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Original Article

MicroRNA-519 suppresses cell growth and invasion by reducing HuR levels in hepatocellular carcinoma

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Abstract: MicroRNAs (miRNAs), which are suppressors of gene expression, participate in diverse biological functions and in carcinogenesis. Aberrant expression of miR-519 has been confirmed in various human cancer tissues and cells, and it represses HuR translation without influencing HuR mRNA level. However, the role of miR-519 in hepatocellular carcinoma (HCC) has yet to be elucidated. Here, we analyzed the miR-519 expression in HCC tissues and cell lines using qRT-PCR. We observed that miR-519 was dramatically downregulated in HCC clinical specimens and cell lines as compared to normal tissues and cells. In addition, enhanced expression of miR-519 resulted in the inhibition of cell growth through suppressing proliferation and promoting apoptosis, and repression of cell invasion. Importantly, we found that miR-519 potently repressed HuR expression through suppressing translation without affecting HuR mRNA level. Meanwhile, our results demonstrated that HuR protein level in the cancer tissues was inversely correlated with miR-519 expression in 25 HCC patients. Furthermore, overexpression of HuR abrogates the suppressive effects of miR-519 upregulation on HCC cell growth and invasion. Taken together, our findings suggested that miR-519 might functions as a tumor suppressor via inhibiting HuR expression in the development of progression of HCC, and implicated the potential application of miR-519 in HCC treatment.

Keywords: Hepatocellular carcinoma, miRNA-519, HuR, cell growth, cell invasion

Introduction

Hepatocellular carcinoma (HCC) is the fifth common solid tumor worldwide with an incidence of approximately 626,000 cases each year [1, 2]. In recent years, the prognosis of patients with HCC has been greatly improved owing to the development of effective surgical techniques and diagnostic methods. However, long-term prognosis is still unsatisfactory largely due to local invasion and intrahepatic metastasis [3, 4]. The molecular mechanisms for aggressive behavior of HCC remain unclear. Therefore, there is an urgent need to identify the new molecular biomarkers in predicting the aggressive biology of HCC and improve the current therapeutic strategies of HCC.

HuR, a ubiquitously expressed member of the ELAV (embryonic lethal abnormal vision) family, is an RNA binding protein (RBP) that stabilizes mRNAs of genes that modulate cell proliferation, angiogenesis, apoptosis, rapid inflammatory response and the stress response [5-7]. It is primarily in nucleus but translocates to the cytoplasm, and HuR stabilizes and/or regulates the translation of target mRNAs in the cytoplasm [8]. One of the RBPs most closely associated with tumorigenesis is HuR, which was upregulated in many human carcinomas including breast, uterine, gastric, prostate, brain and colon carcinomas [9-14]. HuR could function as a main trigger, a strong prognostic marker and a key therapeutic target for tumorigenesis [15].

MicroRNAs (miRNAs) are a class of non-coding RNA molecules (20-23 nucleotides), which represses gene expression through promoting mRNA decay or suppressing translation [16, 17]. As a novel layer of gene regulation, miRNAs harbored diverse functions, such as the regulation of cellular differentiation, proliferation, death and metabolism [18-21]. Furthermore, aberrant expression of miRNAs has been confirmed in a variety of human malignancies [22-26], and is also associated with the clinical outcome of cancer patients [26, 27]. Accumulating evidence indicates that miRNAs may function

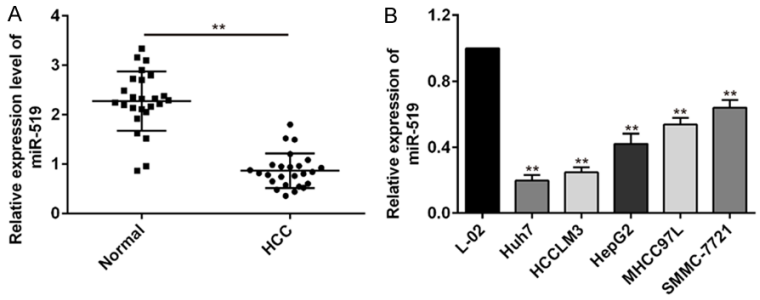

Figure 1. miR-519 expression was downregulated in hepatocellular carcinoma (HCC) tissues and cell lines. A. miR-519 expression was quantified by qRT-TPCR analysis in 25 pairs HCC and their corresponding adjacent nontumorous livers tissues. B. qRT-TPCR analysis of miR-519 expression in HCC cells (MHCC97L, SMMC7721, Huh7, HCCLM3, HepG2) and normal hepatocytes (LO-2). Results were normalized against the expression level of U6 messenger RNA (mRNA) in each sample. ** vs control, $P < 0.01$.

as oncogenes or tumor suppressor genes [28]. A recent report revealed that miR-519 is dramatically downregulated in some human cancer including ovarian, kidney and lung tumor samples and represses tumor growth through reducing HuR levels [29]. Overexpression of miR-519 reduced HuR abundance in several human carcinoma cell lines tested, HeLa (cervical), RKO (colon) and A2780 (ovarian) [30]. However, the expression of miR-519 and its role in HCC are still poorly understood.

In the present study, we explored the biological function and molecular mechanism of miR-519 in HCC. miR-519 was dramatically decreased in HCC clinical specimens and cell lines as compared to normal tissues and cells. Overexpression of miR-519 suppressed cell growth through inhibiting proliferation and promoting apoptosis, and inhibited cell invasion. Further study showed that miR-519 targeted HuR for translational repression via a target site in its 3'-UTR. Furthermore, we found that miR-519 expression inversely correlated with HuR protein in the tested HCC specimens. Meanwhile, suppression of cell growth and invasion by miR-519 was abrogated by HuR upregulation. Our data suggests that miR-519 may be playing a tumor suppressor role in HCC, with HuR as a direct and functional target.

Materials and methods

Patient tissue samples and liver cancer cell lines

Primary tumor tissue samples and their corresponding noncancerous liver samples were obtained from 25 patients (13 males and 12 females) who were diagnosed with HCC during hepatic resection in the Zhongshan Hospital, Fudan University between July 2014 and July 2015. None of the patients had received preoperative radio therapy or chemotherapy prior to surgical resection. Histological diagnosis and differentiation were evaluated independently by three pathologists according to the WHO classification system [31]. Fresh specimens were snap-frozen in liquid nitrogen and stored at -70°C immediately after resection for subsequent RNA extraction. The project protocol was approved by the Institutional Ethics Committee of Fudan University prior to the initiation of the study. All patients provided written informed consent for the use of the tumor tissues for clinical research. The HCC cell lines, MHCC97L, SMMC7721, Huh7, HCCLM3, HepG2, and one normal liver cell line, LO2, were purchased from the Shanghai Cell Bank (Shanghai, China). SMMC7721 and MHCC97L cells were cultured in RPMI-1640 (Gibco BRL, Grand Island, NY). HCCLM3, Huh7, HepG2 and LO2 cells were grown in DMEM (Gibco BRL, Grand Island, NY). All media were supplemented with 10% fetal calf serum (Gibco BRL, Grand Island, NY) and 100 IU/ml penicillin (Sigma, St. Louis, MO).

RNA preparation and quantitative real-time PCR

Total RNA was extracted from HCC tissue samples and culture cells using TRIzol Reagent (Invitrogen) according to the manufacturer's instructions. cDNA synthesis was performed by the High Capacity cDNA Synthesis Kit (Applied Biosystems) with miRNA-specific primers. The miR-519 primers were purchased from Ribobio (Guangzhou, China). The U6 gene was used as a reference control for miR-519. Real-time qRT-PCR was carried out on an Applied Biosystems 7500 Real-Time PCR machine with miRNA-specific primers by TaqMan Gene Expression Assay (Applied Biosystems). All reactions were performed in triplicate. The $2^{-\Delta\Delta Ct}$ method was conducted to analyze the miR-519 relative expression.

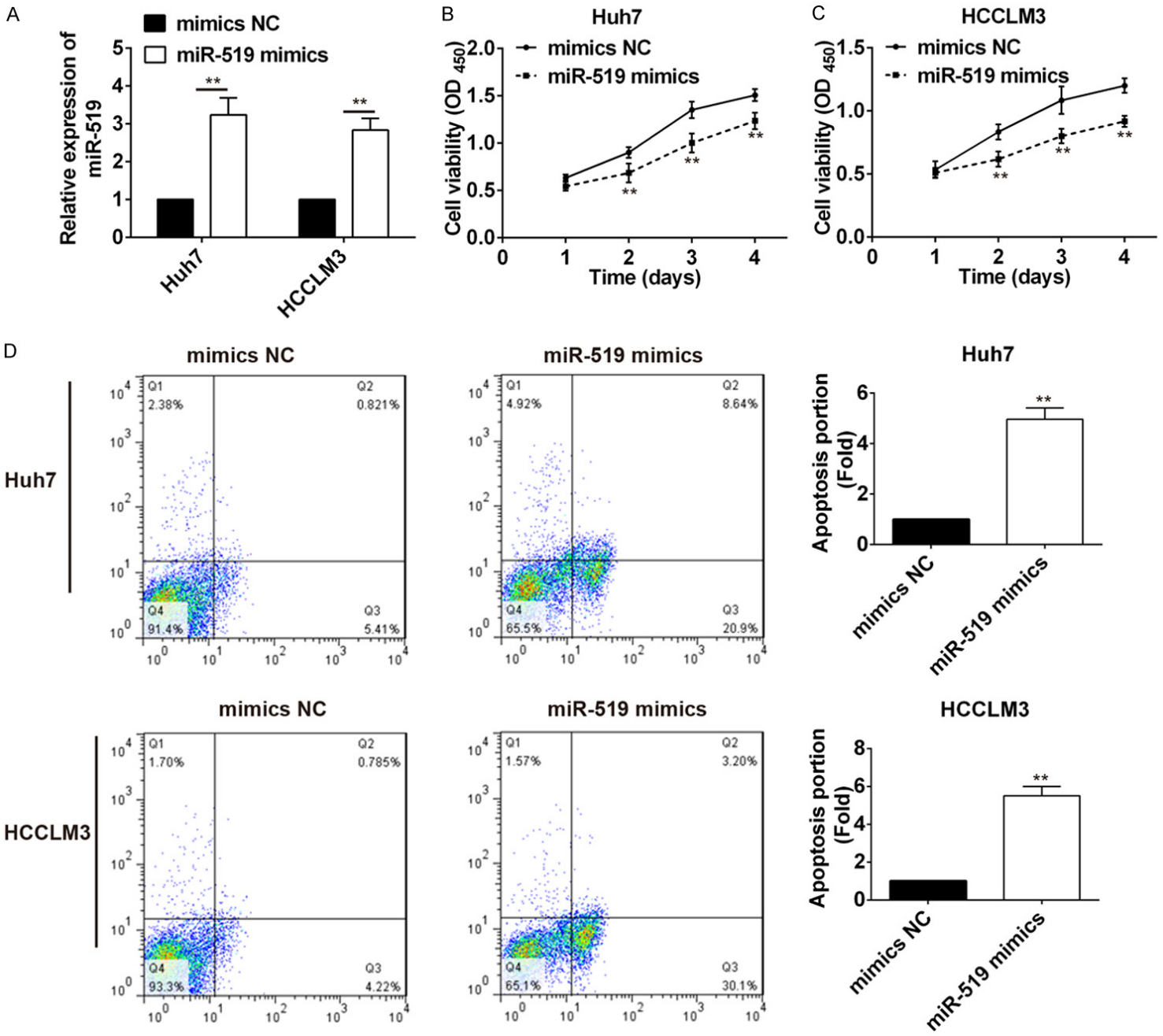
A
mimics NC
miR-519 mimics
Relative expression of miR-519
Huh7
HCCLM3
B
Huh7
mimics NC
miR-519 mimics
Cell viability (OD 450)
Time (days)
C
HCCLM3
mimics NC
miR-519 mimics
Cell viability (OD 450)
Time (days)
D
mimics NC
miR-519 mimics
Huh7
Q1 2.38%
Q2 0.821%
Q4 91.4%
Q3 5.41%
Q1 4.92%
Q2 8.64%
Q4 65.5%
Q3 20.9%
Apoptosis portion (Fold)
mimics NC
miR-519 mimics
HCCLM3
Q1 1.70%
Q2 0.785%
Q4 93.3%
Q3 4.22%
Q1 1.57%
Q2 3.20%
Q4 65.1%
Q3 30.1%
Apoptosis portion (Fold)
mimics NC
miR-519 mimics

Figure 2. Overexpression of miR-519 represses HCC cell growth. A. The Huh7 and HCCLM3 cells were transfected with miR-519 mimics or mimics negative control (NC) at a final concentration of 40 nM, miR-519 expression level were quantified by qRT-TPCR analysis. B and C. The Huh7 and HCCLM3 cells proliferation was measured using cell counting Kit-8 (CCK-8) assay following transfection with miR-519 mimics or mimics NC, respectively. D. Flow cytometry assay was performed to detect cell apoptosis in Huh7 and HCCLM3 cells after transfection with miR-519 mimics or mimics NC. ** vs control, $P < 0.01$.

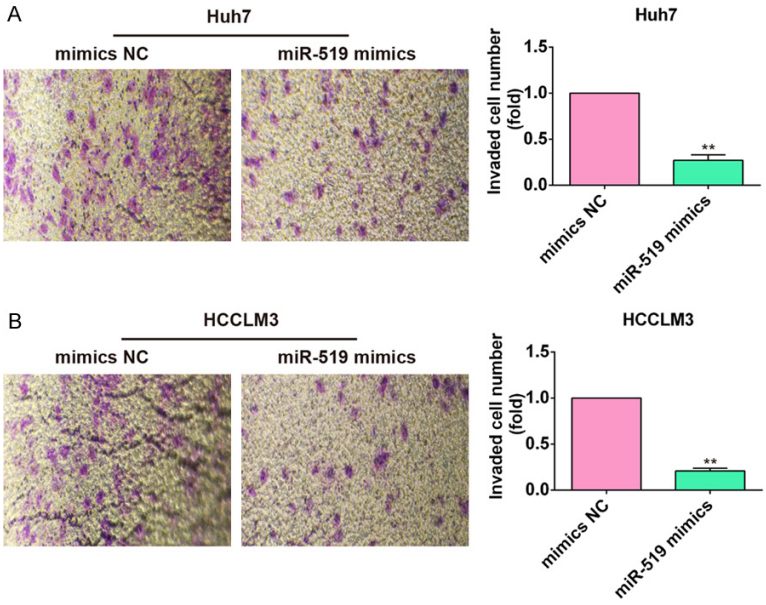

Figure 3. Overexpression of miR-519 represses HCC cell invasion. A and B. After the transfection with miR-519 mimics or mimics NC, the Huh7 and HCCLM3 cells invasion were measured by Transwell chamber assay, respectively. ** vs control, $P < 0.01$.

Cell transfection

The HCC cell lines Huh7 or HCCLM3 were seeded into six-well plates and incubated overnight. When cells were grown to 60-80% confluence, miR-519 mimics or negative control (NC) oligonucleotide (RiboBio, Guangzhou, China) (40 nM) were transfected using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. About 48 h after transfection, the transfection efficiency was assessed. The cells could be used for subsequent analysis when the transfection efficiency was above 80%. HuR rescue experiments, transfection reagent alone (control) or miR-519 mimics was transfected and 24 h later, 1 μg pcDNA-HuR was then transfected in Huh7 or HCCLM3 cells. 48 h after co-transfection, cells were harvested for cell growth and invasion assays.

Cell counting Kit-8 assay

The proliferation of cells was determined by the Cell counting Kit-8 (CCK-8) assay according to the manufacturer's instructions [32]. After transfection with miR-519 mimics or NC oligonucleotide into Huh7 or HCCLM3 cells, cells (5×10^4 cells/well) were seeded in 96-well plate with 100 μl DMEM medium supplemented with 10% FBS. After 48 h incubation, CCK-8 reagent (10 μl) was added to each well and continuously cultured for 1 h in 5% CO_2 (Thermo). The absorbance rate at 450 nm was measured by Microplate Reader (Bio-Rad, USA). All experiments were performed in quintuplicate on three separate occasions.

Apoptosis analysis

About 48 h after transfection, 1×10^6 cells were collected and washed twice with Hepes-buffered saline. After treatment with trypsin, cells were fixed with 70% ice-cold methanol at 4°C for 30 min. Cells were then resuspended in binding buffer and stained with 5 μl of Annexin V-FITC (BD, Mountain View, CA, United States) and 1 μl of propidium iodide (PI, 50 μg/ml) (BD, Mountain View, CA, United States). Flow cytometric evaluation was performed within 5 min. Stained cells were analyzed by flow cytometry (BD, FACSCalibur, CA, United States). The measurements were performed independently for at least three times with similar results.

Cell invasion assay

Huh7 or HCCLM3 cells were transfected with miR-519 mimics or mimic NC for 48 h, cell concentration in each group was adjusted to 2×10^5 cells/ml with serum-free medium. The upper chamber of Transwell chamber (Costar; 24-well insert, pore size: 8 μm) was filled with 200 μl cell suspension, and the lower chamber was filled with 500 μl of medium supplementing 20% FBS. In the invasion assay, polycarbon-

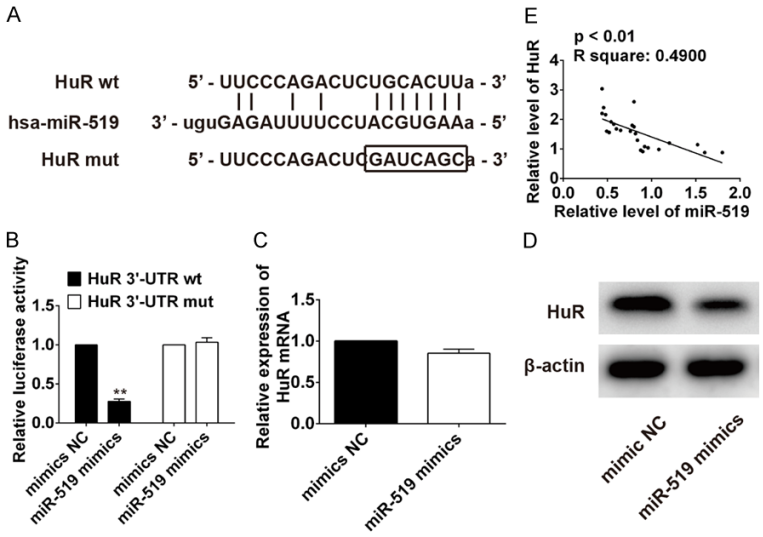

Figure 4. miR-519 represses HuR expression through suppressing translation without affecting HuR mRNA level. A. The HuR 3'-UTR region containing the wild type or mutant binding site for miR-519. B. The relative luciferase activity of HuR wild type or mutant 3'-UTR in Huh7 cells following transfection with the miR-519 mimic or corresponding mimic NC. C. The HuR mRNA level was measured using qRT-PCR in Huh7 cells transfected with the miR-519 mimic or corresponding mimic NC. D. Western blot was conducted to detect the protein level of HuR in Huh7 cells transfected with miR-519 mimic or corresponding mimic NC, β-actin was used as an internal control. E. miR-519 expression and HuR level in HCC tissues showed an inverse correlated trend from 25 HCC patients (R^2 = 0.4900). ** vs control, P < 0.01.

ate filters coated with 50 μl Matrigel (1:9, BD Bioscience) were placed in a Transwell chamber (Costar). The cells were incubated for 24 h and cells that did not migrate through the pores were removed by a cotton swab. Cells on the lower surface of the membrane were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. The migratory cells were visualized and counted in five random visual fields per insert under an inverted microscope at 200 × magnification (Nikon MicrophotFX, Japan).

Luciferase reporter assay

The wild-type HuR-3'-UTR (wt) and mutant HuR-3'-UTR (mut) containing the putative binding site of miR-519 were established (**Figure 4A**) and cloned in the firefly luciferase expressing vector pMIR-REPORT (Ambion, USA). Liver cancer cells Huh7 were seeded into 24-well plates the day before transfection, and transfected with either the pMIR-REPORT-HuR-3'-UTR wt or the pMIR-REPORT-HuR-3'-UTR mut reporter vector, together with miR-519 mimics or mimic NC using Lipofectamine 2000 (Invitrogen). 48 h after transfection, the luciferase activity was measured using the Dual-Light luminescent reporter gene assay system (Applied Biosystems). Each experiment was repeated at least three times in independent experiments. The ratio of Renilla luciferase to Firefly luciferase was calculated for each well.

Western blot analysis

48 h after transfection, total protein of cultured cells was extracted using RIPA buffer with protease inhibitor Cocktail (Pierce, Rockford, IL, USA). BCA protein assay kit (Beyotime, Haimen, China) was used to detect the concentration. Total proteins (20 μg) were separated on 10% SDS-PAGE (Sigma Aldrich, St. Louis, MO) and then transferred onto polyvinylidene fluoride membranes (Millipore, Billerica, MA, USA). After blocking with 5% non-fat milk at 4°C overnight, the membranes were incubated with primary antibodies against HuR (1:1000, Santa Cruz Biotechnology, Santa Cruz, CA) at 4°C overnight, β-actin (1:1000, Sigma, St. Louis, MO) was used as an internal control for protein loading. Horseradish peroxidase-conjugated (HRP) antibodies were used as the secondary antibodies. The bands were scanned using the ChemiDocXRS + Imaging System (Bio-Rad) and quantified using Quantity One v4.6.2 software (Bio-Rad).

Statistical analysis

All statistical analysis was performed using SPSS 14.0 software (Chicago, IL). Numerical data presented as the mean ± standard deviation. The difference between means was analyzed with Student's *t* test. Probability value of < 0.05 was considered significant and < 0.01 was considered very significant.

Results

miR-519 expression is downregulated in HCC tissues and cell lines

To analysis the miR-519 expression level, 25 pairs of HCC tissues and adjacent noncancer-

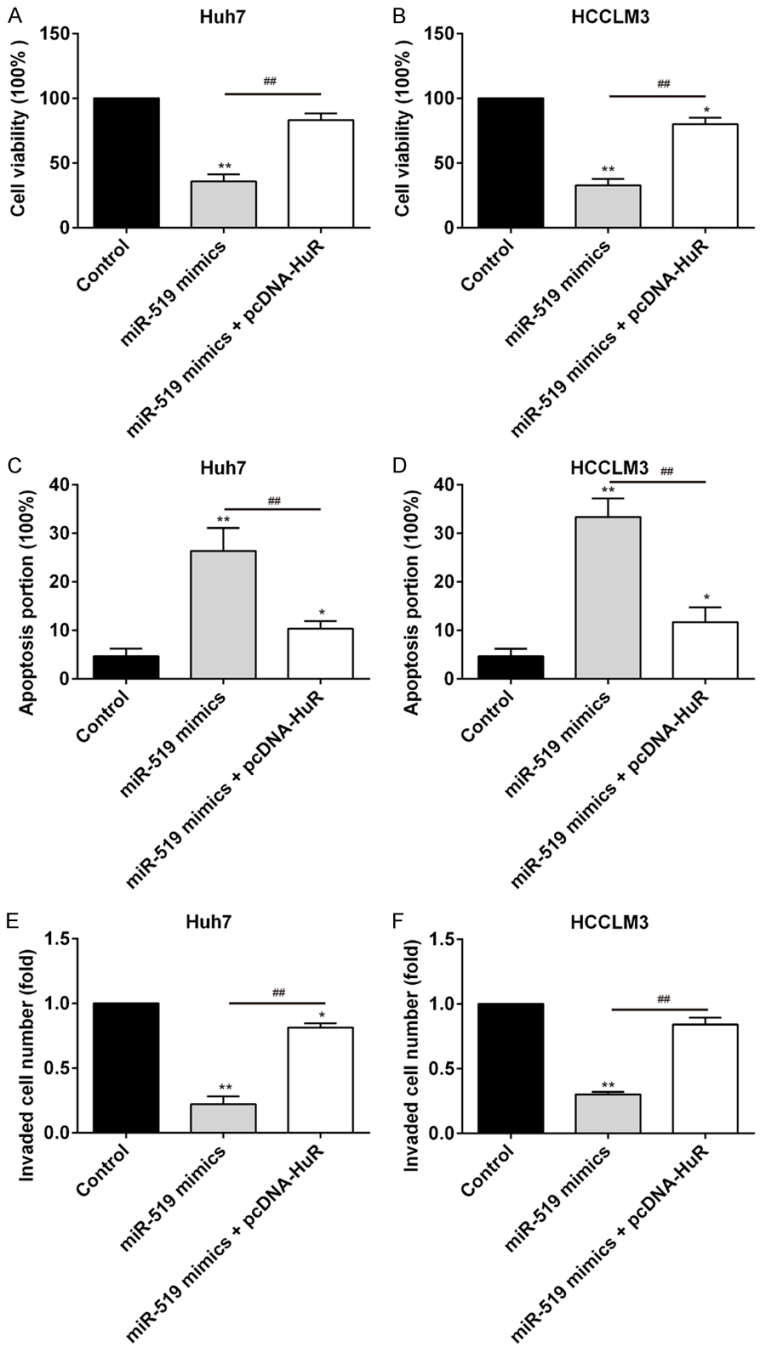

Figure 5. miR-519 suppression of cell growth and invasion via directly targeting HuR. The Huh7 and HCCLM3 cells were transfected with miR-519 mimic (miR-519), or co-transfected with miR-519 mimic and HuR overexpression plasmid (miR-519 mimics + pcDNA-HuR) for 48 h. Huh7 or HCCLM3 did not receive transfection (control group). A and B. The Huh7 and HCCLM3 cells proliferation were assessed by CCK-8 assay, respectively. C and D. The flow cytometric analysis was used to determine apoptotic cells in Huh7 and HCCLM3 cells, respectively. E and F. The Huh7 and HCCLM3 cells invasion were measured using Transwell chamber assay, respectively. ** vs control, $P < 0.01$.

ous liver tissues were detected by qRT-PCR. The result showed that miR-519 downregulation was observed in 25 cases of HCC tissues, which was significantly lower than that in matched non-tumorous tissues ($P < 0.01$; **Figure 1A**). To further verify this differential expression of miR-519, we detected the miR-519 expression in five kinds of HCC cells lines (SMMC7721, MHCC97L, HepG2, HCCLM3 and Huh7) and one normal liver cell line LO2. Consistent with the results in HCC tissues, miR-519 level was significantly downregulated in all HCC cells compared with normal liver cell line LO2 ($P < 0.01$; **Figure 1B**). These results indicated that the miR-519 expression level was downregulated in HCC.

Functional effects of miR-519 upregulation on HCC cells proliferation and apoptosis

Among the five HCC cell lines analyzed, Huh7 and HCCLM3 cells demonstrated relatively lower levels of miR-519 expression. Subsequently, these two cell lines were selected for further study. To explore the role of miR-519 in proliferation and apoptosis of HCC cells *in vitro*, the Huh7 and HCCLM3 cells were transfected with miR-519 mimics or mimics NC. We found that the miR-519 was significantly upregulated in Huh7 and HCCLM3 cells transfected with miR-519 mimics compared with NC ($P < 0.01$; **Figure 2A**). Then CCK-8 assay was performed to determine cells proliferation ability. The results showed that enhancement of the miR-519 expression level in Huh7 and HCCLM3 cells could significantly suppress cancer cell proliferation compared with NC group after transfection for 48 h ($P < 0.01$; **Figure 2B** and **2C**). Consistent with the CCK-8 assay, flow

cytometry demonstrated that Huh7 or HCCLM3 cells with ectopic overexpression of miR-519 showed a significant increase in cell apoptosis portion compared with the NC group ($P < 0.01$; **Figure 2D**). Taken together, these data suggested that miR-519 inhibits HCC cells growth via inducing cell apoptosis.

Ectopic expression of miR-519 inhibits HCC cells invasion

To investigate the function of miR-519 in cell invasion, we performed Transwell chamber assay to exam the HCC cells invasion. We found that the invaded cell number was markedly decreased in Huh7 cells following transfection with miR-519 mimics compared with NC ($P < 0.01$; **Figure 3A**). The same result was also observed in HCCLM3 cells ($P < 0.01$; **Figure 3B**). These results indicated that miR-519 inhibits HCC cells invasion.

HuR is translationally repressed by miR-519

To investigate the mechanism by which miR-519 affects cell growth and invasion in HCC, we performed bioinformatics analysis to predicate the putative targets of miR-519, and found that HuR might be a target gene of miR-519 and the target site located in the 3'-UTR (**Figure 4A**). To verify this bioinformatics predication, the wild type or mutant type of HuR-3'-UTR was constructed and inserted into the firefly luciferase expressing vector pMIR-REPORT. The wild type or mutant reporter plasmid was cotransfected into Huh7 cells along with miR-519 mimics or mimics NC, and measured the luciferase activity. We observed that miR-519 mimics significantly inhibited the luciferase activity compared with the mimic NC in the presence of the wild-type 3'-UTR ($P < 0.01$; **Figure 4B**), whereas miR-519 did not inhibit the luciferase activity of the reporter vector containing 3'-UTR of HuR with mutations in the miR-519-binding site (**Figure 4B**). Subsequently, we performed the Western blot and qRT-PCR analysis to determine the protein and mRNA level for HuR, respectively. We found that overexpression miR-519 has no significant influence on HuR mRNA levels relative to mimics NC (**Figure 4C**), but represses the protein level of HuR (**Figure 4D**), suggesting that miR-519 represses HuR translation but does not decay HuR mRNA. In order to explore the potential clinical significance of miR-519 in HCC patients, we investigated the association between miR-519 and HuR protein expression in HCC patient samples. Our results revealed that HuR protein level in the cancer tissues was inversely correlated with miR-519 expression in 25 HCC patients ($R^2 = 0.4900$, $P < 0.01$; **Figure 4E**). These data indicated that miR-519 suppresses HuR level through translational repression in HCC.

Overexpression of HuR abrogates the suppressive effects of miR-519 upregulation on HCC cells

To investigate whether ectopic expression of HuR could rescue the suppressive effect of miR-519, Huh7 or HCCLM3 cells were transfected with miR-519 mimics (miR-519 mimics group) or were co-transfected with miR-519 mimics and pcDNA-HuR plasmid (miR-519 mimics + pcDNA-HuR group). Huh7 or HCCLM3 cells did not receive transfection are used as control group. Subsequently, we performed CCK-8 assay and flow cytometric analysis to determine the cell proliferation and apoptosis in each group. We found that miR-519 upregulation significantly inhibited cell proliferation and increased apoptotic cell in Huh7 and HCCLM3 cells, but overexpression of HuR increased cell proliferation and decreased apoptotic cell after co-transfection with miR-519 mimics and pcDNA-HuR plasmid (**Figure 5A-D**). Then, the Transwell chamber assay was conducted to measure cell invasion. As expected, HuR upregulation significantly increases invaded cell number in Huh7 and HCCLM3 cells following co-transfection with miR-519 mimics and pcDNA-HuR plasmid compared with miR-519 mimics transfected group (**Figure 5E** and **5F**). Taken together, these data indicated that miR-519 suppresses cell growth and invasion through inhibiting HuR expression.

Discussion

Local invasion and intrahepatic metastasis have been identified as the main causes of the poor clinical outcome in HCC patients [3, 4]. miRNAs acted as oncogenes or tumor suppressor genes involved in various tumor processes, including tumor migration, invasion and development [15, 28, 33]. Previous study uncovered that miR-519 reduces tumorigenesis in murine ovarian tumors [30]. However, the role of miR-519 in HCC has yet to be elucidated. In this

study, we first confirmed that miR-519 was markedly downregulated in human HCC tissues and cell lines. We further demonstrated that miR-519 inhibited cell growth due to a dramatic promotion of cell apoptosis. Moreover, overexpressing miR-519 in Huh7 and HCCLM3 cells inhibited cell invasion. Importantly, we found that miR-519 represses HuR translation through targeting its 3'-UTR but does not affect HuR mRNA level. HuR upregulation abrogated miR-519-engendered repression of cell growth and invasion, suggesting that HuR is a functional target of miR-519 in HCC cells.

It has been well documented that miRNAs can act as tumor suppressors or oncogenes to play important roles in the initiation, promotion and development of various cancers [34] and aberrant expression of miRNAs might be of potential use as a diagnostic and prognostic biomarker for human cancer including HCC. Several miRNAs have been proved to regulate tumor cell growth, migration and invasion in HCC, such as miR-20, miR-125b, miR-182, miR-494, and miR-372 [35-38]. Ectopic expression of miR-519 was reported in various human cancer tissues and cells including ovarian, kidney and lung tumor samples, and HeLa (cervical), RKO (colon) and A2780 (ovarian) cell lines [29, 30]. Moreover, miR-519 might function as suppressor to repress tumor growth in murine ovarian tumors [29]. Therefore, we postulated that miR-519 expression is significant in the underlying mechanisms of tumorigenesis in various cell types. In current study, we performed qRT-PCR to detect miR-519 level in 25 pairs of HCC tissues and adjacent noncancerous liver tissues and found that miR-519 expression was significantly lower in HCC tissues compared with adjacent healthy liver tissues. Similar result was confirmed in different HCC cell lines. Furthermore, our results clearly showed that miR-519 overexpression repressed cell growth through inhibiting proliferation and promoting apoptosis, and suppressed cell invasion. Thus, these data suggested that miR-519 function as a tumor suppressor in HCC through inhibiting cell growth and invasion. However, the molecular mechanisms of miR-519 suppression need further studies to be understood deeply.

Under specific cellular conditions, miRNA-regulated suppression was prevented or reversed, and RBPs modulated inhibitory effect of miRNAs via acting on the same mRNA. Among the RBPs that antagonize or facilitate miRNA-regulated repression, a prominent factor is HuR, which affects stability and the translation of numerous genes implicated in cancer aggressiveness [39]. HuR expression has recently been shown to be modulated by miR-519 through inhibiting its translation, which lead to suppression of cancer cell growth *in vitro* [40]. Abdelmohsen et al. revealed that HuR protein is abundantly expressed in ovarian, lung and kidney carcinomas, while miR-519 levels were upregulated when compared with adjacent nonneoplastic tissues [29]. Our data showed that miR-519 may function as a tumor suppressor for HCC by targeting the HuR for translational repression but does not affect HuR mRNA level. Moreover, we demonstrated that miR-519 level inversely correlated with HuR protein expression in the tested tumor and non-tumor human tissues. Meanwhile, restored expression of HuR abrogated the suppressive effects of miR-519 on HCC cell growth and invasion. Taken together, these results indicated that miR-519 represses HCC cell growth and invasion via inhibiting HuR *in vitro*.

In conclusion, our results uncovered that miR-519 aberrant expression was verified in human HCC tissues and cell lines. We found that miR-519 suppresses cell growth and invasion in HCC cells through targeting HuR for translational repression. These findings indicated that the complex miR-519/HuR may as a novel potential target for the development of therapies aimed at overcoming aggressive disease in HCC patients.

Acknowledgements

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Disclosure of conflict of interest

None.

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References

[1] Colombo M and Hospital M. Natural history of hepatocellular carcinoma. Cancer Imaging 2005; 14: 85-88.

[2] Chen HW, Huang XD, Li HC, He S, Ni RZ, Chen CH, Peng C, Wu G, Wang GH, Wang YY, Zhao YH, Zhang YX, Shen AG and Wang HM. Expression of FOXJ1 in hepatocellular carcinoma: correlation with patients' prognosis and tumor cell proliferation. Mol Carcinog 2013; 52: 647-659.

[3] Block TM, Mehta AS, Fimmel CJ and Jordan R. Molecular viral oncology of hepatocellular carcinoma. Oncogene 2003; 22: 5093-5107.

[4] Feng JT, Shang S and Beretta L. Proteomics for the early detection and treatment of hepatocellular carcinoma. Oncogene 2006; 25: 3810-3817.

[5] Brennan CM and Steitz JA. HuR and mRNA stability. Cell Mol Life Sci 2001; 58: 266-277.

[6] Dixon DA, Tolley ND, King PH, Nabors LB, McIntyre TM, Zimmerman GA and Prescott SM. Altered expression of the mRNA stability factor HuR promotes cyclooxygenase-2 expression in colon cancer cells. J Clin Invest 2001; 108: 1657-1665.

[7] Lal A, Kawai T, Yang X, Mazan-Mamczarz K and Gorospe M. Antiapoptotic function of RNA-binding protein HuR effected through prothymosin α. EMBO J 2005; 24: 1852.

[8] Hinman MN and Lou H. Diverse molecular functions of Hu proteins. Cell Mol Life Sci 2008; 65: 3168.

[9] Denkert C, Weichert W, Winzer KJ, Müller BM, Noske A, Niesporek S, Kristiansen G, Guski H, Dietel M and Hauptmann S. Expression of the ELAV-like protein HuR is associated with higher tumor grade and increased cyclooxygenase-2 expression in human breast carcinoma. Clin Cancer Res 2004; 10: 5580-6.

[10] Lim SJ, Kim HJ, Kim JY, Park K and Lee CM. Expression of HuR is associated with increased cyclooxygenase-2 expression in uterine cervical carcinoma. Int J Gynecol Pathol 2007; 26: 229-34.

[11] Mrena J, Wiksten JP, Thiel A, Kokkola A, Pohjola L, Lundin J, Nordling S, Ristimäki A and Haglund C. Cyclooxygenase-2 is an independent prognostic factor in gastric cancer and its expression is regulated by the messenger RNA stability factor HuR. Clin Cancer Res 2005; 11: 7362-8.

[12] Niesporek S, Kristiansen G, Thoma A, Weichert W, Noske A, Buckendahl AC, Jung K, Stephan C, Dietel M and Denkert C. Expression of the ELAV-like protein HuR in human prostate carcinoma is an indicator of disease relapse and linked to COX-2 expression. Int J Oncol 2008; 32: 341-347.

[13] Nabors LB, Gillespie GY, Harkins L and King PH. HuR, a RNA stability factor, is expressed in malignant brain tumors and binds to adenine- and uridine-rich elements within the 3' untranslated regions of cytokine and angiogenic factor mRNAs. Cancer Res 2001; 61: 2154.

[14] de Silanes IL, Fan J, Yang X, Zonderman AB, Potapova O, Pizer ES and Gorospe M. Role of the RNA-binding protein HuR in colon carcinogenesis. Oncogene 2003; 22: 7146-7154.

[15] Guo X, Wu Y and Hartley RS. MicroRNA-125a represses cell growth by targeting HuR in breast cancer. RNA Biol 2009; 6: 575-583.

[16] Kim VN, Han J and Siomi MC. Biogenesis of small RNAs in animals. Nat Rev Mol Cell Biol 2009; 10: 126-139.

[17] Chekulaeva M and Filipowicz W. Mechanisms of miRNA-mediated post-transcriptional regulation in animal cells. Curr Opin Cell Biol 2009; 21: 452-460.

[18] Chen CZ. MicroRNAs modulate hematopoietic lineage differentiation. Science 2004; 303: 83-86.

[19] Cheng AM, Byrom MW, Shelton J and Ford LP. Antisense inhibition of human miRNAs and indications for an involvement of miRNA in cell growth and apoptosis. Nucleic Acids Res 2005; 33: 1290-1297.

[20] Croce CM and Calin GA. miRNAs, cancer, and stem cell division. Cell 2005; 122: 6-7.

[21] Karp X and Ambros V. Encountering MicroRNAs in cell fate signaling. Science 2005; 310: 1288.

[22] Su H, Yang JR, Xu T, Huang J, Xu L, Yuan Y and Zhuang SM. MicroRNA-101, down-regulated in hepatocellular carcinoma, promotes apoptosis and suppresses tumorigenicity. Cancer Res 2009; 69: 1135.

[23] Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR and Golub TR. MicroRNA expression profiles classify human cancers. Nature 2005; 435: 834-838.

[24] Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M, Stephens RM, Okamoto A, Yokota J, Tanaka T, Calin GA, Liu CG, Croce CM and Harris CC. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. Cancer Cell 2006; 9: 189-198.

[25] Iorio MV, Visone R, Di Leva G, Donati V, Petrocca F, Casalini P, Taccioli C, Volinia S, Liu CG, Alder H, Calin GA, Ménard S and Croce CM. MicroRNA signatures in human ovarian cancer. Cancer Res 2007; 67: 8699.

[26] Murakami Y, Yasuda T, Saigo K, Urashima T, Toyoda H, Okanoue T and Shimotohno K. Com-

prehensive analysis of microRNA expression patterns in hepatocellular carcinoma and nontumorous tissues. Oncogene 2005; 25: 2537-2545.
[27] Calin GA, Ferracin M, Cimmino A, Di Leva G, Shimizu M, Wojcik SE, Iorio MV, Visone R, Sever NI, Fabbri M, Iuliano R, Palumbo T, Pichiorri F, Roldo C, Garzon R, Sevignani C, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M and Croce CM. A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. N Engl J Med 2005; 353: 1793-1801.
[28] Esquela-Kerscher A and Slack FJ. Oncomirs [mdash] microRNAs with a role in cancer. Nat Rev Cancer 2006; 6: 259-269.
[29] Abdelmohsen K, Kim MM, Srikantan S, Mercken EM, Brennan SE, Wilson GM, De Cabo R and Gorospe M. miR-519 suppresses tumor growth by reducing HuR levels. Cell Cycle 2010; 9: 1354-1359.
[30] Abdelmohsen K, Srikantan S, Kuwano Y and Gorospe M. miR-519 reduces cell proliferation by lowering RNA-binding protein HuR levels. Proc Natl Acad Sci U S A 2008; 105: 20297-20302.
[31] Zhou L, Rui JA, Ye DX, Wang SB, Chen SG and Qu Q. Edmondson-Steiner grading increases the predictive efficiency of TNM staging for long-term survival of patients with hepatocellular carcinoma after curative resection. World J Surg 2008; 32: 1748-56.
[32] He Y, Meng XM, Huang C, Wu BM, Zhang L, Lv XW and Li J. Long noncoding RNAs: novel insights into hepatocelluar carcinoma. Cancer Lett 2014; 344: 20-27.
[33] Vanharanta S and Massagué J. Origins of metastatic traits. Cancer Cell 2013; 24: 410-421.
[34] MacFarlane LA and Murphy PR. MicroRNA: biogenesis, function and role in cancer. Curr Genomics 2010; 11: 537-561.
[35] Fan MQ, Huang CB, Gu Y, Xiao Y, Sheng JX and Zhong L. Decrease expression of microRNA-20a promotes cancer cell proliferation and predicts poor survival of hepatocellular carcinoma. J Exp Clin Cancer Res 2013; 32: 21-21.
[36] Zhou JN, Zeng Q, Wang HY, Zhang B, Li ST, Nan X, Cao N, Fu CJ, Yan XL, Jia YL, Wang JX, Zhao AH, Li ZW, Li YH, Xie XY, Zhang XM, Dong Y, Xu YC, He LJ, Yue W and Pei XT. MicroRNA-125b attenuates epithelial-mesenchymal transitions and targets stem-like liver cancer cells through small mothers against decapentaplegic 2 and 4. Hepatology 2015; 62: 801-815.
[37] Du C, Weng X, Hu W, Lv Z, Xiao H, Ding C, Gyabaah OA, Xie H, Zhou L, Wu J and Zheng S. Hypoxia-inducible MiR-182 promotes angiogenesis by targeting RASA1 in hepatocellular carcinoma. J Exp Clin Cancer Res 2015; 34: 67.
[38] Chuang KH, Whitney-Miller CL, Chu CY, Zhou Z, Dokus MK, Schmit S and Barry CT. MicroRNA-494 is a master epigenetic regulator of multiple invasion-suppressor microRNAs by targeting ten eleven translocation 1 in invasive human hepatocellular carcinoma tumors. Hepatology 2015; 62: 466-480.
[39] Abdelmohsen K and Gorospe M. Posttranscriptional regulation of cancer traits by HuR. Wiley Interdiscip Rev RNA 2010; 1: 214-229.
[40] Ristimäki A. Tumor suppressor effect of the microRNA miR-519 is mediated via the mRNA-binding protein HuR. Cell Cycle 2010; 9: 1234-1234.