*Oncology Research*, Vol. 26, pp. 457–466
Printed in the USA. 

0965-0407/18 $90.00 + .00
DOI: https://doi.org/10.3727/096504017X15035025554553
E-ISSN 1555-3906
www.cognizantcommunication.com



# MicroRNA-384 Inhibits the Growth and Invasion of Renal Cell Carcinoma Cells by Targeting Astrocyte Elevated Gene 1

Haitao Song,* Yanwei Rao,† Gang Zhang,* and Xiangbo Kong*

*Department of Urinary Surgery, China–Japan Union Hospital, Jilin University, Changchun, P.R. China
†Department of Critical Care Medicine, Jilin Province People's Hospital, Changchun, P.R. China

MicroRNAs (miRNAs) are emerging as pivotal regulators in the development and progression of various cancers, including renal cell carcinoma (RCC). MicroRNA-384 (miR-384) has been found to be an important cancer-related miRNA in several types of cancers. However, the role of miR-384 in RCC remains unclear. In this study, we aimed to investigate the potential function of miR-384 in regulating tumorigenesis in RCC. Here we found that miR-384 was significantly downregulated in RCC tissues and cell lines. Overexpression of miR-384 significantly inhibited the growth and invasion of RCC cells, whereas inhibition of miR-384 had the opposite effects. Bioinformatic analysis and luciferase reporter assay showed that miR-384 directly targeted the 3′-untranslated region of astrocyte elevated gene 1 (AEG-1). Further data showed that miR-384 could negatively regulate the expression of AEG-1 in RCC cells. Importantly, miR-384 expression was inversely correlated with AEG-1 expression in clinical RCC specimens. Moreover, miR-384 regulates the activation of Wnt signaling. Overexpression of AEG-1 significantly reversed the antitumor effects of miR-384. Overall, these findings suggest that miR-384 suppresses the growth and invasion of RCC cells via downregulation of AEG-1, providing a potential therapeutic target for the treatment of RCC.

**Key words: Astrocyte elevated gene 1 (AEG-1); miR-384; Renal cell carcinoma (RCC)**

## INTRODUCTION

Renal cell carcinoma (RCC) is one of most prevalent urologic malignancies, with increasing morbidity and mortality in recent years[1,2]. Despite advances in cancer therapies, patients with advanced RCC remain incurable[3,4]. However, the molecular mechanism underlying the pathogenesis of RCC remains largely unknown. Therefore, a better understanding of the molecular mechanisms of RCC could help in the identification of novel therapeutic targets for the treatment of RCC.

MicroRNAs (miRNAs) are highly conserved, small noncoding RNAs, which have emerged as novel tools for controlling gene expression[5]. miRNAs can negatively regulate protein expression through binding to the 3′-untranslated regions (3′-UTRs) of their target gene mRNAs[6]. miRNAs are thought to be involved in the regulation of various biological functions, including signal transduction, differentiation, proliferation, and metastasis[7]. A growing body of evidence has suggested that numerous miRNAs, which could function as oncogenes or tumor suppressors, are differently expressed during the development and progression of cancers[8,9]. These miRNAs could serve as potential biomarkers for diagnosis and prognosis as well as therapeutic targets for treatment of cancers[10,11]. Global miRNA expression studies have revealed that various miRNAs are dysregulated and involved in the development and progression of RCC[12–14]. Therefore, a better understanding of the precise role of miRNAs may help in the development of novel and effective therapeutic approaches for RCC.

An increasing number of studies have suggested that astrocyte elevated gene 1 (AEG-1) is a novel oncogene in many types of cancers[15,16]. AEG-1 was originally characterized in primary human fetal astrocytes, which can be induced by human immunodeficiency virus 1 (HIV-1) and tumor necrosis factor-α[17–19]. AEG-1 plays an important role in cancer progression, obesity, and neurodegenerative diseases[20]. AEG-1 is highly expressed in many cancers, including gastric cancer[21], colorectal cancer[22], hepatocellular carcinoma[23], glioma[24], and prostate cancer[25]. AEG-1 regulates the proliferation, metastasis, and drug resistance of cancers through activation of various oncogenic

Address correspondence to Xiangbo Kong, Department of Urinary Surgery, China–Japan Union Hospital, Jilin University, 126 Xiantai Street, Changchun, Jilin 130033, P.R. China. E-mail: kongxiangbo_cju@163.com

signaling pathways, such as the phosphoinositol 3-kinase (PI3K)/Akt and wingless-related integration site (Wnt) signaling pathways[15,26]. Studies have shown that AEG-1 is highly expressed in RCC and that AEG-1 expression level is correlated with tumor grade, clinical staging, and metastasis classification[27]. Knockdown of AEG-1 inhibits its growth and induces apoptosis of RCC cells[28]. Thus, AEG-1 may have the potential to be used as a therapeutic target for RCC.

Several studies have reported that miR-384 is a novel tumor-suppressive miRNA in many types of cancers[29–31]. However, the role of miR-384 in RCC remains unclear. In this study, we aimed to investigate the potential role and molecular mechanism of miR-384 in the development and progression of RCC. Our results showed that miR-384 was significantly downregulated in RCC tissues and cell lines. Overexpression of miR-384 significantly inhibited the growth and invasion of RCC cells, whereas inhibition of miR-384 had the opposite effects. AEG-1 was identified as a potential target gene of miR-384. Importantly, miR-384 expression was inversely correlated with AEG-1 expression in clinical RCC specimens. Moreover, miR-384 regulates the activation of Wnt signaling. Overexpression of AEG-1 significantly reversed the antitumor effects of miR-384. Overall, these findings suggest that miR-384 functions as a tumor suppressor in RCC and serves as a potential therapeutic target for its treatment.

## MATERIALS AND METHODS

### *Tissue Samples*

A total of 20 paired RCC tissues and adjacent normal tissues were obtained from RCC patients who underwent nephrectomy in the China–Japan Union Hospital (Changchun, P.R. China). The samples were immediately frozen in liquid nitrogen and stored at −80°C until use. Written informed consent was obtained from all participants. This study was reviewed and approved by the Institutional Human Experiment and Ethics Committee of the China–Japan Union Hospital. The experimental procedures were conducted in accordance with the Helsinki Declaration.

### *Cell Lines and Cultures*

RCC cell lines including ACHN, A-498, 786-O, and Caki-1 and the human normal kidney tubule epithelial cell line HK-2 were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). HK-2 cells were cultured in keratinocyte serum-free medium (Gibco, Rockville, MD, USA) supplemented with 0.05 mg/ml bovine pituitary extract and 0.5 ng/ml human recombinant epidermal growth factor. ACHN and A-498 cells were cultured in Eagle's minimum essential medium (ATCC); 786-O cells were grown in Roswell Park Memorial Institute (RPMI)-1640 medium (Gibco); and Caki-1 cells were cultured in McCoy's 5a modified medium (ATCC). In addition, 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin mix (Sigma-Aldrich, St. Louis, MO, USA) were added to the media. Cells were grown in a humidified atmosphere of 95% air and 5% $CO_2$ at 37°C.

### *RNA Isolation and Real-Time Quantitative Reverse Transcriptase PCR (qRT-PCR)*

Total RNAs were isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocols. Reverse transcription of miRNA was performed using the TaqMan microRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA), whereas the reverse transcription of mRNA was performed using Moloney murine leukemia virus (M-MLV) reverse transcriptase (Takara Biotechnology Co., Ltd., Dalian, P.R. China). qRT-PCR was performed on an Applied Biosystems AB7500 Real-Time PCR System in a 20-μl reaction containing 1 μl of cDNA, 1 μl of primer, 10 μl of Power SYBR Green PCR Master Mix (Applied Biosystems), and 8 μl of $ddH_2O$ using the following cycling conditions: 95°C for 10 min; 40 cycles of 94°C for 10 s, 58°C for 20 s, and 72°C for 30 s; 72°C for 5 min; and 4°C for 5 min. *U6* small nuclear RNA and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) served as internal controls for normalization of gene expression. Relative gene expression analysis was performed using the comparative $2^{-\Delta\Delta}$Ct method.

### *Transient Transfection*

The mimics, inhibitor, and negative controls (NCs) of miR-384 were synthesized by GenePharma (Shanghai, P.R. China). The open reading frame of AEG-1 was cloned into the pcDNA3.1 vector. The oligonucleotides and vectors were transiently transfected into cells using Lipofectamine 2000 (Invitrogen) following the manufacturer's instructions. After 6 h, the culture medium was replaced by fresh medium, and the cells were incubated for 48 h prior to detection as described below. The transfection efficacy was confirmed by qRT-PCR or Western blot analysis.

### *Cell Counting Kit-8 (CCK-8) Assay*

Cell proliferation was assessed using the CCK-8 assay according to the manufacturer's instructions. Briefly, cells were plated into 96-well plates ($1\times10^4$ cells/well) and cultured overnight. After 48 h of transfection, 10 μl of CCK-8 solution (Sigma-Aldrich) was added to each well and incubated for 2 h at 37°C. The optical density (OD) value was then measured at 450 nm using an auto microplate reader (Bio-Rad, Hercules, CA, USA).

*Colony Formation Assay*

After 48 h of transfection, cells were seeded into six-well plates at a density of 1,000 cells/well. The plates were precoated with growth medium containing 0.7% noble agar. After 14 days in culture at 37°C, cells were then washed with phosphate-buffered saline (PBS), fixed with 4% formaldehyde, and stained with 0.1% crystal violet. Stained colonies (diameter >1 mm) were counted under the microscope.

*Cell Invasion Assay*

Cell invasion was detected using the Transwell invasion assay. Transwell chambers with 8-μm-pore membranes (Millipore, Bedford, MA, USA) were coated with Matrigel (BD Biosciences, San Diego, CA, USA) according to the manufacturer's instructions. A total of 200 μl of serum-free medium containing $1\times10^5$ cells was added to the upper chamber, and 600 μl of medium containing 10% FBS was added to the lower chamber. The cells were cultured for 24 h at 37°C, noninvasive cells on the top surface were removed by a cotton swab, and the invasive cells on the lower surface were fixed with 4% formaldehyde and stained with 0.1% crystal violet. Then cells were observed and counted under the microscope.

*Dual-Luciferase Reporter Assay*

Fragments of the wild-type or mutant AEG-1 3′-UTR containing the putative targeting site of miR-384 were subcloned into the pmirGLO dual-luciferase reporter vector (Promega, Madison, WI, USA). Cells were seeded into 24-well plates ($1\times10^5$ cells/well) and cultured overnight. Thereafter, cells were cotransfected with the pmirGLO-AEG-1 3′-UTR vector and miR-384 mimics or miR-384 inhibitor using Lipofectamine 2000 (Invitrogen) and incubated for 48 h. For measurement of Wnt activity, cells were transfected with miR-384 mimics or miR-384 inhibitor in the presence of TOPFlash firefly luciferase vector and phRL-TK *Renilla* luciferase vector. Luciferase activity was measured with the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions.

*Western Blot Analysis*

Cells were lysed using radioimmunoprecipitation assay lysis buffer (Beyotime Biotechnology, Haimen, P.R. China). The concentrations of proteins were measured using the bicinchoninic acid assay. Equal amounts of proteins were electrophoresed in 10% sodium dodecyl sulfate polyacrylamide gels and transferred onto a polyvinylidene fluoride membrane (Millipore). The membrane was blocked with 5% nonfat dry milk for 1 h at room temperature. The membrane was incubated with primary antibodies including anti-AEG-1 and anti-GAPDH (Cell Signaling Technology, Danvers, MA, USA) at 4°C overnight. Afterward, the membrane was blotted with horseradish peroxidase-conjugated secondary antibody (Beyotime Biotechnology) for 1 h at 37°C. Targeted proteins were visualized using the enhanced chemiluminescence (ECL) system (Pierce, Rockford, IL, USA). Gray scale analysis of protein bands was performed by Image-Pro Plus 6.0 software.

*Data Analysis*

Results are expressed as the means ± standard deviation. Statistical analyses were performed by Student's *t*-test or one-way analysis of variance followed by Bonferroni post hoc analysis using SPSS 18.0 statistical software

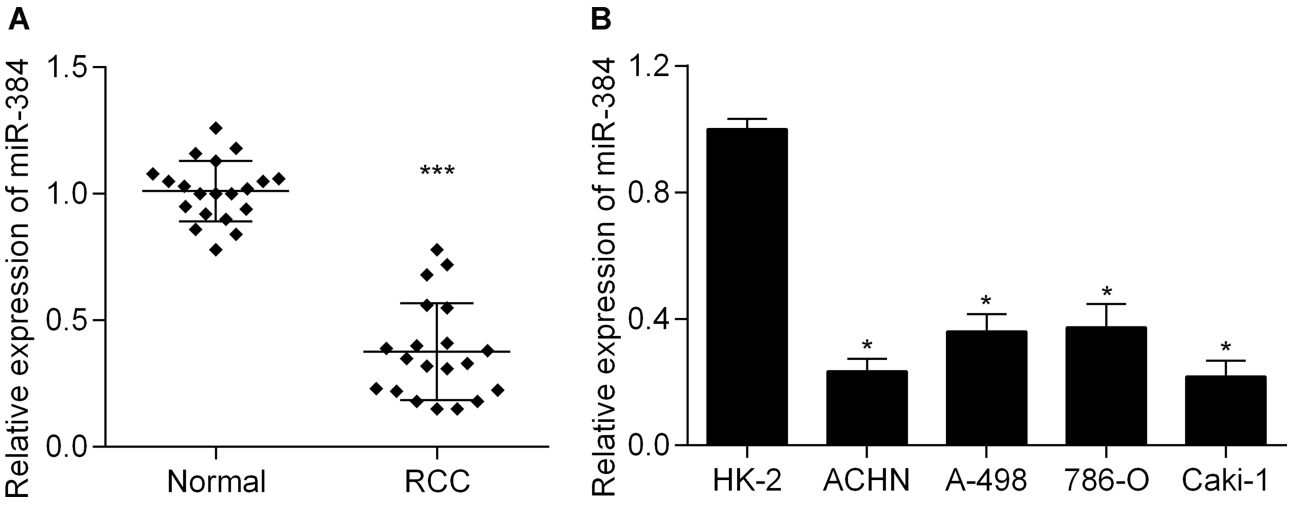


**Figure 1.** Decreased expression of microRNA-384 (miR-384) in renal cell carcinoma (RCC). (A) Relative expression of miR-384 in 20 paired clinical RCC specimens and adjacent normal tissues was detected by real-time qualitative reverse transcriptase polymerase chain reaction (qRT-PCR). ***$p<0.001$ versus normal. (B) Relative expression of miR-384 in RCC cell lines (ACHN, A-498, 786-O, and Caki-1) and normal kidney tubule epithelial cell line HK-2 was detected by qRT-PCR. *$p<0.05$ versus HK-2.

package (SPSS Inc., Chicago, IL, USA). The correlation between miR-384 and AEG-1 expression was determined using Spearman's correlation test. Results were considered statistically significant at a value of $p<0.05$.

## RESULTS

### *miR-384 Is Downregulated in RCC Tissues and RCC Cell Lines*

To investigate whether miR-384 is differently expressed in RCC, we detected the expression of miR-384 in 20 paired RCC specimens and matched adjacent normal renal tissues by qRT-PCR. We found that miR-384 expression was significantly decreased in clinical RCC specimens compared with adjacent normal renal tissues (Fig. 1A). Moreover, we detected the expression of miR-384 in a series of RCC cell lines by qRT-PCR. The results showed that miR-384 expression was markedly downregulated in RCC cell lines compared with normal kidney cells (Fig. 1B). These results indicate that miR-384 may play an important role in the progression of RCC.

### *Overexpression of miR-384 Inhibits the Growth and Invasion of RCC Cell Lines*

To investigate the exact biological function of miR-384 in RCC, we transfected miR-384 mimics or miR-384 inhibitor into ACHN and Caki-1 cell lines. The results

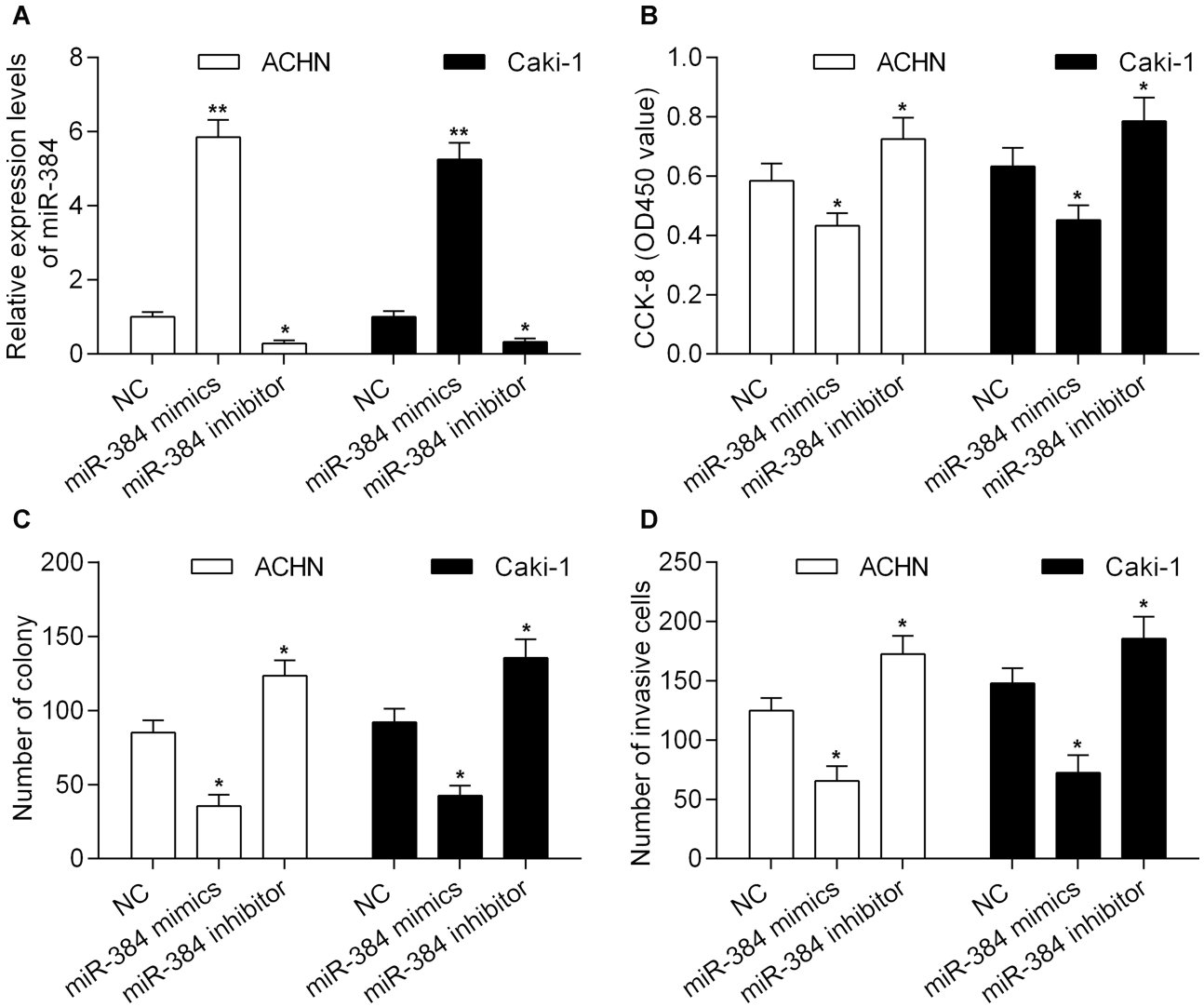


**Figure 2.** miR-384 inhibits the growth and invasion of RCC cell lines. ACHN and Caki-1 cells were transiently transfected with miR-384 mimics or miR-384 inhibitor and incubated for 48 h before subsequent experiments. (A) The relative expression of miR-384 in ACHN and Caki-1 cells was detected by qRT-PCR. The effect of miR-384 overexpression or inhibition on the growth of ACHN and Caki-1 cells was detected by cell counting kit-8 (CCK-8) (B) and colony formation (C) assays. (D) The effect of miR-384 overexpression or inhibition on the invasion of ACHN and Caki-1 cells was detected by Transwell invasion assay. *$p < 0.05$, **$p < 0.01$ versus NC.

showed that miR-384 expression was increased in cells transfected with miR-384 mimics, whereas miR-384 expression was decreased in miR-384 inhibitor-transfected cells (Fig. 2A). We then detected the effect of miR-384 overexpression or inhibition of cell growth of RCC cell lines. The CCK-8 assay showed that overexpression of miR-384 significantly inhibited the proliferation of ACHN and Caki-1 cells (Fig. 2B). The colony formation assay showed that overexpression of miR-384 also inhibited the colony-forming capacity of ACHN and Caki-1 cells (Fig. 2C). Moreover, we investigated the role of miR-384 in regulating RCC cell invasion. The Transwell invasion assay revealed that overexpression of miR-384 significantly suppressed the invasion ability of ACHN and Caki-1 cells (Fig. 2D). In contrast, miR-384 inhibition had the opposite effects (Fig. 2B–D). Overall, these results suggest that miR-384 may exert antitumor effects by inhibiting the growth and invasion of RCC cells.

*miR-384 Inhibits AEG-1 Expression by Directly Targeting its 3′-UTR*

To investigate the molecular mechanism by which miR-384 exerts its antitumor effects, we investigated the predicted target genes of miR-384. Bioinformatics analysis showed that AEG-1, an important oncogene in various cancers[15,16], was predicted as a potential target gene of miR-384. The 3′-UTR of AEG-1 contains putative targeting sites for miR-384 (Fig. 3A). To verify whether miR-384 binds to the AEG-1 3′-UTR, we performed a dual-luciferase reporter assay. The results showed that overexpression of miR-384 significantly decreased the luciferase activity of the reporter vector containing the wild-type AEG-1 3′-UTR, whereas miR-384 inhibition significantly increased the luciferase activity of the wild-type AEG-1 3′-UTR (Fig. 3B and C). However, these effects were obviously abrogated when the binding sites

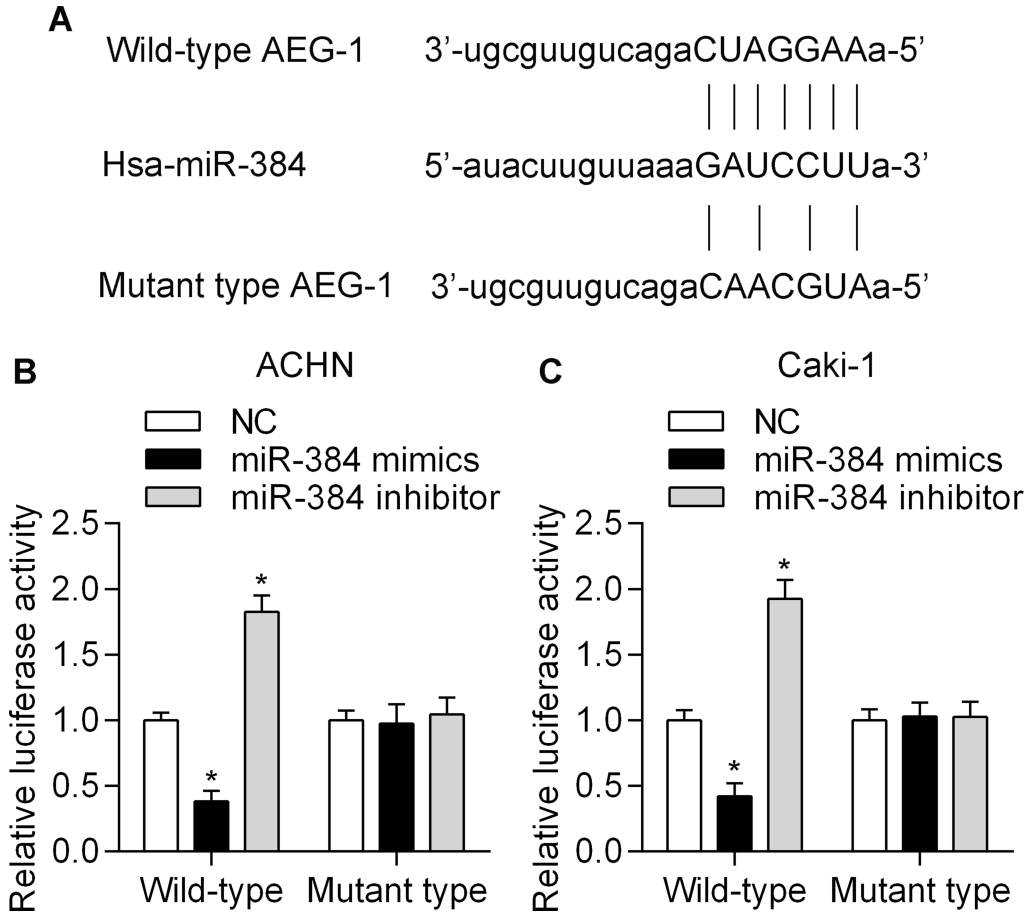


**Figure 3.** miR-384 targets the 3′-untranslated region (UTR) of astrocyte elevated gene 1 (AEG-1). (A) The schematic diagram of miR-384 targeting sites in the 3′-UTR of AEG-1. Dual-luciferase reporter assay was performed using ACHN (B) and Caki-1 (C) cells cotransfected with wild-type or mutant type 3′-UTR reporter vector and miR-384 mimics or miR-384 inhibitor for 48 h. *$p<0.05$ versus NC.

were mutated in the AEG-1 3′-UTR (Fig. 3B and C). These results suggest that miR-384 directly binds to the 3′-UTR of AEG-1. To investigate whether miR-384 directly regulates AEG-1 expression, we detected the effect of miR-384 overexpression or inhibition on AEG-1 expression by qRT-PCR and Western blot analysis. The results showed that overexpression of miR-384 significantly inhibited the mRNA and protein expression of AEG-1, whereas miR-384 inhibition markedly increased the expression of AEG-1 (Fig. 4A and B) in RCC cells. Overall, these results indicate that miR-384 inhibits AEG-1 expression through the direct targeting of its 3′-UTR.

### *miR-384 Expression Is Inversely Correlated With AEG-1 Expression in RCC Specimens*

To further investigate the relationship between miR-384 and AEG-1, we analyzed the correlation between miR-384 and AEG-1 expression in clinical RCC specimens. We found that AEG-1 mRNA expression was significantly increased in RCC specimens (Fig. 5A). Correlation analysis showed that miR-384 expression was inversely correlated with AEG-1 mRNA expression in RCC specimens (Fig. 5B). These results suggest that miR-384 may be negatively associated with AEG-1 in RCC patients in vivo.

### *miR-384 Negatively Regulates Wnt Signaling by Targeting AEG-1 in RCC Cells*

AEG-1 has been suggested as a coactivator of multiple oncogenic signaling pathways, including Wnt signaling[32]. Considering the regulatory effect of miR-384 on AEG-1 expression, we investigated the regulatory effect of miR-384 on Wnt signaling in RCC cells. The results showed that overexpression of miR-384 significantly suppressed the activation of Wnt signaling, whereas miR-384 inhibition enhanced its activation (Fig. 6A). To investigate whether miR-384 regulates Wnt signaling through targeting of AEG-1, we determined the effect of restoring AEG-1 expression on miR-384-induced inhibition of Wnt signaling. Transfection of the pcDNA3.1/AEG-1 vector (without the 3′-UTR) significantly increased the expression of AEG-1 in miR-384 mimic-transfected cells (Fig. 6B). Further experiments showed that overexpression of AEG-1 significantly reversed the inhibitory effects of miR-384 on Wnt signaling in RCC cells (Fig. 6C). Overall, these results suggest that miR-384 negatively regulates Wnt signaling by targeting AEG-1 in RCC cells.

### *Overexpression of AEG-1 Reverses the Inhibitory Effect of miR-384 on RCC Cell Growth and Invasion*

To investigate whether AEG-1 is involved in miR-384-mediated RCC cell growth and invasion, we detected the restoration of AEG-1 expression on miR-384-mediated RCC cell growth and invasion. The results showed that overexpression of AEG-1 significantly reversed the inhibitory effect of miR-384 on RCC cell growth and invasion (Fig. 7A and B). These data indicate that miR-384 inhibits the growth and invasion of RCC cells through downregulation of AEG-1.

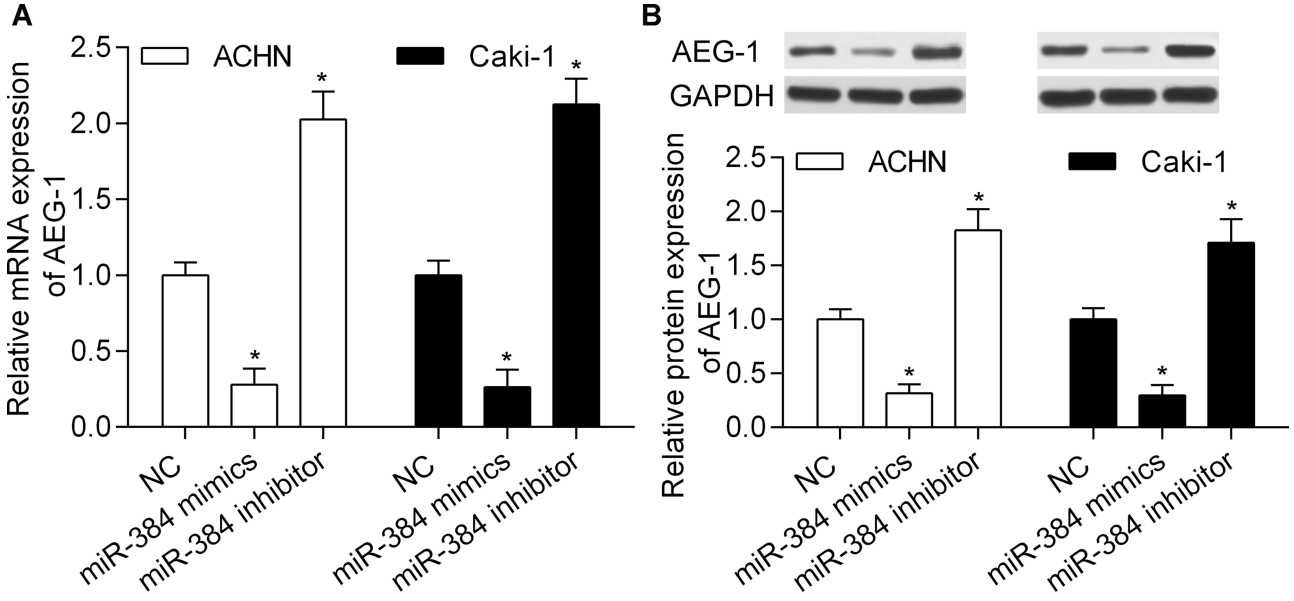


**Figure 4.** miR-384 negatively regulates AEG-1 expression. ACHN and Caki-1 cells were transiently transfected with miR-384 mimics or miR-384 inhibitor and incubated for 48 h. (A) The relative mRNA expression of AEG-1 was detected by qRT-PCR. (B) The relative protein expression of AEG-1 was detected by Western blot analysis. *$p<0.05$ versus NC. GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

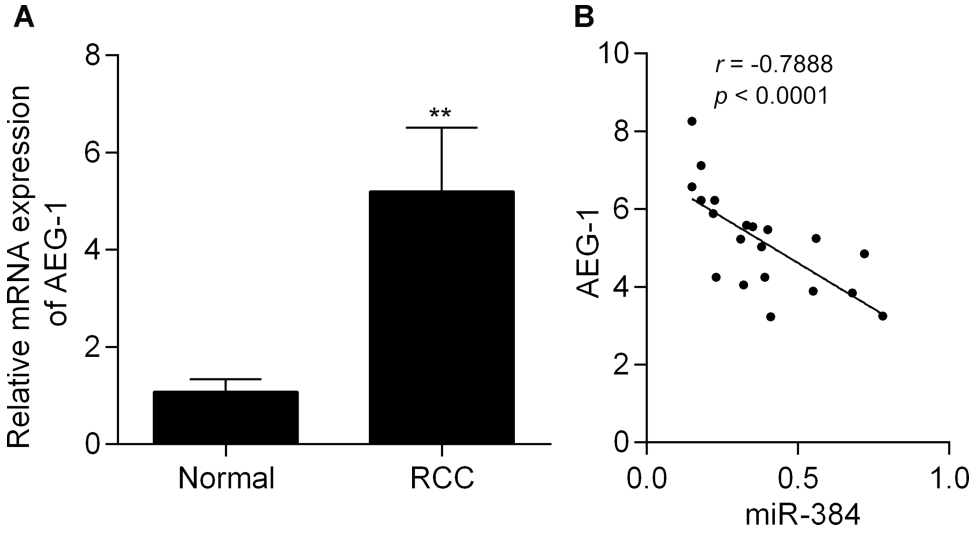


**Figure 5.** miR-384 expression is inversely correlated with AEG-1 expression in RCC specimens. (A) The relative mRNA expression of AEG-1 in 20 paired clinical RCC specimens and adjacent normal tissues was detected by qRT-PCR. $^{**}p<0.01$ versus normal. (B) The relationship between miR-384 and AEG-1 expression was analyzed by Spearman's correlation test. $r=-0.7888$; $p<0.0001$.

## DISCUSSION

In the present study, we demonstrate an important role of miR-384 in the progression of RCC. We found significant downregulation of miR-384 in RCC tissues and cell lines. Functional experiments indicated that miR-384 inhibited the growth and invasion of RCC cells through targeting of AEG-1. Overall, our study reports for the first time that miR-384 functions as a tumor suppressor in RCC.

Recent studies have reported that miR-384 plays an important role in the development and progression of various cancers. It is reported that miR-384 is involved in regulating the tumor metastasis of melanoma cells by targeting histone deacetylase 3[33]. Overexpression of miR-384 results in a decrease of piwi-like RNA-mediated gene silencing 4 (PIWIL4), leading to tumor repression of glioma[31]. In hepatocellular carcinoma, miR-384 is found decreased in hepatocellular carcinoma tissues and cell lines, and overexpression of miR-384 inhibits the proliferation of hepatocellular carcinoma cells by targeting insulin receptor substrate 1[30]. Similarly, Chen et al. reported that inhibition of miR-384 promoted the proliferation, migration, and invasion of hepatocellular carcinoma cells[34]. A recent study showed that suppression of miR-384 leads to overexpression of pleiotrophin, which promotes the proliferation, metastasis, and lipogenesis of hepatitis B virus-related hepatocellular carcinoma cells[35]. Moreover, Wang et al. reported that low expression of miR-384 was correlated with the invasive depth, lymph node, and distant metastasis of colorectal cancer[29]. Furthermore, overexpression of miR-384 inhibited the metastasis of colorectal cancer by targeting Kirsten rat sarcoma viral oncogene homolog (KRas) and cell division cycle 42[29]. These studies suggest a tumor-suppressive role of miR-384. However, little is known about the role of miR-384 in RCC. In this study, we found that miR-384 was significantly downregulated in RCC tissues and cell lines. We also showed that overexpression of miR-384 inhibited the growth and invasion of RCC cells, whereas inhibition of miR-384 promoted the growth and invasion of RCC cells. These results suggest that miR-384 functions as a tumor suppressor in RCC.

AEG-1 has been suggested as a critical oncogene in various cancers[15,16], including RCC. Chen et al. reported that the mRNA and protein levels of AEG-1 were highly upregulated in RCC tissues and RCC cell lines[27]. Statistical analysis showed that high expression of AEG-1 was correlated with tumor grade, clinical staging, T classification, metastasis classification, and shorter survival time in RCC patients[27]. Similarly, another study shows that high expression of AEG-1 is correlated with tumor capsule invasion, lymphovascular invasion, and tumor size[36]. A functional study revealed that knockdown of AEG-1 inhibits cell proliferation and colony formation and arrests cell cycle progression of RCC cells[28]. Moreover, knockdown of AEG-1 increases chemosensitivity to 5-fluorouracil in RCC cells[28]. Therefore, AEG-1 may serve as a promising target for the treatment of RCC. In this study, our results showed that AEG-1 was a target gene of miR-384. We found that miR-384 could negatively regulate AEG-1 expression in RCC cells. In addition, miR-384 was inversely correlated with AEG-1 expression in RCC specimens. Therefore, decreased miR-384 expression may contribute to the high expression of AEG-1 in RCC, which leads to the development and progression of RCC. Our results also showed that

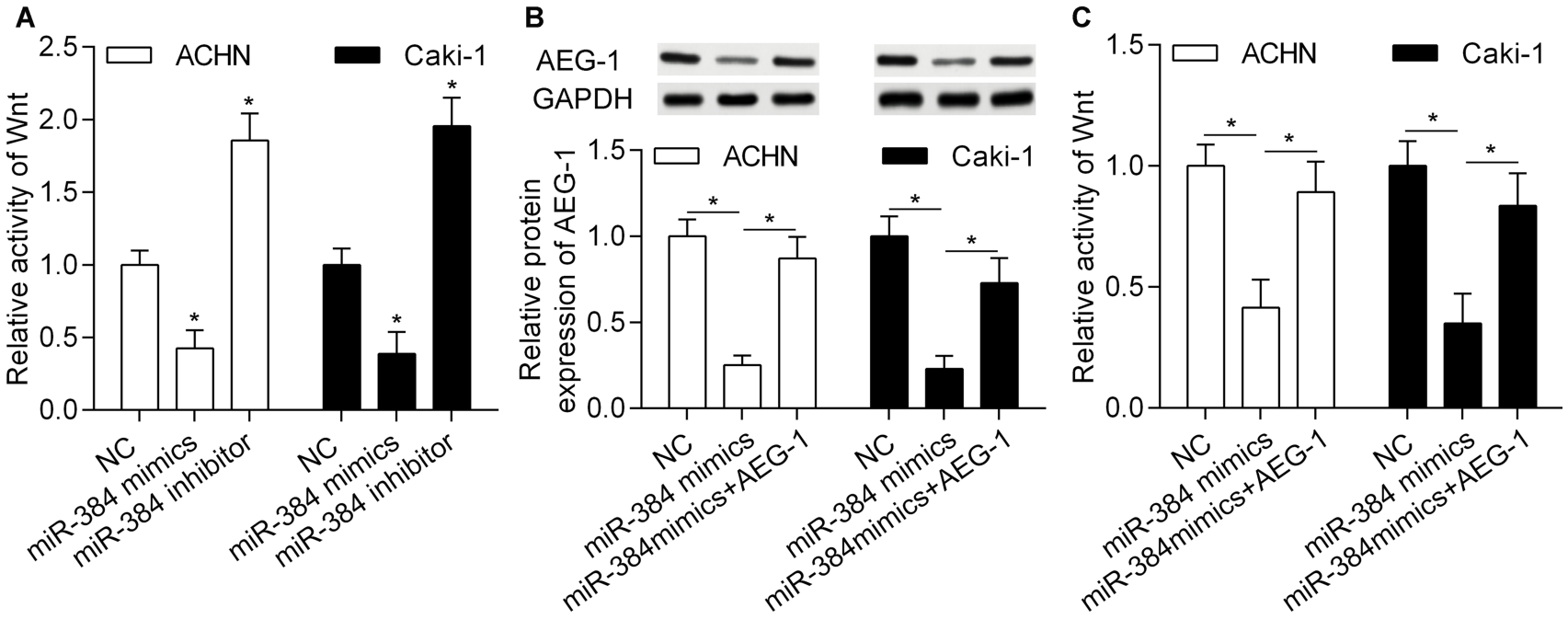


**Figure 6.** miR-384 negatively regulates wingless-related integration site (Wnt) signaling in RCC cells. (A) Relative activity of Wnt was detected by luciferase reporter assay. ACHN and Caki-1 cells were transfected with miR-384 mimics or miR-384 inhibitor in the presence with TOPFlash firefly luciferase vector and phRL-TK *Renilla* luciferase vector and incubated for 48 h. *$p<0.05$ versus NC. (B) Relative protein expression of AEG-1 was detected by Western blotting. ACHN and Caki-1 cells were cotransfected with miR-384 mimics and pcDNA3.1/AEG-1 vector for 48 h. *$p<0.05$. (C) Relative activity of Wnt was detected by luciferase reporter assay in ACHN and Caki-1 cells cotransfected with miR-384 mimics and pcDNA3.1/AEG-1 vector in the presence with TOPFlash firefly luciferase vector and phRL-TK *Renilla* luciferase vector and incubated for 48 h. *$p<0.05$.

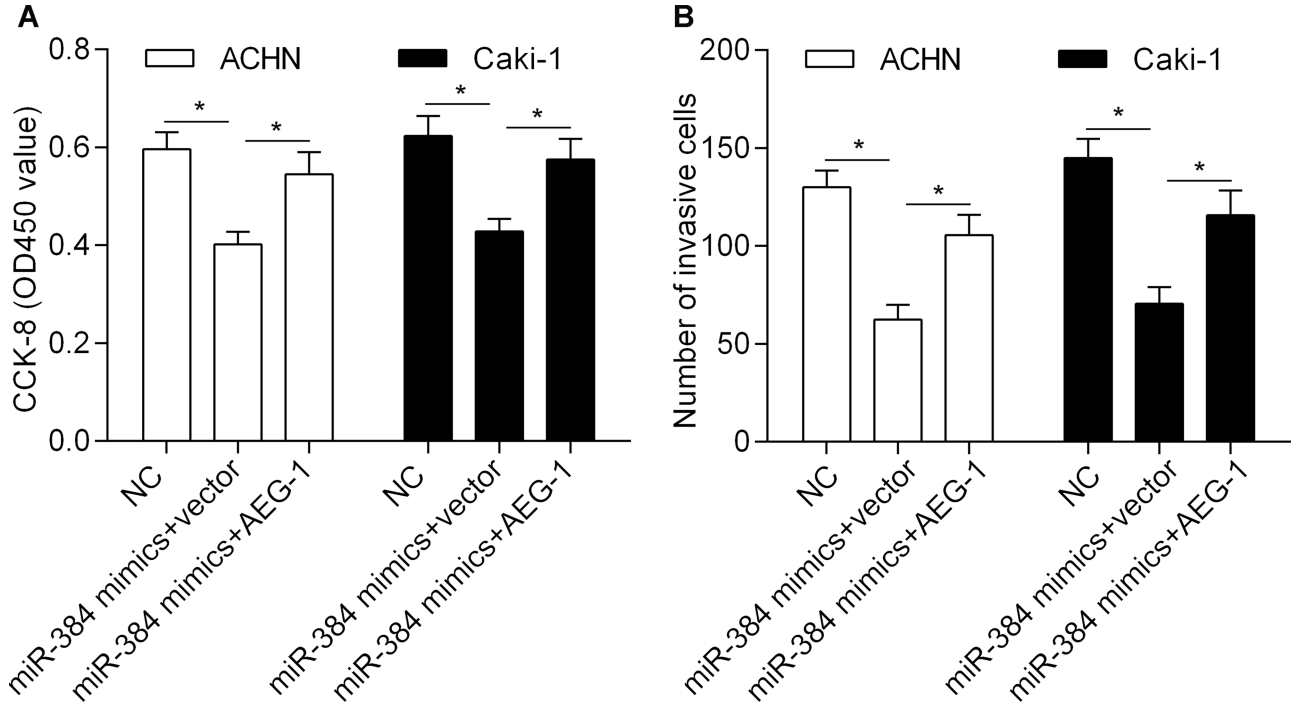


**Figure 7.** Overexpression of AEG-1 reverses the inhibitory effect of miR-384 on RCC cell growth and invasion. ACHN and Caki-1 cells were cotransfected with miR-384 mimics and pcDNA3.1/AEG-1 vector and incubated for 48 h. (A) Cell proliferation was detected by CCK-8 assay. (B) Cell invasion was detected by Transwell invasion assay. $*p<0.05$.

overexpression of AEG-1 significantly reversed the antitumor effect of miR-384, indicating that miR-384 inhibits the growth and invasion of RCC cells through targeting of AEG-1. Our study suggests that manipulating AEG-1 expression by miR-384 may represent a promising therapeutic strategy for the treatment of RCC.

In recent years, a growing body of evidence has revealed that AEG-1 expression is regulated by numerous miRNAs, including miR-375[37], miR-136[38], and miR-217[39]. These studies reveal that AEG-1 expression is posttranscriptionally controlled by specific miRNAs, which may be involved in the development and progression of cancers. However, it is unknown if the dysregulated AEG-1 expression in RCC is associated with miRNA. In this study, we found that AEG-1 was a target gene of miR-384, and its expression was regulated by miR-384 in RCC. Similarly, a recent study reports that miR-30d targets and regulates AEG-1 in RCC[40]. These findings suggest that the high expression of AEG-1 during tumorigenesis is associated with specific miRNA dysregulation. Therefore, miRNA-based methods for inhibiting AEG-1 expression may be promising strategies for the treatment of RCC.

In conclusion, our results demonstrate that miR-384 inhibits the growth and invasion of RCC cells through downregulation of AEG-1, supporting a tumor-suppressive role for miR-384 in RCC. These findings suggest that miR-384/AEG-1 may play novel functional roles in the pathogenesis of RCC. Therefore, miR-384 may serve as a promising and effective target for the development of a novel therapeutic strategy for the treatment of RCC.

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

## REFERENCES

1. Cairns P. Renal cell carcinoma. Cancer Biomark. 2010;9: 461–73.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin. 2017;67:7–30.
3. Albiges L, Choueiri T, Escudier B, Galsky M, George D, Hofmann F, Lam T, Motzer R, Mulders P, Porta C, Powles T, Sternberg C, Bex A. A systematic review of sequencing and combinations of systemic therapy in metastatic renal cancer. Eur Urol. 2015;67:100–10.
4. Inman BA, Harrison MR, George DJ. Novel immunotherapeutic strategies in development for renal cell carcinoma. Eur Urol. 2013;63:881–89.
5. He L, Hannon GJ. MicroRNAs: Small RNAs with a big role in gene regulation. Nat Rev Genet. 2004;5:522–31.
6. Chen K, Rajewsky N. The evolution of gene regulation by transcription factors and microRNAs. Nat Rev Genet. 2007;8:93–103.
7. Manikandan J, Aarthi JJ, Kumar SD, Pushparaj PN. Oncomirs: The potential role of non-coding microRNAs in understanding cancer. Bioinformation 2008;2:330–34.

8. Pang JC, Kwok WK, Chen Z, Ng HK. Oncogenic role of microRNAs in brain tumors. Acta Neuropathol. 2009; 117:599–611.
9. Chen Y, Fu LL, Wen X, Liu B, Huang J, Wang JH, Wei YQ. Oncogenic and tumor suppressive roles of microRNAs in apoptosis and autophagy. Apoptosis 2014;19:1177–89.
10. Chen Y, Gao DY, Huang L. In vivo delivery of miRNAs for cancer therapy: Challenges and strategies. Adv Drug Deliv Rev. 2015;81:128–41.
11. Tutar L, Tutar E, Tutar Y. MicroRNAs and cancer; an overview. Curr Pharm Biotechnol. 2014;15:430–37.
12. Sellitti DF, Doi SQ. MicroRNAs in renal cell carcinoma. Microrna 2015;4:26–35.
13. Grange C, Collino F, Tapparo M, Camussi G. Oncogenic micro-RNAs and renal cell carcinoma. Front Oncol. 2014; 4:49–58.
14. Al-Ali BM, Ress AL, Gerger A, Pichler M. MicroRNAs in renal cell carcinoma: Implications for pathogenesis, diagnosis, prognosis and therapy. Anticancer Res. 2012;32: 3727–32.
15. Hu G, Wei Y, Kang Y. The multifaceted role of MTDH/AEG-1 in cancer progression. Clin Cancer Res. 2009;15: 5615–20.
16. Sarkar D, Fisher PB. AEG-1/MTDH/LYRIC: Clinical significance. Adv Cancer Res. 2013;120:39–74.
17. Su ZZ, Kang DC, Chen Y, Pekarskaya O, Chao W, Volsky DJ, Fisher PB. Identification and cloning of human astrocyte genes displaying elevated expression after infection with HIV-1 or exposure to HIV-1 envelope glycoprotein by rapid subtraction hybridization, RaSH. Oncogene 2002; 21:3592–602.
18. Su ZZ, Kang DC, Chen Y, Pekarskaya O, Chao W, Volsky DJ, Fisher PB. Identification of gene products suppressed by human immunodeficiency virus type 1 infection or gp120 exposure of primary human astrocytes by rapid subtraction hybridization. J Neurovirol. 2003;9:372–89.
19. Kang DC, Su ZZ, Sarkar D, Emdad L, Volsky DJ, Fisher PB. Cloning and characterization of HIV-1-inducible astrocyte elevated gene-1, AEG-1. Gene 2005;353:8–15.
20. Emdad L, Das SK, Hu B, Kegelman T, Kang DC, Lee SG, Sarkar D, Fisher PB. AEG-1/MTDH/LYRIC: A promiscuous protein partner critical in cancer, obesity, and CNS diseases. Adv Cancer Res. 2016;131:97–132.
21. Luo Y, Zhang X, Tan Z, Wu P, Xiang X, Dang Y, Chen G. Astrocyte elevated gene-1 as a novel clinicopathological and prognostic biomarker for gastrointestinal cancers: A meta-analysis with 2999 patients. PLoS One 2015;10: e0145659–e74.
22. Gnosa S, Shen YM, Wang CJ, Zhang H, Stratmann J, Arbman G, Sun XF. Expression of AEG-1 mRNA and protein in colorectal cancer patients and colon cancer cell lines. J Transl Med. 2012;10:109–21.
23. Turner N, Pusey CD. Aortic dissection masquerading as systemic disease—The post-dissection syndrome. Q J Med. 1990;75:525–31.
24. Emdad L, Sarkar D, Lee SG, Su ZZ, Yoo BK, Dash R, Yacoub A, Fuller CE, Shah K, Dent P, Bruce JN, Fisher PB. Astrocyte elevated gene-1: A novel target for human glioma therapy. Mol Cancer Ther. 2010;9:79–88.
25. Kikuno N, Shiina H, Urakami S, Kawamoto K, Hirata H, Tanaka Y, Place RF, Pookot D, Majid S, Igawa M, Dahiya R. Knockdown of astrocyte-elevated gene-1 inhibits prostate cancer progression through upregulation of FOXO3a activity. Oncogene 2007;26:7647–55.
26. Lee SG, Su ZZ, Emdad L, Sarkar D, Franke TF, Fisher PB. Astrocyte elevated gene-1 activates cell survival pathways through PI3K-Akt signaling. Oncogene 2008;27: 1114–21.
27. Chen W, Ke Z, Shi H, Yang S, Wang L. Overexpression of AEG-1 in renal cell carcinoma and its correlation with tumor nuclear grade and progression. Neoplasma 2010;57:522–9.
28. Wang P, Yin B, Shan L, Zhang H, Cui J, Zhang M, Song Y. RNA interference-mediated knockdown of astrocyte elevated gene-1 inhibits growth, induces apoptosis, and increases the chemosensitivity to 5-fluorouracil in renal cancer Caki-1 cells. Mol Cells 2014;37:857–64.
29. Wang YX, Chen YR, Liu SS, Ye YP, Jiao HL, Wang SY, Xiao ZY, Wei WT, Qiu JF, Liang L, Liao WT, Ding YQ. MiR-384 inhibits human colorectal cancer metastasis by targeting KRAS and CDC42. Oncotarget 2016;7:84826–38.
30. Lai YY, Shen F, Cai WS, Chen JW, Feng JH, Cao J, Xiao HQ, Zhu GH, Xu B. MiR-384 regulated IRS1 expression and suppressed cell proliferation of human hepatocellular carcinoma. Tumour Biol. 2016;37:14165–71.
31. Zheng J, Liu X, Wang P, Xue Y, Ma J, Qu C, Liu Y. CRNDE promotes malignant progression of glioma by attenuating miR-384/PIWIL4/STAT3 axis. Mol Ther. 2016; 24:1199–215.
32. He W, He S, Wang Z, Shen H, Fang W, Zhang Y, Qian W, Lin M, Yuan J, Wang J, Huang W, Wang L, Ke Z. Astrocyte elevated gene-1 (AEG-1) induces epithelial-mesenchymal transition in lung cancer through activating Wnt/beta-catenin signaling. BMC Cancer 2015;15:107–19.
33. Eom S, Kim Y, Park D, Lee H, Lee YS, Choe J, Kim YM, Jeoung D. Histone deacetylase-3 mediates positive feedback relationship between anaphylaxis and tumor metastasis. J Biol Chem. 2014;289:12126–44.
34. Chen Z, Yu C, Zhan L, Pan Y, Chen L, Sun C. LncRNA CRNDE promotes hepatic carcinoma cell proliferation, migration and invasion by suppressing miR-384. Am J Cancer Res. 2016;6:2299–309.
35. Bai PS, Xia N, Sun H, Kong Y. Pleiotrophin, a target of miR-384, promotes proliferation, metastasis and lipogenesis in HBV-related hepatocellular carcinoma. J Cell Mol Med. 2017;21(11):3023–3043.
36. Erdem H, Oktay M, Yildirim U, Uzunlar AK, Kayikci MA. Expression of AEG-1 and p53 and their clinicopathological significance in malignant lesions of renal cell carcinomas: A microarray study. Pol J Pathol. 2013;64:28–32.
37. Yan JW, Lin JS, He XX. The emerging role of miR-375 in cancer. Int J Cancer 2014;135:1011–18.
38. Yang Y, Wu J, Guan H, Cai J, Fang L, Li J, Li M. MiR-136 promotes apoptosis of glioma cells by targeting AEG-1 and Bcl-2. FEBS Lett. 2012;586:3608–12.
39. Wang B, Shen ZL, Jiang KW, Zhao G, Wang CY, Yan YC, Yang Y, Zhang JZ, Shen C, Gao ZD, Ye YJ, Wang S. MicroRNA-217 functions as a prognosis predictor and inhibits colorectal cancer cell proliferation and invasion via an AEG-1 dependent mechanism. BMC Cancer 2015;15:437–47.
40. Wu C, Jin B, Chen L, Zhuo D, Zhang Z, Gong K, Mao Z. MiR-30d induces apoptosis and is regulated by the Akt/FOXO pathway in renal cell carcinoma. Cell Signal. 2013; 25:1212–21.