Oncology Research, Vol. 26, pp. 557–563
Printed in the USA. All rights reserved.
Copyright © 2018 Cognizant, LLC.

0965-0407/18 $90.00 + .00
DOI: https://doi.org/10.3727/096504017X15000784459799
E-ISSN 1555-3906
www.cognizantcommunication.com

MicroRNA-374a Promotes Hepatocellular Carcinoma Cell Proliferation by Targeting Mitogen-Inducible Gene 6 (MIG-6)

Hui Li,*[1] Huicheng Chen,†[1] Haibin Wang,‡ Yilong Dong,† Min Yin,† Liang Zhang,§ and Jia Wei*

*Department of Liver and Infectious Diseases, Liver Disease Research Center, The Second People's Hospital of Yunnan Province, Kunming, P.R. China
†School of Medicine, Yunnan University, Kunming, P.R. China
‡The Second Department of Liver Diseases, The Third People's Hospital of Kunming City, Kunming, P.R. China
§Liver Disease Research Center, The Second People's Hospital of Yunnan Province, Kunming, P.R. China

Hepatocellular carcinoma (HCC) is a disease with poor prognosis rates and ineffective therapeutic options. Previous studies have reported the involvement of mitogen-inducible gene 6 (MIG-6) as a negative regulator in tumor formation. MicroRNAs (miRNAs) play crucial roles in the development of different types of cancer. However, the underlying mechanisms of miRNAs in HCC are poorly understood. This study was aimed to investigate the role of miR-374a in HCC and its role in the regulation of expression of MIG-6. The results showed that MIG-6 overexpression significantly inhibited cell viability of HepG2 cells after 4 days post-transfection. Moreover, MIG-6 was a direct target of miR-374a, and the expression of MIG-6 was remarkably downregulated by the overexpression of miR-374a in HepG2 cells. Furthermore, we found that overexpression of miR-374a promoted cell viability; however, the protective effect was abolished by MIG-6 overexpression. In addition, overexpression of miR-374a activated the EGFR and AKT/ERK signaling pathways by regulation of MIG-6. Our findings suggest that miR-374a could promote cell viability by targeting MIG-6 and activating the EGFR and AKT/ERK signaling pathways. These data provide a promising therapeutic strategy for HCC treatment.

Key words: MicroRNA-374a; Mitogen-inducible gene 6 (MIG-6); Hepatocellular carcinoma (HCC); Cell viability; EGFR/AKT/ERK pathways

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver malignant disease and is among the leading causes of cancer-related deaths worldwide[1,2]. Like other cancers, the development of HCC is a complex, multistep process with genetic aberrations and epigenetic changes[3]. It has a poor prognosis, and the currently available therapeutic options are largely ineffective[4]. Therefore, it is necessary to understand the underlying mechanism of HCC tumor dissemination and metastasis in order to develop effective treatment strategies.

MicroRNAs (miRNAs) constitute a large class of short RNAs (20–24 nucleotides in length), which play key roles in cell development and differentiation by mediating the posttranscriptional regulation of protein-coding genes[5,6]. miRNAs also play important roles in a variety of biological processes such as cell proliferation, migration, and invasion[7]. Accumulating evidence indicated that the expressions of several miRNAs were dysregulated in HCC cells and were involved in the regulation of cellular processes[8,9]. In this aspect, miRNAs have emerged as novel molecules or targets for tumor therapy, and HCC represents an excellent model for their testing.

Recent studies have demonstrated that miR-374a is upregulated in many types of cancer, such as head and neck squamous cell carcinoma, follicular lymphoma, osteosarcoma, and bladder urothelial carcinoma[10–13]. These studies indicate that miR-374a may play vital roles in cancer tumorigenesis. Cai et al. demonstrated that miR-374a was markedly upregulated in breast cancer cells and was considered to be associated with poor metastasis-free survival[14]. In addition, Xu et al. demonstrated that miR-374a could promote cell proliferation, migration, and invasion by targeting SRCIN1 in gastric cancer[15]. Also, He et al.

[1]These authors provided equal contribution to this work.
Address correspondence to Jia Wei, Department of Liver and Infectious Diseases, Liver Disease Research Center, The Second People's Hospital of Yunnan Province, No. 176 Qingnian Road, Kunming 650021, P.R. China. E-mail: wejia@aliyun.com

reported that miR-374a promoted osteosarcoma cell proliferation by targeting FOXO1 expression[16]. However, the effects of miR-374a on HCC remain unclear.

Mitogen-inducible gene 6 (MIG-6), also known as gene 33, ERRFI1, or RALT[17,18], has been mapped to human chromosome 1p36. MIG-6 is an immediate early response gene that can be induced by stressful stimuli and mitogens, including hormones and growth factors[19]. MIG-6 protein can directly interact with all four members of the ErbB family, including epidermal growth factor receptor (EGFR) and ErbB2-4, and it acts as a negative feedback regulator of the ErbB/RTK pathway[20]. Recently, it has been reported that downregulated expression of the MIG-6 gene is observed in a variety of human cancers[20], indicating the tumor-suppressive functions of MIG-6 in these cancers.

Hence, in our study, we aimed to investigate the role of miR-374a on HCC cell viability as well as explore the relationship between miR-374a and MIG-6. Our study will provide a new insight into the treatment of HCC.

MATERIALS AND METHODS

Cell Culture

Human liver cancer cell line HepG2 used in this study was purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). These cells were grown in Dulbecco's modified Eagle's medium (DMEM; Gibco-BRL, Breda, Netherlands) supplemented with 10% fetal bovine serum (FBS; Gibco-BRL) and 1% penicillin/streptomycin (Gibco-BRL) at 37°C in a humidified incubator under 5% CO_2 condition.

Cell Transfection

MIG-6 expression vector was constructed by subcloning the full-length MIG-6 coding sequence into pcDNA3.1 plasmid to overexpress MIG-6, and the empty pcDNA-3.1 plasmid was used as a negative control. In addition, the miR-374a mimic and scrambled negative control RNA (mimic NC) were purchased from GenePharma (Shanghai, P.R. China). Cells were seeded in six-well plates; after incubation for 24 h, cells were transfected with the miR-347a mimic or MIG-6 expression vector. All transfections were conducted by Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. These cells were collected after 48 h of transfection for subsequent analyses.

Cell Viability Assay

For analysis of cell viability, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay was performed. In brief, the transfected HepG2 cells were seeded in 96-well plates. After incubation for 1, 2, 3, 4, and 5 days, 50 μg of MTT (Sigma-Aldrich, St. Louis, MO, USA) was added to each well, and this mix was incubated for 4 h at 37°C. After removing the medium, 150 μl of dimethyl sulfoxide (DMSO; Sigma-Aldrich) was added to terminate the reaction. Plates were read on a microplate reader (Molecular Devices, Sunnyvale, CA, USA) at a wavelength of 570 nm. Triplicate readings for each sample were averaged.

Western Blot

Protein samples were extracted with radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime Biotechnology, Shanghai, P.R. China). Proteins (20 μg) were loaded into each lane and separated with 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Subsequently, the bands on the gel were transferred to a polyvinylidene fluoride membrane (Roche, Basel, Switzerland). The membranes were blocked with 5% nonfat milk for 1 h at room temperature and incubated with specific primary antibodies of MIG-6 (#2440), p-EGFR (#4407), EGFR (#8504), p-phosphoinositol 3-kinase/protein kinase B (AKT) (#4060), p-extracellular signal-regulated kinases 1/2 (ERK1/2) (#4370), and GAPDH (#5174; all purchased from Cell Signaling Technology, Beverly, MA, USA). The membranes were

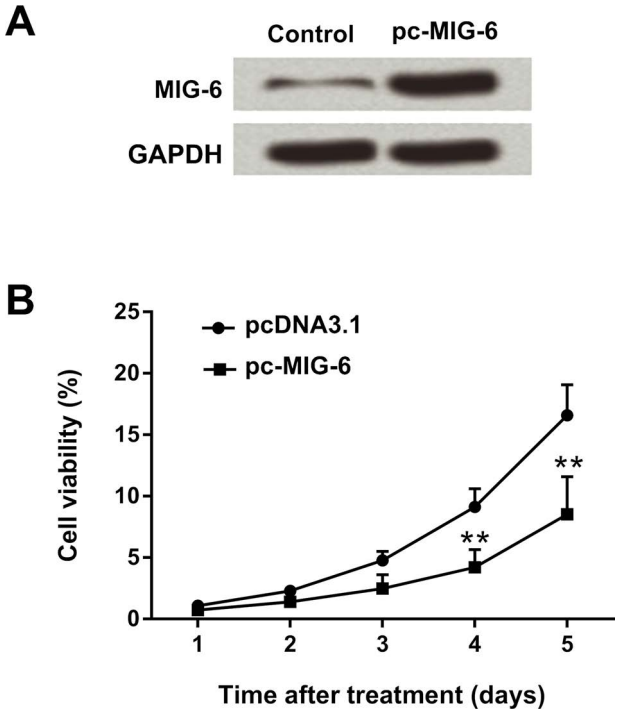

Figure 1. Mitogen-inducible gene 6 (MIG-6) suppressed HepG2 cell viability. HepG2 cells were transfected with pc-MIG-6 to overexpress MIG-6 expression. (A) The protein level of MIG-6 was detected by Western blot assay. (B) Cell viability was examined by 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) assay. $^{**}p<0.01$.

then incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h. The proteins were detected by enhanced chemiluminescence (ECL Plus; Amersham Pharmacia Biotech, Piscataway, NJ, USA) method.

Luciferase Activity Assay

The 3′-untranslated region (3′-UTR) segment of the MIG-6 gene containing the miR-374a binding site was amplified through polymerase chain reaction (PCR) and then inserted into the pmirGLO Dual-Luciferase miRNA Target Expression Vector (Promega, Madison, WI, USA). HepG2 cells were cotransfected with MIG-6 3′-UTR and miR-374a mimic or mimic NC using Lipofectamine 2000 (Invitrogen). After posttransfection for 48 h, the luciferase activity was analyzed using the Dual-Luciferase Reporter Assay System (Promega). For each transfection, the luciferase activity was averaged from three replicates.

Real-Time Polymerase Chain Reaction (RT-PCR)

Total cellular RNA was extracted with TRIzol reagent (Invitrogen, Grand Island, NY) according to the manufacturer's protocol. RNA (500 ng) was reverse transcribed to cDNA using an NCode miRNA First-Strand cDNA Synthesis Kit (Invitrogen). RT-PCR was conducted using an Applied Biosystems real-time detection system (Applied Biosystems, Darmstadt, Germany). The thermocycling parameters were 95°C for 3 min and 40 cycles of 95°C for 15 s followed by 60°C for 30 s. Each sample was run in triplicate and was normalized to U6 snRNA levels. Data were analyzed by the $2^{-\Delta\Delta}$Ct method.

Statistical Analysis

Statistical differences between groups were analyzed by Student's *t*-test and a one-way analysis of variance (ANOVA) for parametric data. Data are expressed as mean ± standard error of measurement (SEM). A value of $p < 0.05$ was set as the level of statistical significance.

RESULTS

MIG-6 Overexpression Inhibits Cell Viability of HepG2 Cells

HepG2 cells were transfected with pc-MIG-6 to increase MIG-6 expression. Western blot assay was performed to analyze the expression of MIG-6 in HepG2 cells transfected with pc-MIG-6. The results revealed that MIG-6 expression was significantly upregulated in HepG2 cells transfected with pc-MIG-6 compared to the control group (Fig. 1A). To explore the effect of MIG-6 on cell viability, we carried out the MTT assay at 1–5 days after pc-MIG-6 transfection. The results demonstrated that cell viability was obviously decreased by overexpression of MIG-6 at 4 and 5 days posttransfection compared to the control group ($p < 0.01$) (Fig. 1B). These data suggested that overexpression of MIG-6 could suppress cell viability of HepG2 cells.

MIG-6 Is a Direct Target of miR-347a in HepG2 Cells

To confirm whether MIG-6 was a direct target of miR-347a, TargetScan (http://www.targetscan.org/) and miRNA database (http://www.microrna.org/) were used. As shown in Figure 2A, MIG-6 was predicated as a

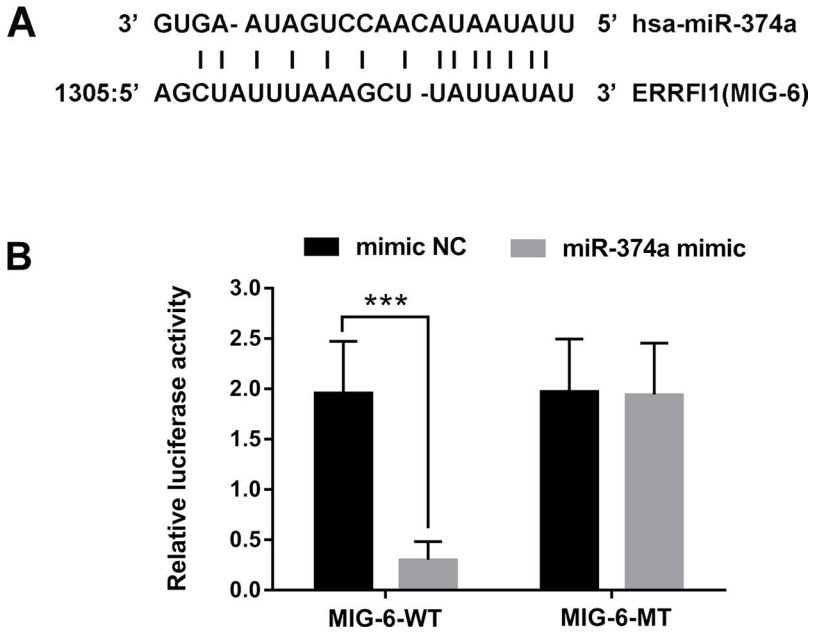

Figure 2. MIG-6 was a direct target of miR-374a in HepG2 cells. (A) MIG-6 was predicated as a target of miR-374a using TargetScan and microRNA database. (B) MIG-6 as a direct target of miR-374a was further confirmed by dual-luciferase reporter assay in HepG2 cells. ***$p < 0.001$.

direct target of miR-347a. Furthermore, the dual-luciferase reporter assay revealed that overexpression of miR-347a remarkably decreased the luciferase activity of the reporter gene, which fused to the 3′-UTR-WT of MIG-6 ($p<0.001$). However, there was no effect of the reporter fused to the 3′-UTR-MT of MIG-6 (Fig. 2B). Taken together, these data indicated that MIG-6 was a direct target of miR-347a in HepG2 cells.

miR-374a Overexpression Decreases MIG-6 Expression in HepG2 Cells

To explore the relationship between miR-374a and MIG-6, the miR-374a mimic and mimic NC were transfected into HepG2 cells. RT-PCR analysis results demonstrated that the expression of miR-374a was significantly upregulated in the miR-374a mimic group compared to the mimic NC group ($p<0.001$) (Fig. 3A). Western blot analysis revealed that the protein level of MIG-6 was reduced by the overexpression of miR-374a compared to its control group (Fig. 3B). Similarly, the mRNA expression of MIG-6 was prominently downregulated in the miR-374a mimic group compared to the control group ($p<0.01$) (Fig. 3C). These data demonstrated that miR-374a could regulate MIG-6 expression in HepG2 cells.

miR-374a Overexpression Promotes Cell Viability by Regulation of MIG-6 in HepG2 Cells

To explore the cross-regulation effect between miR-374a and MIG-6 on cell viability, HepG2 cells were transfected with the miR-374a mimic, pc-MIG-6, and corresponding controls. After transfection, cell viability was determined using MTT at 1, 2, 3, 4, and 5 days, respectively. The results showed that overexpression of miR-374a significantly promoted cell viability at 4 and 5 days compared to the mimic NC group ($p<0.01$). However, the promoting effect of miR-374a overexpression on cell viability was obviously abolished by MIG-6 overexpression ($p<0.05$ or $p<0.01$) (Fig. 4). These results revealed that miR-374a could promote cell viability by regulation of MIG-6 in HepG2 cells.

miR-374a Activates the EGFR and AKT/ERK Signaling Pathways by Regulation of MIG-6 in HepG2 Cells

To explore whether miR-374a and MIG-6 were involved in the regulation of the EGFR and AKT/ERK signaling pathways, HepG2 cells were transfected with the miR-374a mimic, pc-MIG-6, and their corresponding controls. Western blot results displayed that overexpression of miR-374a significantly upregulated p-EGFR, EGFR, p-AKT, and p-ERK1/2 expressions. Cotransfection with the miR-374a mimic and pc-MIG-6 was remarkably downregulated in these four factor expressions (Fig. 5A). The mRNA expressions of p-EGFR, EGFR, p-AKT, and p-ERK1/2 were in line with the protein levels ($p<0.05$ or $p<0.01$) (Fig. 5B). The results indicated that miR-374a activated the EGFR and AKT/ERK signaling pathways by regulation of MIG-6 in HepG2 cells.

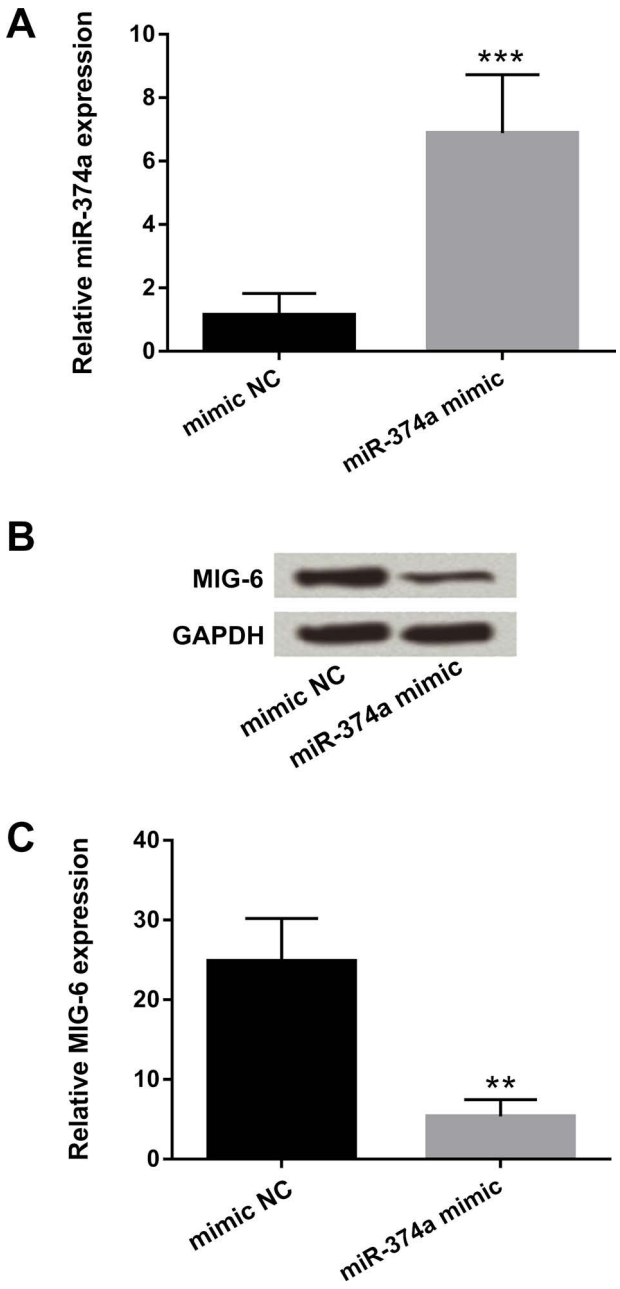

Figure 3. miR-374a overexpression decreases MIG-6 expression in HepG2 cells. The miR-374a mimic and scrambled negative control RNA (mimic NC) were transfected into HepG2 cells. (A) The expression of miR-374a was examined by quantitative real-time polymerase chain reaction (qRT-PCR). (B, C) The protein and mRNA expressions of MIG-6 were determined by Western blot and RT-PCR. $^{**}p<0.01$; $^{***}p<0.001$.

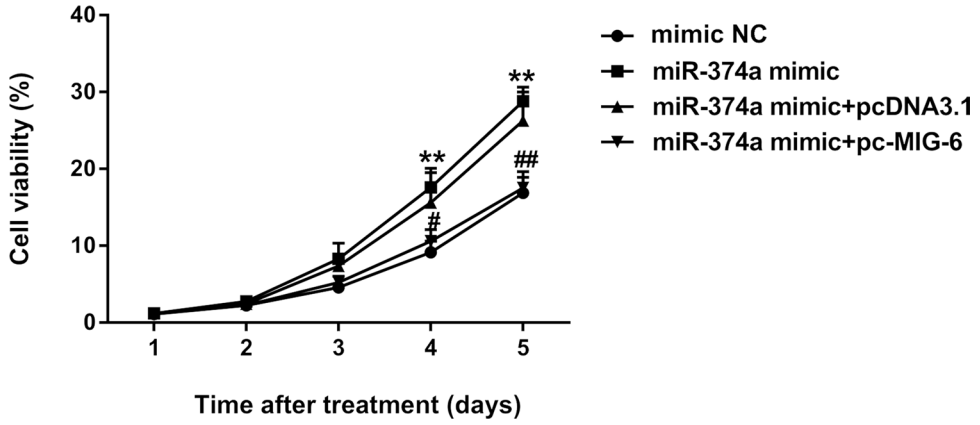

Figure 4. miR-374a overexpression promotes cell viability by regulation of MIG-6 in HepG2 cells. miR-374a mimic, pc-MIG-6, and corresponding controls were transfected into HepG2 cells. After transfection, cell viability was detected by MTT assay at 1, 2, 3, 4, and 5 days in HepG2 cells. $^{**}p<0.01$, versus mimic NC; $\#p<0.05$; $\#\#p<0.01$, versus miR-374a mimics + pcDNA3.1.

DISCUSSION

In the present study, we demonstrated that MIG-6 overexpression inhibited viability of HepG2 cells. MIG-6 was a direct target of miR-374a, and MIG-6 expression was significantly negatively regulated by miR-374a overexpression. Furthermore, overexpression of miR-374a promoted viability of HepG2 cells. However, the protective effect of miR-374a on cell viability was abolished by MIG-6 overexpression. In addition, overexpression of miR-374a activated the EGFR and AKT/ERK signaling pathways by regulation of MIG-6. Collectively, these findings indicated that miR-374a could promote HCC cell proliferation by targeting MIG-6.

MIG-6 is a multiadaptor protein implicated in the regulation of the HER family of receptor tyrosine kinases[21]. Several studies reported that MIG-6 is an important mediator to induce tumor formation and regulate biological processes in various tissues[22,23]. Li et al. demonstrated that MIG-6 was downregulated in HCC cells and suppressed HCC cell proliferation by regulation of the p-ERK/cyclin D1 pathway[24]. Reschke et al. also proved that MIG-6 as a tumor suppressor inhibited HCC cell proliferation[20]. Similar to these previous studies, our study revealed that overexpression of MIG-6 could reduce viability of HepG2 cells.

It has been reported that miRNAs negatively regulated the expressions of their target mRNAs in a sequence-specific manner[25]. Growing evidence demonstrated that MIG-6 expression was regulated by different miRNAs in various cancers. Okada et al. found that miR-214 overexpression decreased the expression of MIG-6 in HCC cells[26]. Kim et al. reported that miR-148a could modulate EGFR and cell growth in glioblastoma by targeting MIG-6[27]. However, to the best of our knowledge, there has been no information about the relationship between miR-374a and MIG-6 in HCC. By using bioinformatic analysis and dual-luciferase activity assay, we first confirmed that MIG-6 was a genuine target of miR-374a. Similar to the study of Okada et al.[26], we also found that overexpression of miR-374 obviously downregulated MIG-6 expression in HepG2 cells.

Mounting evidence has demonstrated miR-374a as an oncogene or tumor suppressor that participated in the regulation of various biological processes in different cancers[14,28]. In terms of HCC, miR-374a may be a useful diagnostic marker for HCC, and it was associated with tumorigenesis and tumor progression[29]. However, the cross-regulation effects of miR-374a and MIG-6 on cell viability in HCC remain unclear. In the present study, our study demonstrated that miR-374a could increase HepG2 cell viability, whereas MIG-6 overexpression significantly abolished the promotive effect. These data indicated that miR-374a induced HCC cell viability by regulation of MIG-6.

To date, the EGFR signaling pathway has been widely reported in various cancers, including HCC[30]. One study demonstrated that EGFR and its ligands were frequently expressed in HCC, thereby contributing to tumor development of HCC[31]. Wang et al. demonstrated miR-203a as an antioncogene that inhibits HCC cell progression by regulation of the EGFR signaling pathway[32]. As important signaling molecules, the AKT/ERK signaling pathway also plays a critical role in the regulation of cell proliferation, differentiation, and survival[33]. Bao et al. reported that miR-21 led to the activation of the AKT/ERK pathways and finally enhanced HCC cell proliferation and tumor growth[34]. A recent study demonstrated MIG-6 as a tumor suppressor of the EGFR signaling pathway in HCC[20]. Moreover, suppression of MIG-6 led

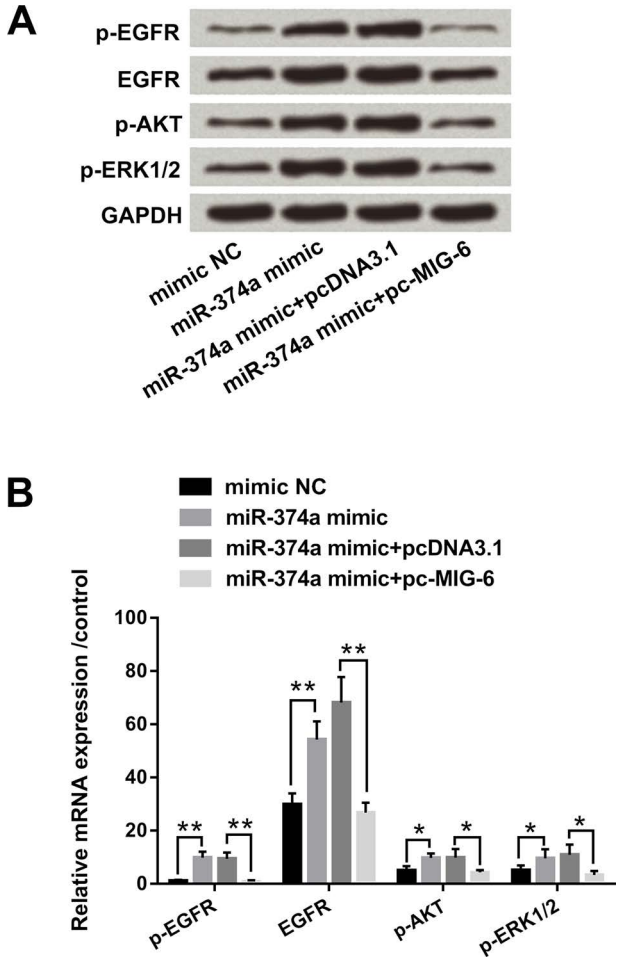

Figure 5. miR-374a activates epidermal growth factor receptor (EGFR) and phosphoinositol 3-kinase/protein kinase B (AKT)/extracellular signal-regulated kinase (ERK) signaling pathways by regulation of MIG-6 in HepG2 cells. miR-374a mimic, pc-MIG-6, and their corresponding controls were transfected into HepG2 cells. (A) The protein levels of p-EGFR, EGFR, p-AKT, and p-ERK1/2 were measured by Western blot. (B) The mRNA expressions of p-EGFR, EGFR, p-AKT, and p-ERK1/2 were determined by RT-PCR. *$p < 0.05$; **$p < 0.01$.

to a marked increase in the levels of activated EGFR, AKT, and ERK1/2[20]. However, the moderating effects of miR-374a and MIG-6 on the EGFR and AKT/ERK signaling pathways in HCC remain unclear. In our study, we demonstrated that miR-374a activated the EGFR and AKT/ERK signaling pathways by regulation of MIG-6 in HepG2 cells.

Taken together, on the basis of our finding in vitro, we demonstrated that miR-374a could promote cell proliferation and activate the EGFR and AKT/ERK signaling pathways by targeting MIG-6 in HCC cells. These data provided a new idea that miR-374a has a critical role in HCC development. Therefore, miR-374a deserves further exploration for the treatment of HCC.

ACKNOWLEDGMENT: This study was supported by the Provincial Science and Technology Department: Innovative Team of Viral Hepatitis and Related Liver Diseases of The Second People's Hospital of Yunnan Province (No. 2015HC019). The authors declare no conflicts of interest.

REFERENCES

1. Coleman WB. Mechanisms of human hepatocarcinogenesis. Curr Mol Med. 2003;3(3):573–88.
2. Bruix J, Boix L, Sala M, Llovet JM. Focus on hepatocellular carcinoma. Cancer Cell 2004;5(3):215–9.
3. Kanda M, Sugimoto H, Kodera Y. Genetic and epigenetic aspects of initiation and progression of hepatocellular carcinoma. World J Gastroenterol. 2015;21(37):10584–97.
4. Chen Y, Wang D, Guo Z, Zhao J, Wu B, Deng H, Zhou T, Xiang H, Gao F, Yu X. Rho kinase phosphorylation promotes ezrin-mediated metastasis in hepatocellular carcinoma. Cancer Res. 2011;71(5):1721–9.
5. Zhao T, Li J, Chen AF. MicroRNA-34a induces endothelial progenitor cell senescence and impedes its angiogenesis via suppressing silent information regulator 1. Am J Physiol Endocrinol Metab. 2010;299(299):E110–6.
6. Zhang L, Liu X, Jin H, Guo X, Xia L, Chen Z, Bai M, Liu J, Shang X, Wu K. miR-206 inhibits gastric cancer proliferation in part by repressing cyclinD2. Cancer Lett. 2013; 332(1):94–101.
7. Tu Y, Gao X, Li G, Fu H, Cui D, Liu H, Jin W, Zhang Y. MicroRNA-218 inhibits glioma invasion, migration, proliferation, and cancer stem-like cell self-renewal by targeting the polycomb group gene Bmi1. Cancer Res. 2013; 73(19):6046.
8. Tsukamoto Y, Nakada C, Noguchi T, Tanigawa M, Nguyen LT, Uchida T, Hijiya N, Matsuura K, Fujioka T, Seto M. MicroRNA-375 is downregulated in gastric carcinomas and regulates cell survival by targeting PDK1 and 14-3-3zeta. Cancer Res. 2010;70(6):2339–49.
9. Zou CD, Zhao WM, Wang XN, Qiang L, Hui H, Cheng WP, Jin JF, He Z, Wu MJ, Sheng T. MicroRNA-107: A novel promoter of tumor progression that targets the CPEB3/EGFR axis in human hepatocellular carcinoma. Oncotarget 2016;7(1):266–78.
10. Huang Y, Chuang A, Hao H, Talbot C, Sen T, Trink B, Sidransky D, Ratovitski E. Phospho-ΔNp63α is a key regulator of the cisplatin-induced microRNAome in cancer cells. Cell Death Differ. 2011;18(18):1220–30.
11. Wang W, Corrigan-Cummins M, Hudson J, Maric I, Simakova O, Neelapu SS, Kwak LW, Janik JE, Gause B, Jaffe ES. MicroRNA profiling of follicular lymphoma identifies microRNAs related to cell proliferation and tumor response. Haematologica 2011;97(4):586–94.
12. Namløs HM, Mezazepeda LA, Barøy T, Østensen IHG, Kresse SH, Kuijjer ML, Serra M, Bürger H, Cletonjansen AM, Myklebost O. Modulation of the osteosarcoma expression phenotype by microRNAs. Plos One 1932;7(10):e48086.
13. Zhang ZC, Huang Y, Wang XJ, Wang M, Ma LL. Expression of circulating microRNAs in patients with bladder urothelial carcinoma [in Chinese]. Beijing Da Xue Xue Bao Yi Xue Ban 2013;45(4):532–6.
14. Cai J, Guan H, Fang L, Yang Y, Zhu X, Yuan J, Wu J, Li M. MicroRNA-374a activates Wnt/β-catenin signaling to promote breast cancer metastasis. J Clin Invest. 2013;123 (2):566–79.

15. Xu X, Wang W, Su N, Zhu X, Yao J, Gao W, Hu Z, Sun Y. miR-374a promotes cell proliferation, migration and invasion by targeting SRCIN1 in gastric cancer. FEBS Lett. 2014;589(3):407.
16. He W, Feng L, Xia D, Han N. MiR-374a promotes the proliferation of human osteosarcoma by downregulating FOXO1 expression. Int J Clin Exp Med. 2014;8(3):3482–9.
17. Hackel PO, Gishizky M, Ullrich A. Mig-6 is a negative regulator of the epidermal growth factor receptor signal. Biol Chem. 2005;382(12):1649–62.
18. Anastasi S, Fiorentino LM, Fraioli R, Sala G, Castellani L, Alema S, Alimandi M, Segatto O. Feedback inhibition by RALT controls signal output by the ErbB network. Oncogene 2003;22(27):4221–34.
19. Poisson J, Rossi E, Young S, Gehrig PA, Baejump VL. Abstract 362: Comparison of Mig-6 and hormone receptor expression to BMI as predictors of responsiveness to progestin therapy for endometrial hyperplasia and cancer. Cancer Res. 2011;71(8 Suppl):362.
20. Reschke M, Ferby I, Ewa S, Seitzer N, Horst D, Wagner EF, Ullrich A. Mitogen-inducible gene-6 is a negative regulator of epidermal growth factor receptor signaling in hepatocytes and human hepatocellular carcinoma. Hepatology 2010;51(4):1383–90.
21. Ferby I, Reschke M, Kudlacek O, Knyazev P, Pantè G, Amann K, Sommergruber W, Kraut N, Ullrich A, Fässler R. Mig6 is a negative regulator of EGF receptor-mediated skin morphogenesis and tumor formation. Nat Med. 2006; 12(5):568–573.
22. Li Z, Qu L, Zong H, Xu K, Qiu X, Wang E. Low expression of Mig-6 is associated with poor survival outcome in NSCLC and inhibits cell apoptosis via ERK-mediated upregulation of Bcl-2. Oncol Rep. 2014;31(4):1707–14.
23. Kim TH, Kim BG, Yoo JY, Lee JH, Castrillon DH, Jeong JW. Abstract 2069: Stromal-epithelial crosstalk of Mig-6 has an important role for tumor suppression via progesterone in endometrial cancer. Cancer Res. 2015;75(15 Suppl):2069.
24. Li Z, Qu L, Luo W, Tian Y, Zhai H, Zhong H, Xu K. Mig-6 is down-regulated in HCC and inhibits the proliferation of HCC cells via the P-ERK/Cyclin D1 pathway. Exp Mol Pathol. 2017;102(3):492–9.
25. Yamamoto K, Ito S, Hanafusa H, Shimizu K, Ouchida M. Uncovering direct targets of miR-19a involved in lung cancer progression. PLos One 2015;10(9):e0137887.
26. Okada H, Honda M, Campbell JS, Takegoshi K, Sakai Y, Yamashita T, Shirasaki T, Takabatake R, Nakamura M, Tanaka T. Inhibition of microRNA-214 ameliorates hepatic fibrosis and tumor incidence in platelet-derived growth factor C transgenic mice. Cancer Sci. 2015;106(9):1143–52.
27. Kim J, Zhang Y, Skalski M, Hayes J, Kefas B, Schiff D, Purow B, Parsons S, Lawler S, Abounader R. microRNA-148a is a prognostic oncomiR that targets MIG6 and BIM to regulate EGFR and apoptosis in glioblastoma. Cancer Res. 2014;75(5):1541–53.
28. Wang Y, Xia H, Zhuang Z, Miao L, Chen X, Cai H. Axl-altered microRNAs regulate tumorigenicity and gefitinib resistance in lung cancer. Cell Death Dis. 2014;5(5):e1227.
29. Zhao Q, Li T, Qi J, Liu J, Qin C. The miR-545/374a cluster encoded in the Ftx lncRNA is overexpressed in HBV-related hepatocellular carcinoma and promotes tumorigenesis and tumor progression. PLoS One 2014;9(10):e109782.
30. Zhang N, Zeng Y, Du W, Zhu J, Shen D, Liu Z, Huang JA. The EGFR pathway is involved in the regulation of PD-L1 expression via the IL-6/JAK/STAT3 signaling pathway in EGFR-mutated non-small cell lung cancer. Int J Oncol. 2016;49(4):1360.
31. Berasain C, Castillo J, Prieto J, Avila MA. New molecular targets for hepatocellular carcinoma: The ErbB1 signaling system. Liver Int. 2007;27(2):174–85.
32. Wang L, Sun H, Wang X, Hou N, Zhao L, Tong D, He K, Yang Y, Song T, Yang J. EGR1 mediates miR-203a suppress the hepatocellular carcinoma cells progression by targeting HOXD3 through EGFR signaling pathway. Oncotarget 2016;7(29):45302–16.
33. Florian E, Dominik N, Astrid G, Johanna B, Christiane H, Hofmann BT, Björn N, Manfred J. Vertical targeting of AKT and mTOR as well as dual targeting of AKT and MEK signaling is synergistic in hepatocellular carcinoma. J Cancer 2015;6(12):1195–205.
34. Bao L, Yan Y, Xu C, Ji W, Shen S, Xu G, Zeng Y, Sun B, Qian H, Chen L. MicroRNA-21 suppresses PTEN and hSulf-1 expression and promotes hepatocellular carcinoma progression through AKT/ERK pathways. Cancer Lett. 2013;337(2):226–36.