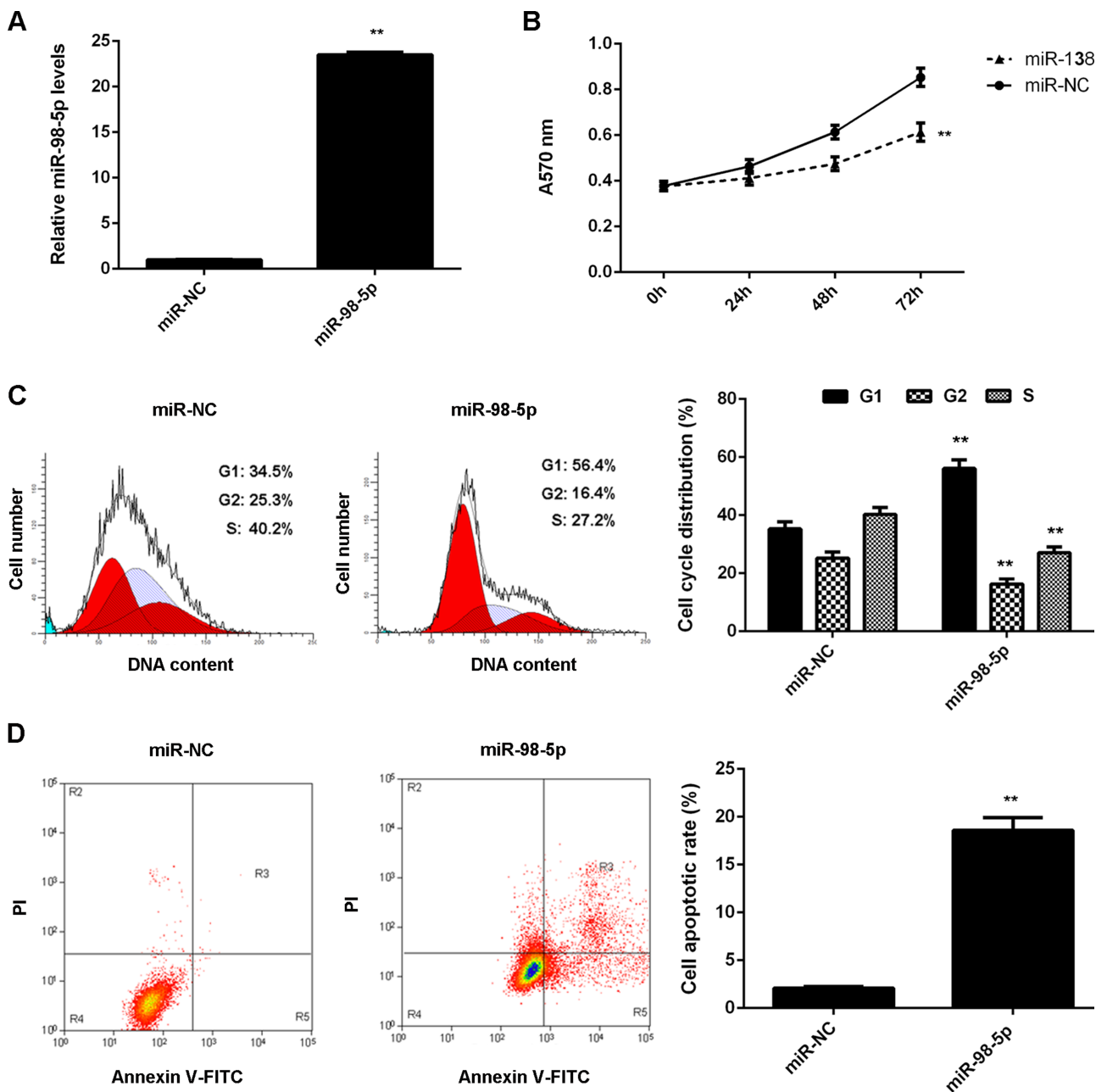
LM3 cells showed the most significant decrease in miR-98-5p expression, and thus we used them in the following experiments to study the regulatory mechanism of miR-98-5p underlying HCC growth in vitro. miR-98-5p mimic was used to transfect LM3 cells to upregulate its



**Figure 1.** miR-98-5p is downregulated in hepatocellular carcinoma (HCC). (A) Real-time PCR was used to conduct the miR-98-5p levels in HCC tissues compared to matched adjacent nontumor tissues. (B) Real-time PCR was used to determine the miR-98-5p levels in HCC cell lines compared to normal human liver THLE-3 cells.  $**p < 0.01$  versus THLE-3. (C) The HCC patients with low expression of miR-98-5p showed shorter survival time compared with those with high miR-98-5p levels.

expression. Transfection with miR-98-5p mimic significantly increased the miR-98-5p levels compared with the miR-NC group (Fig. 2A). MTT assay was then conducted to examine cell proliferation. MTT assay data showed that overexpression of miR-98-5p significantly inhibited LM3 cell proliferation (Fig. 2B). We then examined cell cycle distribution using flow cytometry and found that the percentage of LM3 cells at the G<sub>1</sub> stage was higher in

the miR-98-5p group compared with the miR-NC group, suggesting that miR-98-5p plays a suppressive role in HCC cell proliferation via inducing cell cycle arrest (Fig. 2C). We then examined the cell apoptosis rate in each group. The apoptosis of LM3 cells was higher in the miR-98-5p group compared with the miR-NC group (Fig. 2D). Based on the above data, we suggest that miR-98-5p may play a suppressive role in HCC growth by



**Figure 2.** miR-98-5p overexpression inhibits LM3 cell proliferation and induces cell cycle arrest and cell apoptosis. LM3 cells transfected with miR-98-5p mimic or scramble miR (miR-NC), respectively. (A) Real-time PCR was used to determine the miR-98-5p expression. (B) MTT assay was used to determine the cell proliferation. (C) Cell cycle distribution and (D) cell apoptosis were examined using flow cytometry. \*\**p*<0.01 versus miR-NC.

inhibiting HCC cell proliferation and inducing cell cycle arrest and cell apoptosis.

#### *IGF2BP1 Is a Novel Target of miR-98-5p in LM3 Cells*

As miRs function through mediation of their targets, the putative target genes of miR-98-5p were analyzed using TargetScan. IGF2BP1 was predicted to be a target gene of miR-98-5p (Fig. 3A). To confirm this targeting relationship, the luciferase vectors containing WT or MT of IGF2BP1 3'-UTR were constructed (Fig. 3B). Luciferase reporter assay was then conducted. Luciferase activity was significantly decreased in LM3 cells cotransfected with miR-98-5p mimics and WT-IGF2BP1-3'-UTR plasmid, when compared to the control group, which was eliminated when transfected with MT-IGF2BP1-3'-UTR plasmid (Fig. 3C). Accordingly, IGF2BP1 is a novel target gene of miR-98-5p.

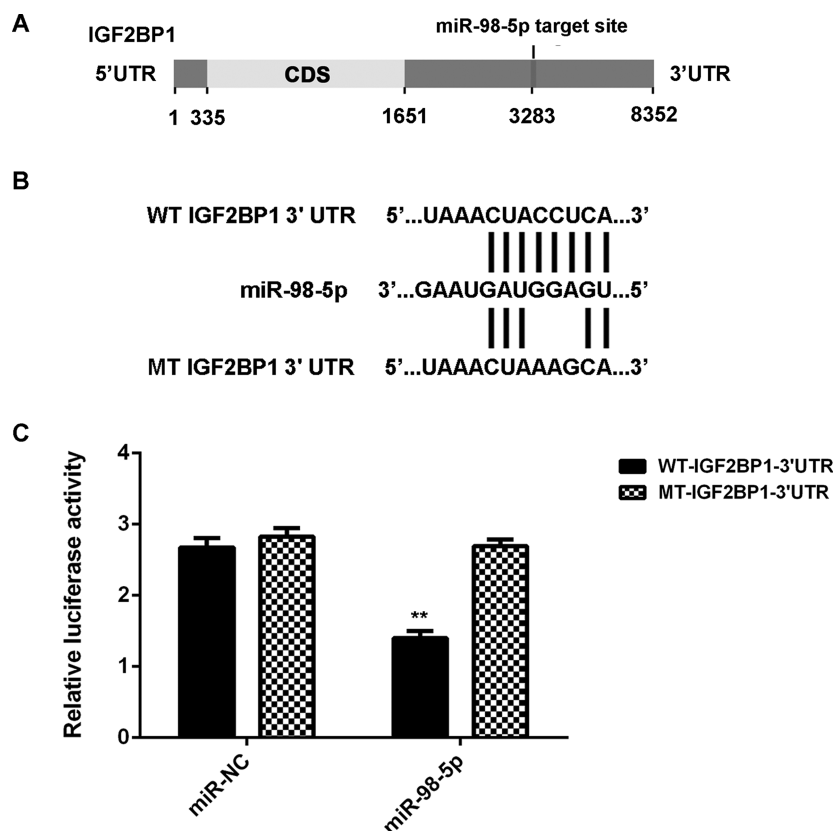
#### *The Protein Expression of IGF2BP1 Is Negatively Regulated by miR-98-5p in LM3 Cells*

As miRs negatively regulate the expression of their target genes at the posttranscriptional level, we examined

the effect of miR-98-5p expression on the protein expression of IGF2BP1 in LM3 cells. Overexpression of miR-98-5p reduced the protein level of IGF2BP1 in LM3 cells compared to the miR-NC group (Fig. 4A). LM3 cells were then transfected with the miR-98-5p inhibitor or the NC inhibitor. miR-98-5p levels were significantly reduced in the miR-98-5p group compared with the NC inhibitor group (Fig. 4B). Western blot data then indicated that the protein expression of IGF2BP1 was increased in the miR-98-5p inhibitor group compared with the NC inhibitor group (Fig. 4C). Therefore, miR-98-5p negatively regulates the protein expression of IGF2BP1 in LM3 cells.

#### *Overexpression of IGF2BP1 Attenuates the Effect of miR-98-5p on HCC Cells*

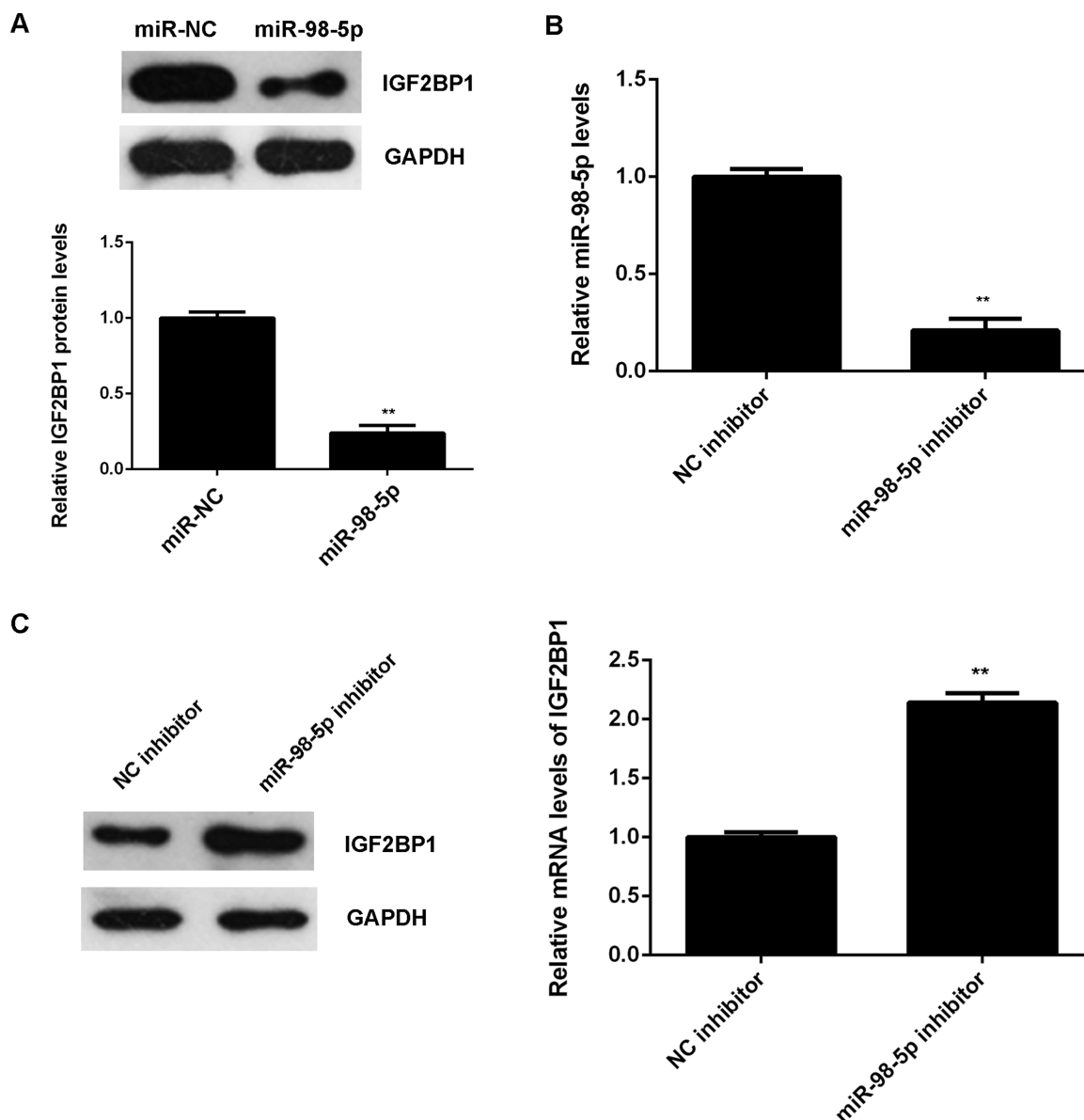
As IGF2BP1 was previously reported to act as an oncogene in HCC and was negatively regulated by miR-98-5p in LM3 cells, we speculated that IGF2BP1 might be involved in miR-98-5p-mediated LM3 cell proliferation, cell cycle progression, and cell apoptosis. To clarify this speculation, miR-98-5p-overexpressing LM3



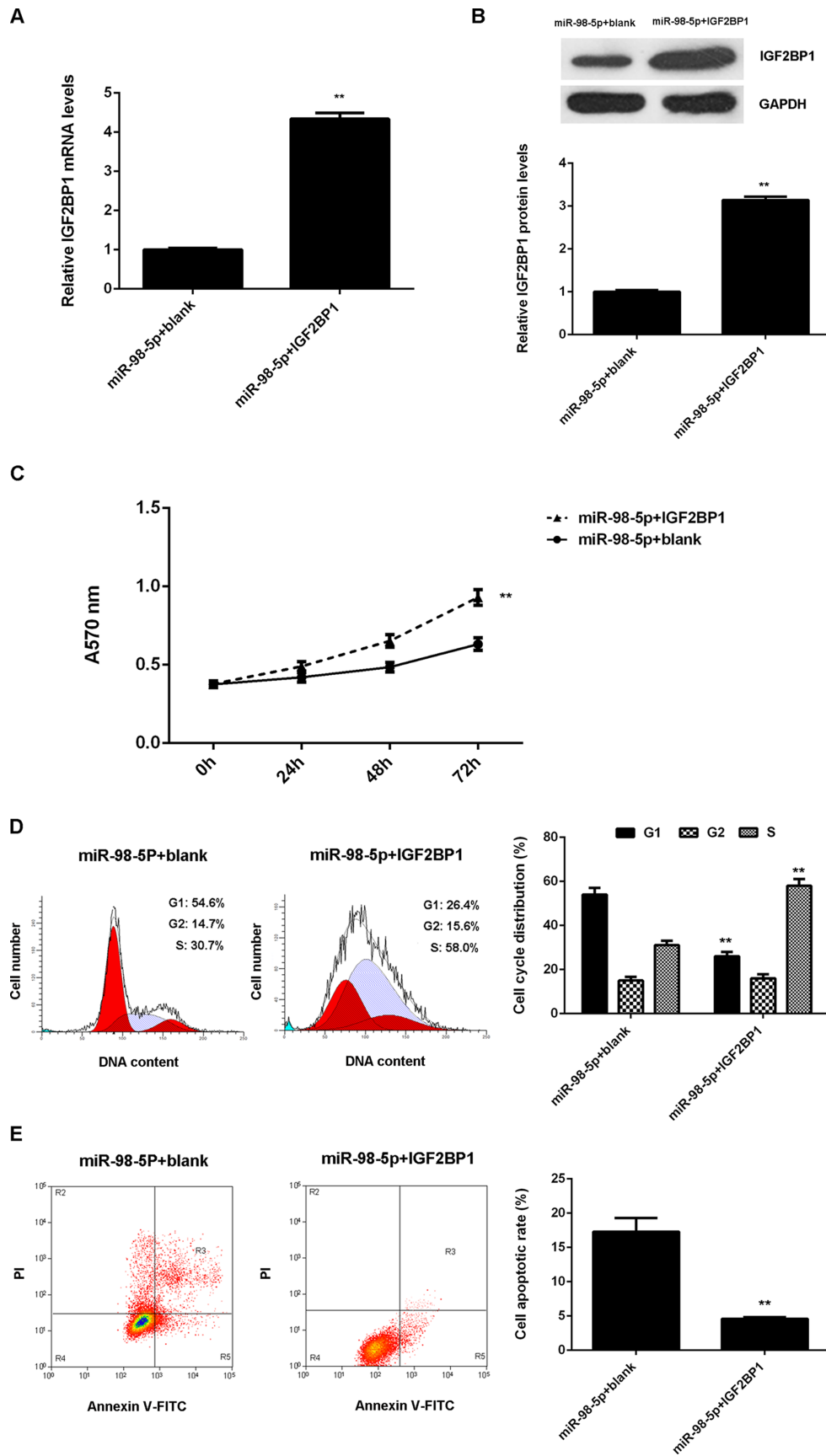
**Figure 3.** Insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1) is a novel target of miR-98-5p in LM3 cells. (A) TargetScan indicated that IGF2BP1 was a putative target of miR-98-5p. (B) The luciferase reporter vectors containing wild type (WT) or mutant type (MT) of IGF2BP1 3'-untranslated region (3'-UTR) were constructed. (C) Luciferase reporter assay was conducted in LM3 cells. \*\* $p < 0.01$  versus miR-NC.

cells were transfected with pcDNA3.1-IGF2BP1 plasmid or blank pcDNA3.1 vector as the control group. After transfection, the mRNA and protein levels of IGF2BP1 were significantly upregulated in the miR-98-5p+IGF2BP1 group compared with the miR-98-5p+blank group (Fig. 5A and B). MTT assay was then conducted, and the proliferation of LM3 cells was significantly increased in the miR-98-5p+IGF2BP1 group compared to the miR-98-5p+blank group (Fig. 5C).

Flow cytometry was then conducted to examine cell cycle distribution and cell apoptosis. The percentage of LM3 cells at the G<sub>1</sub> stage was less in the miR-98-5p+IGF2BP1 group than in the miR-98-5p+blank group (Fig. 5D). In addition, the cell apoptosis rate was lower in the miR-98-5p+IGF2BP1 group compared to the miR-98-5p+blank group (Fig. 5E). Accordingly, overexpression of IGF2BP1 attenuates the effects of miR-98-5p of HCC cells.



**Figure 4.** The protein expression of IGF2BP1 is negatively regulated by miR-98-5p in LM3 cells. (A) Western blot was used to detect the protein expression of IGF2BP1 in LM3 cells transfected with miR-98-5p mimic or scramble miR (miR-NC), respectively. \*\* $p < 0.01$  versus miR-NC. LM3 cells were transfected with miR-98-5p inhibitor or negative control (NC) inhibitor, respectively. After transfection, (B) real-time PCR was used to determine the miR-98-5p levels, and (C) Western blot was conducted to examine the protein expression of IGF2BP1. \*\* $p < 0.01$  versus NC inhibitor.





*IGF2BP1 Is Upregulated in HCC and Inversely Correlates to miR-98-5p Levels*

The expression of IGF2BP1 was then examined in HCC tissues and cell lines. mRNA and protein levels of IGF2BP1 were significantly higher in HCC tissues compared to matched adjacent nontumor tissues (Fig. 6A and B). IGF2BP1 was also upregulated in HCC cell lines compared with normal liver THLE-3 cells (Fig. 6C and D). More interestingly, IGF2BP1 levels were inversely correlated to the miR-98-5p levels in HCC tissues (Fig. 6E), suggesting that reduced miR-98-5p levels may contribute to the upregulation of IGF2BP1 in HCC tissues.

### DISCUSSION

The molecular mechanism of miR-98-5p underlying HCC growth has not been fully uncovered. Herein we showed that miR-98-5p was significantly downregulated in HCC tissues and cell lines and that reduced miR-98-5p levels were significantly associated with HCC progression as well as a poor prognosis for HCC patients. Upregulation of miR-98-5p suppressed HCC cell proliferation while inducing cell cycle arrest and cell apoptosis. IGF2BP1 was then indentified as a novel target of miR-98-5p in LM3 cells, and overexpression of IGF2BP1 attenuated the effect of miR-98-5p on LM3 cells. In addition, IGF2BP1 was found to be upregulated in HCC tissues and cell lines and inversely correlated to the miR-98-5p levels.

In recent years, miR-98-5p has been reported to act as a tumor suppressor in several human cancers, such as NSCLC<sup>13,21</sup>, glioma<sup>22</sup>, and salivary adenoid cystic carcinomas<sup>23</sup>. It is downregulated in NSCLC and suppresses cell proliferation and invasion by directly targeting PAK1 and ITGB3<sup>13,21</sup>. Fan et al. reported that miR-98-5p had a suppressive effect on the invasion of glioma cells via inhibiting the protein expression of IKKε<sup>22</sup>. In addition, it plays an inhibitory role in salivary adenoid cystic carcinomas through downregulating the activities of PI3K/AKT and MAPK/ERK signaling pathways<sup>23</sup>. Recently, miR-98 has been reported to play a suppressive role in HCC, and several targets have been identified. For instance, Wang et al. showed that miR-98 suppressed HCC cell proliferation, migration, and invasion by targeting CTHRC1<sup>16</sup>. Zhou et al. reported that miR-98 acted as a tumor suppressor in HCC via targeting SALL4<sup>6</sup>. In the present study, we found that miR-98-5p was significantly downregulated in HCC tissues and cell lines when compared with that

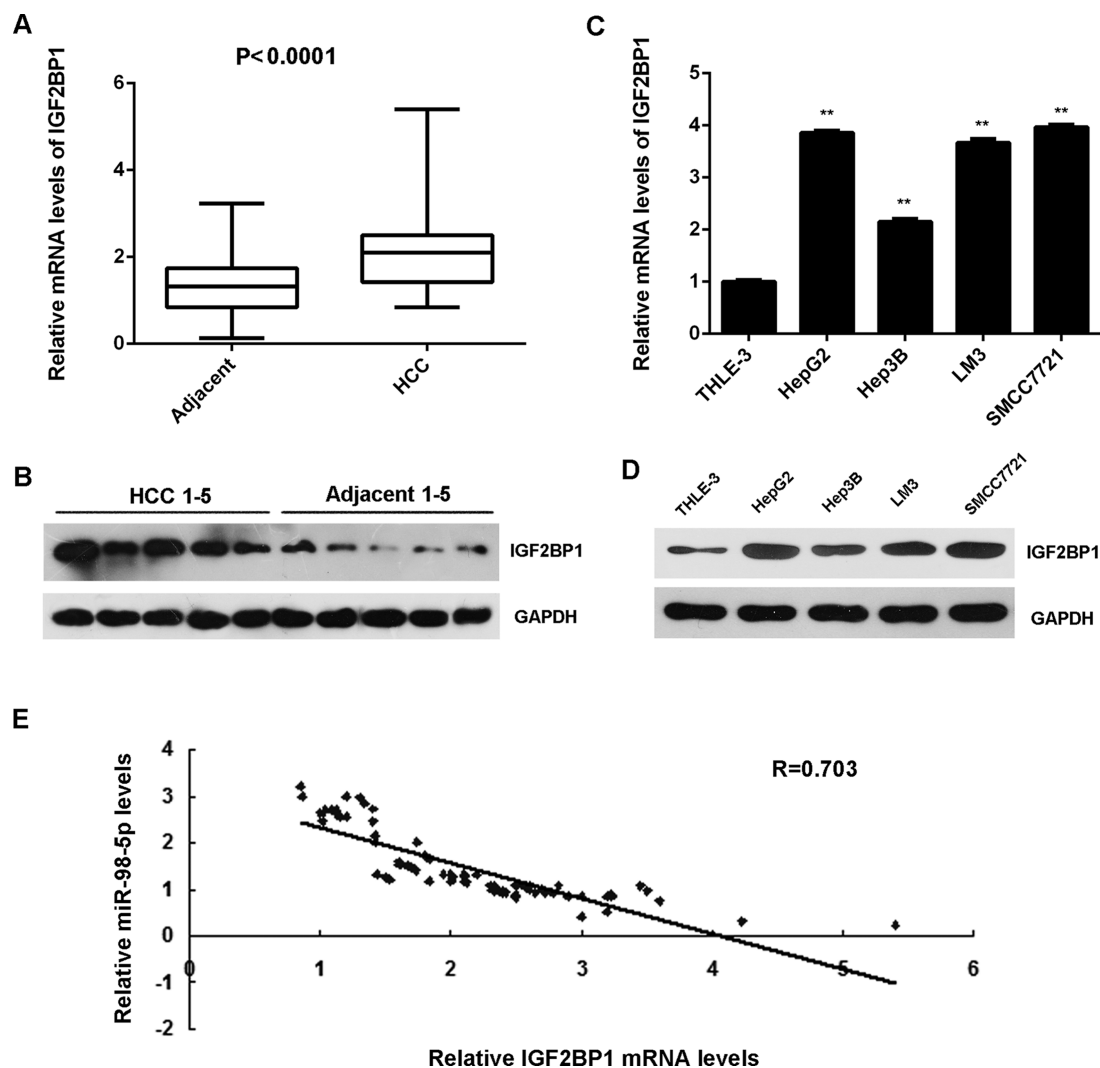
in matched adjacent nontumor tissues and a normal liver cell line. Moreover, we found that low expression levels of miR-98-5p were significantly associated with tumor size, portal vein tumor embolus, vascular invasion, node metastasis, and clinical stage in HCC, but not correlated to age, sex, HBV infection, or AFP levels, which is consistent with previous findings<sup>6</sup>. Further investigation indicated that overexpression of miR-98-5p significantly reduced LM3 cell proliferation via induction of cell cycle arrest at the G<sub>1</sub> stage as well as cell apoptosis.

As CTHRC1 and SALL4 have been identified as target genes of miR-98-5p in HCC cells, other targets of miR-98-5p may also exist and play important roles in HCC. In this study, we conducted a bioinformatics analysis and found that IGF2BP1 was a putative target gene of miR-98-5p, which was confirmed by luciferase reporter gene assay. IGF2BP1 has recently been demonstrated to be significantly upregulated in HCC and functions as an oncogene<sup>19</sup>. IGF2BP1 can bind to and stabilize the mRNAs of c-MYC and MKI67, increasing the protein expression of c-Myc and Ki-67, two potent regulators of cell proliferation and apoptosis<sup>19</sup>. Stable depletion of IGF2BP1 significantly inhibited the tumor growth of HCC cells in a murine xenograft assay<sup>19</sup>, and abrogation of the interplay between IGF2BP1, 2, and 3 and IGF1R can arrest HCC growth<sup>24</sup>, suggesting that IGF2BP1 may be used as a potential therapeutic target for HCC. In this study, we found that the protein expression of IGF2BP1 was negatively affected by miR-98-5p in LM3 cells, and overexpression of IGF2BP1 attenuated the suppressive effects of miR-98-5p on LM3 cell proliferation, as well as its promoting effects on cell cycle arrest and cell apoptosis. Therefore, we suggest that miR-98-5p inhibits cell proliferation and induces cell apoptosis in HCC, partly at least, via directly targeting IGF2BP1. In addition, we found that IGF2BP1 was significantly upregulated in HCC tissues and cell lines when compared with that in matched adjacent nontumor tissues and normal liver cell line. Moreover, IGF2BP1 levels were inversely correlated to the miR-98-5p levels in HCC tissues, suggesting that the increased expression of IGF2BP1 may be caused by the decreased expression of miR-98-5p in HCC.

Except for miR-98-5p, several other miRs were reported to directly target IGF2BP1 in HCC and play a suppressive role in HCC cells. For instance, Zhou et al. found that miR-625 suppressed HCC cell migration and invasion by targeting IGF2BP1<sup>25</sup>. Fawzy et al. showed that miR-1275

### FACING PAGE

**Figure 5.** Overexpression of IGF2BP1 attenuates the effects of miR-98-5p of HCC cells. miR-98-5p-overexpressing LM3 cells were transfected with pcDNA3.1-IGF2BP1 plasmid or blank pcDNA3.1 vector, respectively. (B) MTT assay was used to determine the cell proliferation. (C) Cell cycle distribution and (D) cell apoptosis were examined using flow cytometry. \*\**p*<0.01 versus miR-98-5p+blank.



**Figure 6.** IGF2BP1 is upregulated in HCC and inversely correlates to the miR-98-5p levels. (A) Real-time PCR and (B) Western blot were used to detect the mRNA and protein levels of IGF2BP1 in HCC tissues compared to matched adjacent nontumor tissues. (C) Real-time PCR and (D) Western blot were used to detect the mRNA and protein levels of IGF2BP1 in HCC cell lines compared to normal human liver THLE-3 cells.  $**p < 0.01$  versus THLE-3. (E) An inverse correlation was found between IGF2BP1 mRNA levels and miR-98-5p levels in HCC tissues.

could inhibit HCC tumor growth partially through simultaneously targeting the oncogenic IGF2BPs and IGF1R<sup>26</sup>. In addition, miR-9 plays a tumor-suppressive role partially through a functional miR-9/IGF2BP1/AKT&ERK axis<sup>27</sup>.

In summary, to our knowledge, this is the first study demonstrating that miR-98-5p, downregulated in HCC, inhibits proliferation while inducing apoptosis in HCC LM3 cells, partly at least, through directly targeting IGF2BP1, suggesting that miR-98-5p may become a potential therapeutic candidate for HCC treatment.

**ACKNOWLEDGMENT:** The authors declare no conflicts of interest.

## REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin.* 2011;61:69–90.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J Clin.* 2015;65:5–29.
3. Ambros V. MicroRNAs: Tiny regulators with great potential. *Cell* 2001;107:823–6.
4. Moss EG. MicroRNAs: Hidden in the genome. *Curr Biol.* 2002;12:R138–40.
5. Ambros V. The functions of animal microRNAs. *Nature* 2004;431:350–5.
6. Zhou W, Zou B, Liu L, Cui K, Gao J, Yuan S, Cong N. MicroRNA-98 acts as a tumor suppressor in hepatocellular carcinoma via targeting SALL4. *Oncotarget* 2016;7:74059–73.

7. Sun J, Zheng G, Gu Z, Guo Z. MiR-137 inhibits proliferation and angiogenesis of human glioblastoma cells by targeting EZH2. *J Neurooncol.* 2015;122:481–9.
8. Song H, Zhang Y, Liu N, Wan C, Zhang D, Zhao S, Kong Y, Yuan L. miR-92b regulates glioma cells proliferation, migration, invasion, and apoptosis via PTEN/Akt signaling pathway. *J Physiol Biochem.* 2016;72:201–11.
9. Zhu J, Zeng Y, Xu C, Qin H, Lei Z, Shen D, Liu Z, Huang JA. Expression profile analysis of microRNAs and down-regulated miR-486-5p and miR-30a-5p in non-small cell lung cancer. *Oncol Rep.* 2015;34:1779–86.
10. Wu DC, Zhang MF, Su SG, Fang HY, Wang XH, He D, Xie YY, Liu XH. HEY2, a target of miR-137, indicates poor outcomes and promotes cell proliferation and migration in hepatocellular carcinoma. *Oncotarget* 2016;7:38052–63.
11. Zhang W, Liu K, Liu S, Ji B, Wang Y, Liu Y. MicroRNA-133a functions as a tumor suppressor by targeting IGF-1R in hepatocellular carcinoma. *Tumour Biol.* 2015;36:9779–88.
12. Siragam V, Rutnam ZJ, Yang W, Fang L, Luo L, Yang X, Li M, Deng Z, Qian J, Peng C, Yang BB. MicroRNA miR-98 inhibits tumor angiogenesis and invasion by targeting activin receptor-like kinase-4 and matrix metalloproteinase-11. *Oncotarget* 2012;3:1370–85.
13. Yang G, Zhang X, Shi J. MiR-98 inhibits cell proliferation and invasion of non-small cell carcinoma lung cancer by targeting PAK1. *Int J Clin Exp Med.* 2015;8:20135–45.
14. Li F, Li XJ, Qiao L, Shi F, Liu W, Li Y, Dang YP, Gu WJ, Wang XG. miR-98 suppresses melanoma metastasis through a negative feedback loop with its target gene IL-6. *Exp Mol Med.* 2014;46:e116.
15. Xiang Q, Tang H, Yu J, Yin J, Yang X, Lei X. MicroRNA-98 sensitizes cisplatin-resistant human lung adenocarcinoma cells by upregulation of HMGA2. *Pharmazie* 2013;68:274–81.
16. Wang CY, Zhang JJ, Hua L, Yao KH, Chen JT, Ren XQ. MicroRNA-98 suppresses cell proliferation, migration and invasion by targeting collagen triple helix repeat containing 1 in hepatocellular carcinoma. *Mol Med Rep.* 2016;13:2639–44.
17. Bell JL, Wachter K, Muhleck B, Pazaitis N, Kohn M, Lederer M, Hüttelmaier S. Insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs): Post-transcriptional drivers of cancer progression? *Cell Mol Life Sci.* 2013;70:2657–75.
18. Luo Y, Sun R, Zhang J, Sun T, Liu X, Yang B. miR-506 inhibits the proliferation and invasion by targeting IGF2BP1 in glioblastoma. *Am J Transl Res.* 2015;7:2007–14.
19. Gutschner T, Hammerle M, Pazaitis N, Bley N, Fiskin E, Uckelmann H, Heim A, Grobeta M, Hofmann N, Geffers R, Skawran B, Longerich T, Breuhahn K, Schirmacher P, Mühleck B, Hüttelmaier S, Diederichs S. Insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1) is an important protumorigenic factor in hepatocellular carcinoma. *Hepatology* 2014;59:1900–11.
20. Qu Y, Pan S, Kang M, Dong R, Zhao J. MicroRNA-150 functions as a tumor suppressor in osteosarcoma by targeting IGF2BP1. *Tumour Biol.* 2016;37:5275–84.
21. Ni R, Huang Y, Wang J. miR-98 targets ITGB3 to inhibit proliferation, migration, and invasion of non-small-cell lung cancer. *OncoTargets Ther.* 2015;8:2689–97.
22. Fan YH, Ye MH, Wu L, Lv SG, Wu MJ, Xiao B, Liao CC, Ji QK, Chai Y, Zhu XG. Overexpression of miR-98 inhibits cell invasion in glioma cell lines via downregulation of IKKepsilon. *Eur Rev Med Pharmacol Sci.* 2015;19:3593–604.
23. Liu X, Zhang W, Guo H, Yue J, Zhuo S. miR-98 functions as a tumor suppressor in salivary adenoid cystic carcinomas. *OncoTargets Ther.* 2016;9:1777–86.
24. Fawzy IO, Hamza MT, Hosny KA, Esmat G, Abdelaziz AI. Abrogating the interplay between IGF2BP1, 2 and 3 and IGF1R by let-7i arrests hepatocellular carcinoma growth. *Growth Factors* 2016;34:42–50.
25. Zhou X, Zhang CZ, Lu SX, Chen GG, Li LZ, Liu LL, Yi C, Fu J, Hu W, Wen JM, Yun JP. miR-625 suppresses tumor migration and invasion by targeting IGF2BP1 in hepatocellular carcinoma. *Oncogene* 2015;34:965–77.
26. Fawzy IO, Hamza MT, Hosny KA, Esmat G, El Tayebi HM, Abdelaziz AI. miR-1275: A single microRNA that targets the three IGF2-mRNA-binding proteins hindering tumor growth in hepatocellular carcinoma. *FEBS Lett.* 2015;589:2257–65.
27. Zhang J, Cheng J, Zeng Z, Wang Y, Li X, Xie Q, Jia J, Yan Y, Guo Z, Gao J, Yao M, Chen X, Lu F. Comprehensive profiling of novel microRNA-9 targets and a tumor suppressor role of microRNA-9 via targeting IGF2BP1 in hepatocellular carcinoma. *Oncotarget* 2015;6:42040–52.