

MicroRNA-526a targets p21-activated kinase 7 to inhibit tumorigenesis in hepatocellular carcinoma

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Abstract. MicroRNAs belong to a series of noncoding RNAs and have diverse roles in several biological processes. The association between aberrant microRNA expression and tumorigenesis is complex and remains to be fully elucidated. The present study investigated whether microRNA (miR)-526a can suppress the progression of hepatocellular carcinoma (HCC) *in vitro* and *in vivo*. Reverse transcription-quantitative polymerase chain reaction, luciferase reporter assay, invasion assay, western blotting and *in vivo* implantation were used to investigate the potential function of miR-526a. The present study observed that the level of miR-526a was downregulated in HCC tissues and well-established cell lines. In addition, the ectopic introduction of miR-526a into Huh7 and HepG2 cells significantly attenuated HCC tumorigenesis, including proliferation, migration and invasion. The growth of tumor xenografts was also inhibited following transfection with miR-526a. Using overlapping strategies, p21-activated kinase 7 (PAK7) was predicted to be a target for miR-526a, and this was verified experimentally. An inverse correlation was found between miR-526a and PAK7 in HCC tissues. The results of the present study revealed a novel function of miR-526a and may provide crucial insight into therapeutic interventions targeting microRNAs.

Introduction

The occurrence of tumors poses a serious threat to life. The third leading cause of mortality is reported to be hepatocellular carcinoma (HCC), particularly in China (1). Patients with chronic hepatitis are more at risk for the onset of HCC, and this may have resulted in the significant increase of HCC in

previous years (2). Due to substantial deficiencies in healthcare and physiological conditions, only a fraction of patients are eligible for treatments, including liver transplantation and resection. Therefore, the overall survival rate for patients with HCC is relatively low (3). The majority of patients with HCC are diagnosed during the late stages and, therefore, their prognosis is poor, primarily due to the lack of early biomarkers. As a result, identifying efficient and sensitive markers for HCC is urgently required.

The heterogeneous nature of HCC also complicates current understanding and effective treatment. Previous evidence suggested that the variations in HCC may depend on stochastic alterations during the period of carcinogenesis (4). The abnormal activation of several pathways, including insulin growth factor and Akt signaling, are also major causes of tumorigenesis (5,6). In-depth knowledge of the determinant molecular mechanisms in different HCC backgrounds presents a challenge in clinical and experimental investigations. An effective therapeutic option cannot be developed unless the genomic background of individuals has been clearly characterized.

MicroRNAs are short-length, noncoding RNAs, which can suppress gene expression through base-pairing to the 3'-untranslated region (3'-UTR) of targets (7,8). Increasing evidence has shown that microRNAs may be pivotal in the development of HCC (9-13). For example, microRNA (miR)-148b can regulate cancer stem cell properties in HCC by directly targeting Neuropilin-1, which is a transmembrane receptor critically implicated in initiating angiogenesis and metastasis (13). miRNA-26a can suppress the progression of HCC primarily by targeting the c-Met pathway (14). The microRNA-based classification of HCC has also been demonstrated and three clusters have been identified (2). miR-517a and miR-520c have been shown to be significantly positively correlated with the malignant characteristics of HCC (2). Other microRNAs, including the let-7 family, miR-29, miR-183 and miR-122, showed reduced expression in tumor tissues suggesting tumor suppressive roles for specific microRNAs (15-18). Therefore, the role of microRNAs in tumor development is complex and warrants further investigation.

In the present study, the novel function of a less well-known microRNA, miR-526a, in the tumorigenesis of HCC was investigated. It was found that miR-526a was predominantly downregulated in HCC cell lines and human specimens. The

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ectopic expression of miR-526a significantly attenuated the proliferation, migration and invasion of HCC cell lines *in vitro*. Furthermore, miR-526a transfection decreased tumor volume in an implantation assay *in vivo*. In addition, p21-activated kinase 7 (PAK7) was identified as a potential miR-526a target in HCC. The inverse correlation between PAK7 and miR-526a was significant in tumor specimens. These results are the first, to the best of our knowledge, to show that miR-526a can serve as a candidate tumor suppressor by targeting PAK7 in HCC, providing crucial insight into effective therapeutic treatment, possibly by targeting microRNAs.

Materials and methods

Cell culture and human specimens. All HCC cell lines (Huh7, SK-Hep1, SMMC-7721, Bel7402, HepG2, Hep3B and MHCC97-H), together with the control cell line (L02), were purchased from American Type Culture Collection (Rockville, MD, USA). The culture media for these cell lines comprised RPMI-1640 medium (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The medium was further supplemented with 5% fetal bovine serum (FBS) and penicillin (100 U/ml) (Gibco; Thermo Fisher Scientific, Inc.) in a humidified atmosphere of 5% CO₂ at 37°C. The HCC specimens included were all surgical archives from patients at The Affiliated Hospital of Guizhou Medical University (Guiyang, China) between September 2013 and July 2015. All specimens were maintained in liquid nitrogen at -80°C following resection prior to experiments. All patients signed informed consent forms. The protocols of experimental procedures involving the use of human samples were formally approved by the Human Research Ethics Committee of The Affiliated Hospital of Guizhou Medical University (no. 2013-CF-0010).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. The gene expression levels of miR-526a and PAK7 in HCC were measured using RT-qPCR analysis. In brief, RNA was extracted from the HCC cells and HCC human specimens using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and corresponding cDNA was obtained with a SYBR Premix Ex Taq™ kit (Takara Bio, Inc., Otsu, Japan) according to the manufacturer's protocol. Briefly, 5 μm sections were cut from tissue archives and placed in a micro-centrifuge tube. A total of 1 ml AutoDewaxer™ was added to the sample and the tube was incubated for 15 min at 95°C. The sample was then centrifuged for 1 min at room temperature at 10,000 x g. The incubatory and centrifugation steps were then repeated three times. A total of 300 μl lysis buffer was added to the deparaffinized tissue and it was vortexed for 1 min to obtain a homogeneous solution. A TaqMan miRNA RT-qPCR kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used and GAPDH was used as an internal control. For PAK7 detection, the SYBR-Green PCR Master Mix kit was used (Applied Biosystems; Thermo Fisher Scientific, Inc.). All kinetic reactions for RT-qPCR were performed using the ABI PRISM® 7000 sequence detection system (Applied Biosystems; Thermo Fisher Scientific, Inc.). A total of 2 mg total RNA template was annealed with 1 ml (500 ng) random primer in a sterile RNase-free micro-centrifuge tube and heated at 70°C for 5 min. The following reagents

were then added: First-strand 5xbuffer (4 ml), 0.1 M DTT (2 ml), 10 mM dNTP mix (2.5 mM each; 2 ml), RNasin™ (1 ml) and SuperScript™ II (2 ml). PCR amplification was then carried out for 35 cycles in an ABI 9700 machine (Applied Biosystems; Thermo Fisher Scientific, Inc.). Reactions were carried out at 50°C for 2 min and 95°C for 10 min followed by 35 cycles of 95°C for 15 sec and 60°C for 1 min. Gene expression was quantified as fold-change. A total of 1 μl of the RNA was added to the Nanodrop™ to automatically measure RNA at wavelengths of 260 and 280 nm (19). The primer sequences were as follows: PAK7, forward 5'-ACGTACCGA CAGTGCTGG-3' and reverse 5'-TACGCCAATCGATGC AGGAGAA-3'; miR-526a, forward 5'-ACACTCGAGCTG GGGCTTGTTCGCTTGCCTT-3' and reverse 5'-TGATGC TTCTGAGTCG-3'; GAPDH, forward 5'-CTCTAGGTCTAT CGGT-3' and reverse 5'-ATGTTATGCCGTAAGCAGT-3'.

Generation of stably transfected cell lines. A lentiviral system was used to ectopically overexpress miR-526a in the Huh7 and HepG2 cells in the present study. The lentiviruses containing miR-526a mimics (miR-526a) and negative controls were synthesized and purchased from Sigma-Aldrich; Merck Millipore (Darmstadt, Germany). The Lipofectamine® 2000 system (Invitrogen; Thermo Fisher Scientific, Inc.) was used for viral transfection. Cells (1x10⁵ cells/ml) were seeded into 12-well plates in 100 μl growth medium (RPMI-1640, Thermo Fisher Scientific, Inc.) at the time of transfection. Then, 50 μl transfection lipoplexes were added to each well. The cells were incubated in RPMI-1640 under 5% CO₂ at 37°C in a humidified incubator for 5 h. The cells were then washed with PBS, medium replaced and cells were cultured for another 48 h. At 24 h post-transfection, the culture medium was replaced with fresh medium. All plasmids were experimentally verified using RT-qPCR analysis.

Dual-luciferase reporter assay. The PAK7 gene was obtained from a cDNA library (LIBEST_017384; www.ncbi.nlm.nih.gov/nucest/DR000640.1) and verified using DNA sequencing. The 3'-untranslated region (UTR) of PAK7 with the predicted binding site with hsa-miR-526a was cloned into a Renilla luciferase reporter plasmid (pRL-TK; Sigma-Aldrich; Merck Millipore) leading to the wild-type PAK7 luciferase reporter plasmid (PAK7 3'-UTR WT). The has-miR-526a binding site for the 3'-UTR region of PAK7 was further mutated using a Quick-Change™ Site-Directed Mutagenesis kit (Stratagene; Agilent Technologies, Inc., Santa Clara, CA, USA). The mutated PAK7 3'-UTR was then inserted into the pRL-TK plasmid to produce the mutated luciferase reporter plasmid for PAK7 (PAK7 3'-UTR MUT). Co-transfection was performed for 24 h. The relative luciferase units were determined using a dual-luciferase reporter assay (Promega Corporation, Madison, WI, USA) according to the manufacturer's protocol.

Invasion assay. The chemotaxis 96-well Transwell assay (Chemicon, Temecula, CA, USA) was used to quantify the invasion of cells. The upper chamber of the Transwell was covered with Matrigel (Sigma-Aldrich; Merck Millipore) overnight. The lentivirus-treated Huh7 and HepG2 cells were seeded within the upper chambers (10⁵ cells per well) in RPMI-1640 medium without serum (Gibco; Thermo Fisher Scientific,

Inc.). The lower chambers were then filled with RPMI-1640 medium containing 2% FBS. After 24 h, the migrated cells, which had migrated into the lower chambers were fixed with 5% PFA (Sigma-Aldrich; Merck Millipore) and stained with crystal violet (Sigma-Aldrich; Merck Millipore) at 20°C. Images were captured through a Leica inverted fluorescent microscope (DM-IRB; Leica Microsystems GmbH, Wetzlar, Germany). The invasion was determined as fold-change relative to the control.

Prediction of miR-526a target. The present study used algorithms for target gene prediction: TargetScan (www.targetscan.org), PicTar (pictar.mdc-berlin.de) and miRDB (www.mirdb.org) as previously described (20,21). The predicted targets were ranked by Z scores. Top ranking and overlapping targets were selected for further verification.

Proliferation assay. The Huh7 and HepG2 cells were seeded in 96-well plates (10^4 cells per well) for 5 days. A Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Inc., Kumamoto, Japan) was used. At each 24 h interval, MTT solution was added into culture medium at a final concentration of 5 mg/ml. After 4 h, the medium was removed and the crystalline formazan was dissolved in 100 μ l SDS (15%; Sigma-Aldrich; Merck Millipore) solution for 24 h. The plate was shaken for 5 min leading to complete solubilization. Finally, the optical density at 490 nm was evaluated using a Spectramax M5 microplate reader (Molecular Devices LLC, Sunnyvale, CA, USA) according to the manufacturer's protocols.

Migration assay. Boyden Chambers (BD Biosciences, San Jose, CA, USA) were used in a 12-well plate. At 24 h post-transfection, 10^5 cells from the RPMI-1640 medium were seeded in the upper chamber. RPMI-1640 with 15% FBS was used as an attractant. After 24 h, the non-migrated cells were removed, loaded into 5% PFA and stained using crystal violet. The image was visualized by Leica DM-IRB inverted microscope (Leica Microsystems GmbH, Wetzlar, Germany).

In vivo implantation assay and immunohistochemical staining. BALB/c nude mice (6-week-old, average weight, 15.2 g; 5 male and 5 female) were purchased from the Model Animal Research Center (Nanjing, China) and were maintained in SPF conditions for an additional week. Mice were housed at 20°C, with 55-60% humidity and a light-dark cycle of 10-12 h. *Ad libitum* access to food and water was provided. Animal manipulation and experiments were performed in accordance with the General Guide for the Use of Laboratory Animals and approved by the Animal Experimental Ethics Committee of The Affiliated Hospital of Guizhou Medical University (no. 2013-AF-0035). The HepG2 cells transduced with lentivirus for 12 h were continuously cultured for a further 24 h. The cells were then resuspended in serum-free medium and 10^6 cells were implanted subcutaneously into the null mice. Tumor volume *in vivo* was recorded each week. After 30 days, all mice were sacrificed by an overdose of sodium pentobarbital (5%, 250 mg/kg with intraperitoneal injection; catalog no. 1507002; Sigma-Aldrich; Merck KGaA) and implants were immunostained with Ki-67 (Sigma-Aldrich; Merck Millipore)

for 30 min at 37°C. A rabbit monoclonal anti-mouse/human Ki-67 antibody was used (catalog no. P6834; 1:1,000; Sigma-Aldrich; Merck Millipore) for 15 min at 20°C. The immunohistochemistry was performed with an enhanced biotin-free polymer one-step staining technique with goat anti-rabbit secondary antibody (catalog no. K5007; 1:1,000; Dako, Glostrup, Denmark) for 1 h at 37°C, according to the manufacturer's protocols.

Western blot analysis. The Huh7 and HepG2 cells were resuspended and harvested with cell lysis buffer containing 10% glycerol and 2% NP-40 (Sigma-Aldrich; Merck Millipore). The protein extracts (150 μ g) were then resolved on a 7% SDS-PAGE gel and transferred onto a nitrocellulose membrane (Sigma-Aldrich; Merck Millipore). The protein concentration was measured by Bradford protein assay (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The primary antibody against human PAK7 (catalog no. K3265; 1:1,000; Sigma-Aldrich, Merck KGaA) was used at 4°C overnight, and HRP-conjugated antibodies (catalog no. A9044; 1:1,000; Sigma-Aldrich, Merck KGaA) at 20°C for 4 h. The blots were monitored using a chemiluminescence kit (Sino-American Biotechnology Co., Ltd., Shanghai, China).

Statistical analysis. All experiments were performed with three replicates. The results are presented as the mean \pm standard error of the mean. Statistical differences were determined using Student's t-test (SPSS 16.0; SPSS, Inc., Chicago, IL, USA). $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Expression of miR-526a is frequently downregulated in HCC. To determine the expression of miR-526a in HCC tissues, RT-qPCR analysis was performed and the results showed that the levels of miR-526a were significantly downregulated in HCC specimens, compared with normal adjacent tissues ($n=135$; $P < 0.01$; Fig. 1A). Low expression of miR-526a was associated with histological grade ($P=0.01$), vascular invasion ($P=0.002$) and metastasis ($P < 0.001$), as shown in Table I. However, no significant correlations were found between the expression of miR-526a and age or gender (Table I). The expression of miR-526a was further quantified in seven HCC cell lines. The results of the RT-qPCR analysis also suggested that miR-526a was reduced in tumor cells (Fig. 1B). Taken together, these results suggested that miR-526a may be involved in regulating the progression of HCC. It was found that the levels of miR-526a in Huh7 and HepG2 cells were relatively low, compared with those in other cell lines. Therefore, Huh7 and HepG2 cells were selected for subsequent analyses.

miR-526a inhibits the proliferation, migration and invasion of HCC cell lines. As miR-526a was implicated in the regulation of HCC tumorigenesis, the present study investigated the role of miR-526a in the malignant characteristics of HCC cells. The Huh7 and HepG2 cells were stably transfected with miR-526a precursor or a control vector, and the transfection efficiency was determined using RT-qPCR. The results showed that miR-526a transfection substantially elevated the

Table I. Correlation between miR-526a and clinicopathological features in hepatocellular carcinoma.

Clinicopathological feature	n	Expression of miR-526a		P-value
		High, n (%)	Low, n (%)	
Age (years)				
<60	67	36 (53.7)	31 (46.3)	0.493
≥60	68	32 (47.1)	36 (52.9)	
Gender				
Male	74	40 (54.1)	34 (45.9)	0.389
Female	61	28 (45.9)	33 (54.1)	
Histological grade				
Well/moderate	72	44 (61.1)	28 (38.9)	0.01
Poor	63	24 (38.1)	39 (61.9)	
Lymph node metastasis				
Positive	38	9 (23.7)	29 (76.3)	<0.001
Negative	97	59 (60.8)	38 (39.2)	
Vascular invasion				
Positive	70	27 (38.6)	43 (61.4)	0.002
Negative	65	41 (63.1)	24 (36.9)	

miR, microRNA.

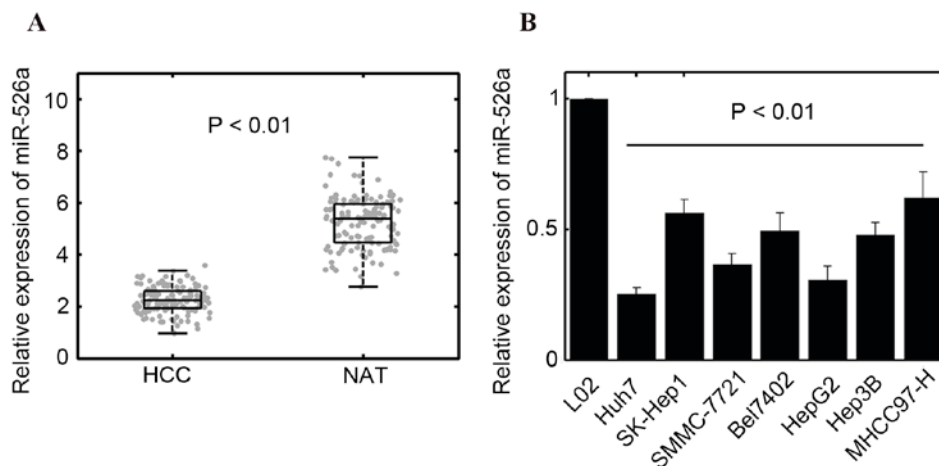


Figure 1. Expression of miR-526a in HCC. (A) Expression of miR-526a in 135 HCC specimens, compared with those in NAT specimens ($P < 0.01$). (B) Expression of miR-526a in a normal liver cell line (L02) and HCC cell lines (Huh7, SK-Hep1, SMMC-7721, Bel7402, HepG2, Hep3B and MHCC97-H). The expression of miR-526a was normalized by endogenous *GAPDH*. HCC, hepatocellular carcinoma; NAT, normal adjacent tissues; miR, microRNA.

intrinsic expression of miR-526a in Huh7 and HepG2 cells with an increase of ~4-5 fold (Fig. 2A; $P < 0.01$). Following miR-526a transfection, the proliferation rates of the Huh7 and HepG2 cells were significantly inhibited (Fig. 2B). The migration assays also confirmed that miR-526a had a suppressive role (Fig. 2C). The effect was more evident in the HepG2 cells (Fig. 2C). The invasive capacities of Huh7 and HepG2 cells were also consistently attenuated with the overexpression of miR-526a (Fig. 2D). These results suggested that miR-526a had a tumor suppressive role in Huh7 and HepG2 cell lines.

miR-526a can inhibit HCC tumor growth in vivo. To further consolidate the role of miR-526a *in vivo*, xenograft

experiments were performed. A total of 10^6 HepG2 cells stably transfected with miR-526a or vector controls were subcutaneously injected into nude mice, and the growth of solid tumors was evaluated at intervals of 3 days. It was found that the size of solid tumors were significantly decreased, compared with those in the control vector transfection group ($P < 0.01$; Fig. 3A). miR-526a transfection significantly decreased tumor volume as early as 9 days post-injection (Fig. 3B). The Ki-67 immunostaining results showed that the growth of HCC solid tumors was markedly inhibited by inducing the overexpression of miR-526a (Fig. 3C). Collectively, these results showed that miR-526a also inhibited tumor development *in vivo*.

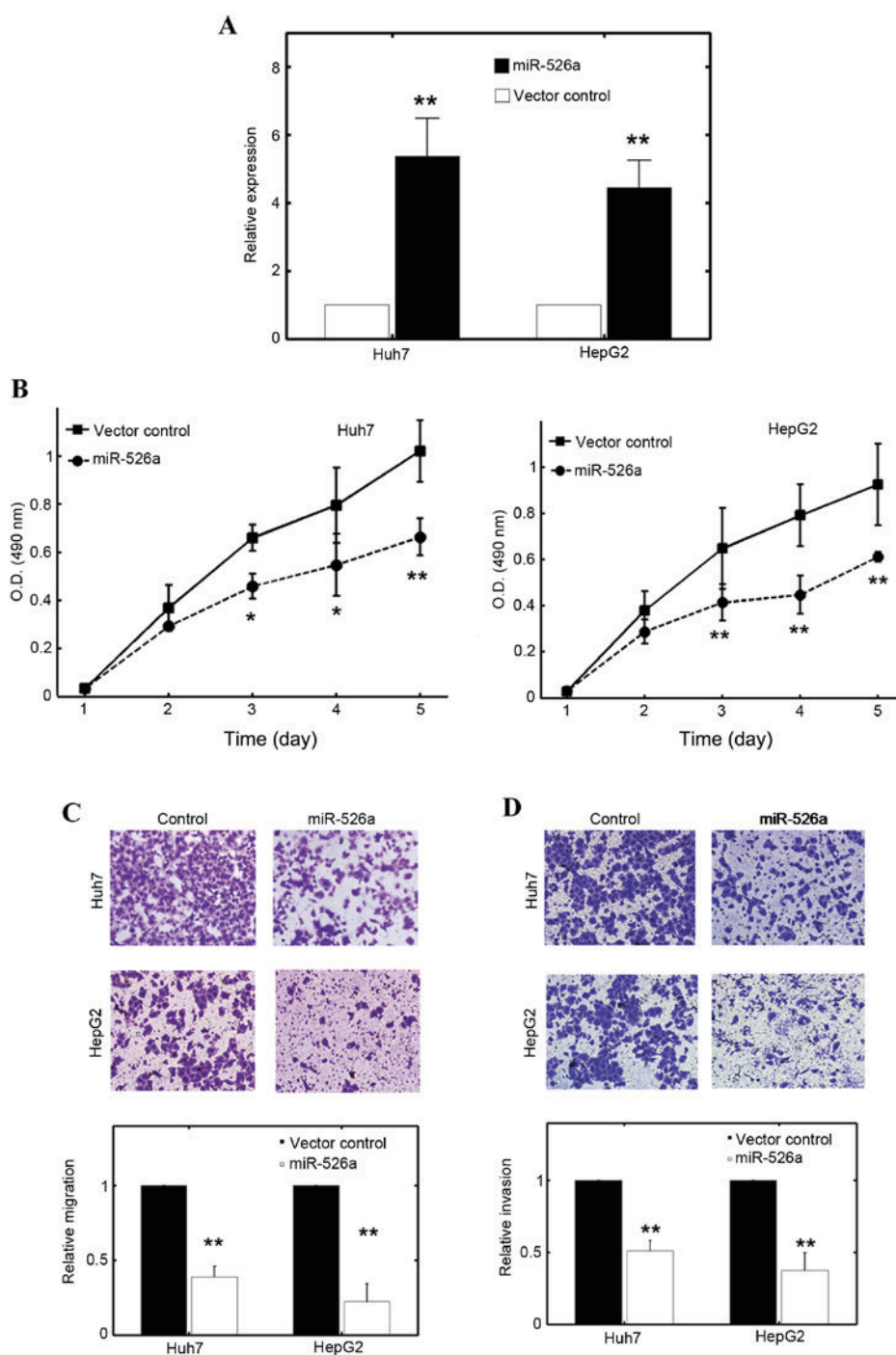


Figure 2. miR-526a inhibits HCC *in vitro*. (A) Levels of miR-526a quantified by reverse transcription-quantitative polymerase chain reaction analysis in Huh7 and HepG2 cells transfected with miR-526a or control vector. (B) Proliferation of Huh7 (above) and HepG2 (below) cells transfected with either control vector or miR-526a-expressing vector. (C) Migration assays for Huh7 and HepG2 cells transfected in control- or miR-526a vector-transfected conditions (above, magnification x100). Quantification of the results is shown below. (D) Representative images of Huh7 and HepG2 cells following Transwell invasion assays. * $P < 0.05$ and ** $P < 0.01$. miR, microRNA; O.D., optical density.

miR-526a suppresses the progression of HCC by targeting PAK7. To determine the potential target of miR-526a, several online databases were used including TargetScan (www.targetscan.org) PicTar (pictar.mdc-berlin.de) and miRDB (www.mirdb.org). The cross verification of several databases indicated that the oncogene, PAK7, may be a promising candidate (Fig. 4A). Using a luciferase reporter assay, it was further shown that miR-526a transfection effectively decreased the luciferase activities of the PAK7 group (Fig. 4B). However, if

the base-pairing between PAK7 and miR-526a was mutated, the inhibitory effect of miR-526a was absent (Fig. 4B). Furthermore, the expression of PAK7 was markedly reduced at the mRNA level by transfecting miR-526a precursor plasmids into the Huh7 and HepG2 cells (Fig. 4C). Consistently, the results of the western blot analysis showed that the protein expression of PAK7 was inhibited by miR-526a transfection (Fig. 4D). These results suggested that miR-526a directly targeted PAK7 in the HCC cell lines.

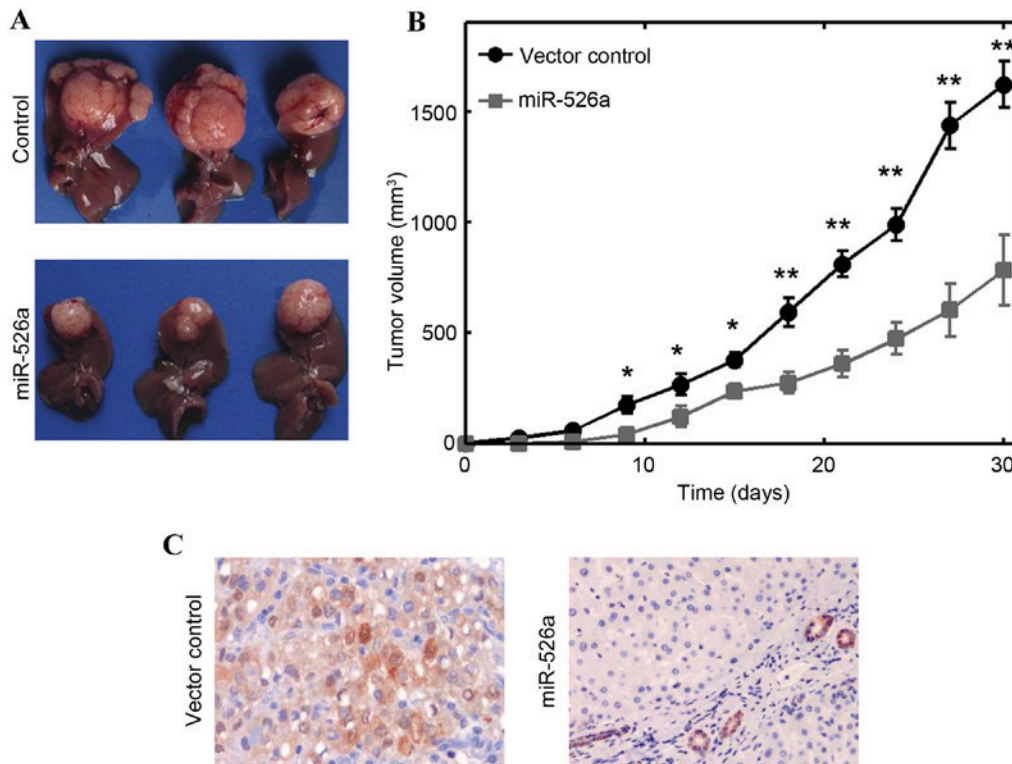


Figure 3. miR-526a inhibits HCC *in vivo*. (A) Representative xenografts of nude mice. (B) Growth of solid tumors in mice injected with HepG2 cells transfected with either vector control or miR-526a. (C) At the end of the implantation period, solid tumors were resected and subject to Ki-67 immunostaining on paraffin-embedded sections. * $P < 0.05$ and ** $P < 0.01$. miR, microRNA (magnification x200).

As the results showed that miR-526a was significantly downregulated in HCC specimens and cell lines (Fig. 1), the present study determined whether the expression of PAK7 correlated with HCC phenotype. The results of the RT-qPCR analysis revealed that the levels of *PAK7* were markedly increased in the HCC specimens, compared with the normal adjacent tissues ($n=135$; Fig. 4E; $P < 0.01$). A reverse correlation was found between *PAK7* and miR-526a in the HCC specimens (Fig. 4F; $R = -0.4596$; $P < 0.01$). These results suggested that PAK7 may be a tumorigenic factor in HCC and negatively correlate with the expression of miR-526a.

Discussion

HCC is a heterogeneous disease, in which multiple factors collaborate with each other. Aberrant microRNA expression has been reported to be involved in the tumorigenesis of several types of cancer (22-24). Tipping the balance of microRNA expression in tumor cells usually favors the uncontrolled proliferation of tumor cells in addition to other malignant characteristics (25). The fragile nature of microRNA gene locus may increase the possibilities of gene alterations and, therefore, increases the likelihood of carcinogenesis (26). Therefore, identifying the missing link between microRNA regulation and tumorigenesis may provide critical insight into the pharmacological intervention of cancer.

In the present study, it was found that miR-526a functioned as a putative tumor suppressor in HCC, at least partially by the fact that the expression of miR-526a was usually downregulated in HCC specimens and cell lines (Fig. 1). Stable

transfection with miR-526a inhibited the malignant phenotypes of HCC, including proliferation, migration and invasion (Figs. 2 and 3). PAK7 was predicted to be the miR-526a target in HCC cell lines using overlapping examinations from three independent databases. In addition, a reverse correlation was found between PAK7 and miR-526a in HCC specimens. p21-activated kinases (PAKs) are serine/threonine kinases, which can be activated by GTPases (27). Two subgroups have been identified for PAKs, termed group I PAKs (PAK1-3) and group II PAKs (PAK4-6). PAK7 belongs to a different type of PAK, compared with group I and group II PAKs (28). The expression of PAK7 is usually elevated in several types of tumor, including lung cancer, osteosarcoma, hepatocellular carcinoma, colorectal cancer and pancreatic cancer (29-32). Therefore, PAK7 may be implicated in the development of various cancer types. A previous report showed that PAK7 promoted cell mobility and proliferation by co-activating different survival pathways (33). PAK7 can also suppress apoptosis, possibly via altered subcellular localization (34). The anti-apoptotic effect may also be ascribed to post-translational modification of anti-apoptotic proteins, including B cell lymphoma-2 family members (33). Collectively, these findings suggested that PAK7 may function as an oncogenic factor in several tumor types. The post-transcriptional inhibition of the expression of PAK7 by miR-526a may serve as an effective method of releasing the intrinsic load of PAK7 in cancer cells, resulting in tumor suppression.

Few reports have focused on miR-526a, particularly in HCC. A previous study suggested that the decreased expression of miR-526 was associated with increased viral infection

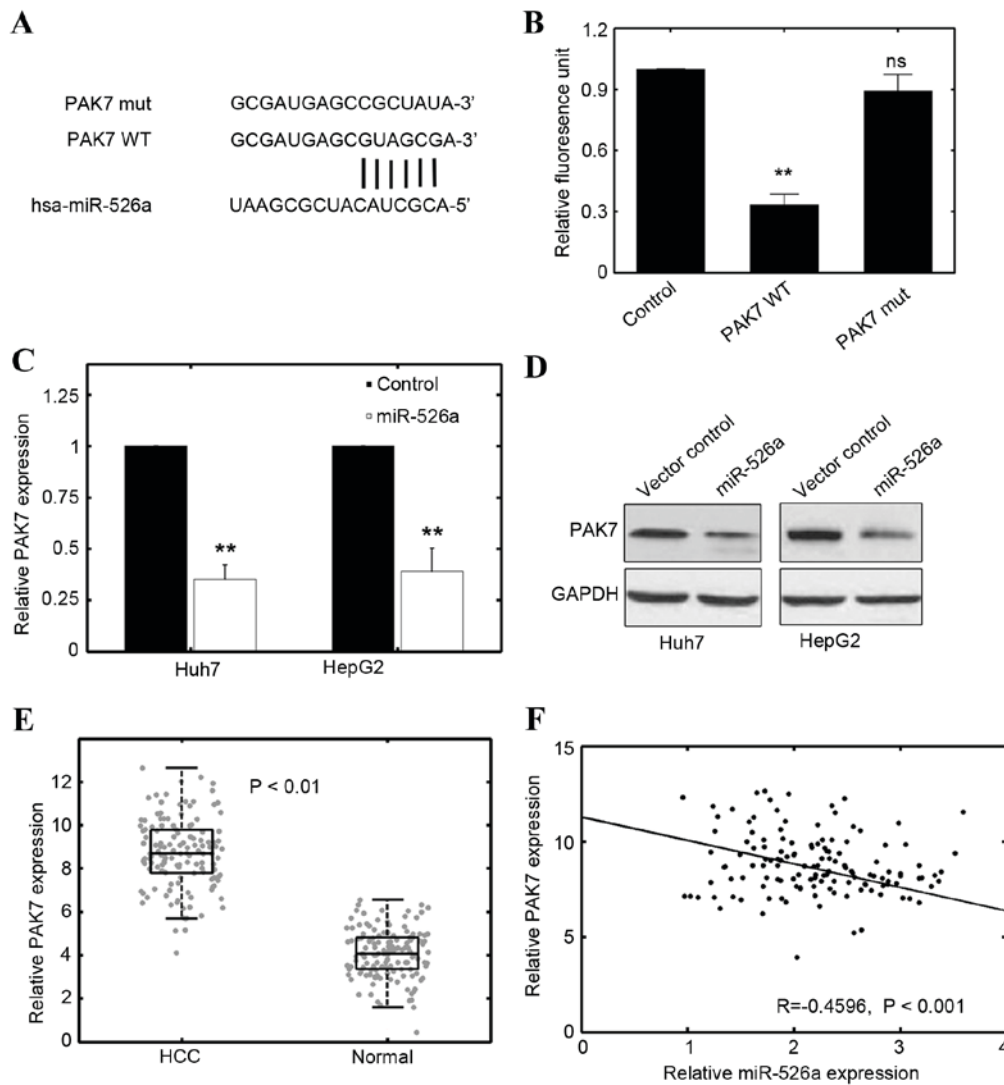


Figure 4. miR-526a targets PAK7 and inhibits carcinogenesis. (A) Prediction of 3'-untranslated region as the binding target of miR-526a. The mutant sequence is shown above. (B) Luciferase activity of HEK293T cells transfected with control vector, or WT or mut PAK7 plasmids. (C) Relative expression of PAK7 in Huh7 and HepG2 cells transfected with either control or miR-526a-expressing vectors. $**P < 0.01$. (D) Protein levels of PAK7 were quantified using western blot analysis in Huh7 and HepG2 cells. The results shown are representative of triplicate experiments. (E) Expression levels of PAK7 in 135 HCC samples and corresponding normal adjacent tissues measured using reverse transcription-quantitative polymerase chain reaction analysis. (F) Pearson correlation between the expression of miR-526a and PAK7 transcripts in 135 HCC samples. $R = -0.4596, P < 0.001$; miR, microRNA; mut, mutant; WT, wild-type; PAK7, p21-activated kinase; HCC, hepatocellular carcinoma; ns, not significant.

and attenuated apoptosis (35). Consistently, another study argued that miR-526a may favor RIG-I-dependent antiviral signaling (36). miR-526a may positively regulate the production of type I interferon and suppress viral infection (36). In addition, Fu *et al* found that patients with decreased expression of miR-526a correlated with active pulmonary tuberculosis (37). Therefore, miR-526a may also inhibit infection and progression of tuberculosis (37). In a tumor-associated study, Zhang *et al* (38) showed that miR-526b can target Ku80 and suppress the incidence of non-small cell lung cancer. The present study is the first, to the best of our knowledge, to reveal a novel function of miR-526a in HCC. It was found that miR-526a had a tumor suppressive role, possibly by targeting PAK7, at least in the HCC cell lines assessed in the present study. These preliminary findings may assist further investigations on the role of miR-526a, particularly in tumor-associated investigations.

In conclusion, the present study found a novel function for miR-526a in HCC. The tumor suppressive effect of miR-526a may function via targeting PAK7. As PAK7 has been implicated in the progression of various tumor types, silencing the expression of PAK7 via pairing with miR-526a may be an effective strategy in tumor suppression. These results may improve current understanding of microRNAs in modulating carcinogenesis and may shed light on determining the heterogeneity in HCC.

Acknowledgements

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