

## Original Article

# MicroRNA-186 regulates cell malignancy by targeting ROCK1 in chronic myeloid leukemia

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**Abstract:** According to recent reports, aberrant miRNA expression has been observed in human cancers, and their alterations contribute to multiple cancer development and progression. In the present study, we indentified miR-186 by analyzing quantitative real-time PCR data of chronic myeloid leukemia (CML). miR-186 was found to be markedly reduced in CML samples compared with that in corresponding normal MSCs. However, little is known about its molecular mechanism in CML development. Here, we reported the effect of miR-186 on CML cell proliferation and epithelial-mesenchymal transition (EMT). MTT, Transwell and Western blot assay were used to assess the effect of miR-186 on A562 and KCL-22 cell proliferation and EMT. Target prediction and luciferase reporter assays were performed to investigate the targets of miR-186. Tumor formation assay in vivo was performed to investigate the antitumor effect of miR-186. We found an inverse correlation between miR-186 and CML cell proliferation, EMT, migration, and invasion. Moreover, we validated that rho-associated protein kinase 1 (ROCK1) was a direct target of miR-186, by which miR-186 regulated CML cell proliferation, EMT, migration, and invasion. The animal experiments showed that overexpression of miR-186 inhibited the growth of CML tumors. In conclusion, miR-186 functions as a tumor suppressor in the progression of malignant CML, which can be recommended as a potential target.

**Keywords:** MiR-186, ROCK1, CML

## Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative disease originating from a constitutively active tyrosine kinase, BCR-ABL [1]. Understanding the molecular pathogenesis of CML may enable advancements in the diagnosis and treatment of this disease. CML progression depends on the capacity to invade and to metastasize to distant sites. Tumor cell metastasis is thought to be controlled by molecular processes that are different from those that control tumor initiation and growth [2]. Support for this hypothesis comes from the observation of human cancer lesions as well as several mouse models in which tissue-specific oncogene expression led to tumor initiation, yet tumor progression was not observed [3-5]. Understanding the molecular mechanisms of CML biology may provide some useful targets or biomarkers in the early stage for patients with CML.

MiRNA acts as a kind of small and non-coding RNAs, and regulates the expression of genes by binding to complementary sequences of 3'UTR of mRNA, leading to the inhibition of miRNA translation or degradation of target mRNA [6]. It has been reported that miRNA regulates the expression of about 90% of all human genes, and plays an essential role in different biological and pathological processes, involving cell proliferation, apoptosis, invasion and migration [7, 8]. Emerging evidence shows that the deregulated expression of miRNAs was found in various types of cancers, which act as a kind of tumor oncogene or suppressor [9, 10]. Latest advances demonstrated that miRNAs serve as a critical regulator for EMT [11-13]. The function of miRNAs in CML development has been extensively reported, and microarray assays also identified many abnormally expressed miRNAs [14, 15]. It should be noted that the expression level of miR-186 in CML cells is also decreased, but the molecular mechanisms of miR-186 in

the development of CML is still poorly understood.

In this study, we determined the expression of miR-186 in primary CML samples and cells, and explored the correlation between the expression of miR-186 and CML cell proliferation, EMT, and invasion. And then, we investigated the regulatory effect of miR-186 on CML cell proliferation, EMT, and invasion. Finally, our team conducted the animal experiments to investigate anti-cancer function of miR-186 in vivo. Our data will provide a new therapeutic target for CML patients.

### Materials and methods

#### *Tissue samples*

22 cases of CML samples and matched normal samples were collected from The First Affiliated Hospital of Harbin Medical University. Eligible samples were obtained from the patients with primary CML who had not received any preoperative chemotherapy or radiotherapy. In addition, there were no coexisting diseases in these patients. This study and the use of human cell lines were performed with the approval of the Medical Ethical Committee of The First Affiliated Hospital of Harbin Medical University and written informed consent was obtained from all patients. All tissue samples were immediately flash-frozen in liquid nitrogen after resection and then stored at  $-80^{\circ}\text{C}$  until use.

#### *Cell lines and cell transfection*

The human A562 and KCL-22 cells were obtained from American Type Culture Collection (Manassas, VA, USA). Cells were grown in RPMI-1640 medium (Thermo Fisher Scientific, Waltham, MA, USA) containing 10% fetal bovine serum (Thermo Fisher Scientific) at  $37^{\circ}\text{C}$  with 5%  $\text{CO}_2$ . miR-186 mimics and negative control (NC) mimics, miR-186 inhibitors (anti-miR-186) and NC inhibitors (anti-NC), and ROCK1 small interfering RNAs (siRNAs) were purchased from GenePharma Company. Cell transfection was carried out using Lipofectamine 2000 (Thermo Fisher Scientific) following the manufacturer's protocol.

#### *RNA extraction and quantitative real-time PCR*

Total RNA was extracted from frozen samples or cultured cells using TRIzol Reagent (Thermo

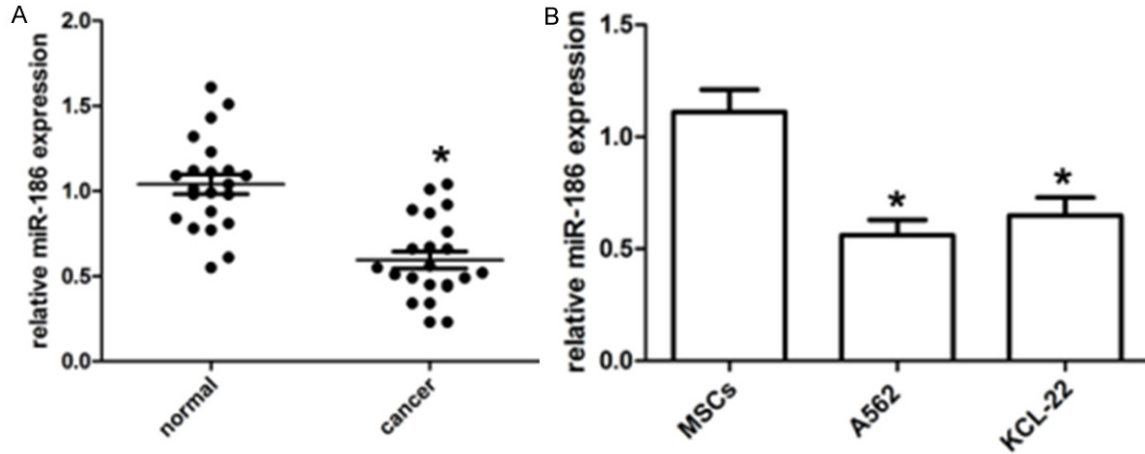
Fisher Scientific) following the manufacturer's suggestions. miR-186 expression was determined using TaqMan miRNA assays (Thermo Fisher Scientific) following the manufacturer's protocol, and U6 small nuclear RNA (GeneCopoeia, Carlsbad, CA, USA) was used as endogenous control. The expression of ROCK1 was determined using SYBR green qPCR assay (Takara, Dalian, People's Republic of China) and normalized with GAPDH (GeneCopoeia).

#### *MTT assay*

Cell suspensions ( $2 \times 10^4$  cells per well) were seeded in 96-well plates and cultured overnight in a humidified incubator at  $37^{\circ}\text{C}$  and 5%  $\text{CO}_2$ . Cell growth was monitored every day for a period of 4 days. Briefly, 20  $\mu\text{L}$  of MTT (5 mg/mL; Sigma-Aldrich Co., St Louis, MO, USA) was added to each well and further incubated for 4 hours. Then, the culture medium in the wells was removed, and 150  $\mu\text{L}$  of dimethyl sulfoxide (Sigma-Aldrich Co.) was added to dissolve the MTT-formazan crystals. The absorbance of each well was read by a microplate reader (Thermo Fisher Scientific) at 570 nm.

#### *Western blot analysis*

Tissue samples were ground to powder in liquid nitrogen. Total proteins were extracted from tissue powder or treated cells using sodium dodecyl sulfate lysis buffer (Beyotime, Shanghai, People's Republic of China) for 30 minutes at  $4^{\circ}\text{C}$ , and an equal amount of protein was separated using 10% polyacrylamide sodium dodecyl sulfate gels. Then, the proteins were transferred to polyvinylidene fluoride membranes (Thermo Fisher Scientific) and were probed with primary antibodies against E-cadherin (Abcam, Cambridge, UK), vimentin (Gene-Tex, San Antonio, TX, USA), ROCK1 (Abcam), N-cadherin (GeneTex), matrix metalloproteinase (MMP)-2 (Abcam), MMP-9 (Abcam), or GAPDH (Gene-Tex). The membranes were incubated overnight at  $4^{\circ}\text{C}$ , followed by incubation with secondary antibody peroxidase-conjugated anti-IgG (Abcam), and detected using a chemiluminescent detection system (Pierce ECL Substrate Western blot detection system; Thermo Fisher Scientific). Quantity One 4.5.0 software (Bio-Rad Laboratories Inc., Hercules, CA, USA) was used to quantify the integrated density of the protein bands.



**Figure 1.** miR-186 is down-regulated in CML samples and cells. A. miR-186 was frequently downregulated in CML samples compared with matched normal MSCs. B. Downregulated miR-186 was detected in CML cell lines A562 and KCL-22 compared with human MSCs. \*P<0.001.

*Cell migration and invasion assays*

Transwell chambers (Corning Incorporated, Corning, NY, USA) with a pore size of 8 μm were used for migration and invasion assays. Briefly, cells were harvested and resuspended in serum-free medium. For migration assay, 5×10<sup>4</sup> cells in serum-free medium were directly added into the upper chamber. For invasion assay, Matrigel (BD Biosciences, San Jose, CA, USA) was coated on the upper surface of the transwell chamber before use, and then 2×10<sup>5</sup> cells in serum-free medium were added into the upper chamber. RPMI-1640 medium containing 10% fetal bovine serum was added into the bottom chamber and served as a chemoattractant. After incubation for 18 hours at 37°C in 5% CO<sub>2</sub>, the cells on the upper surface of the membrane were wiped out with cotton swab. The cells that migrated or invaded to the lower surface of the membrane were fixed with 100% methanol, stained with hematoxylin, and counted (five high-power fields per chamber) under a microscope (Olympus Corporation, Tokyo, Japan).

*Fluorescent reporter assay*

Wild-type 3'UTRs of ROCK1 containing predicted miR-186 target sites were amplified by polymerase chain reaction (PCR) from SW1353 cell genomic DNA, and mutant 3'UTRs were obtained by overlap extension PCR method. SW-1353 cells were transfected with Lipofectamine 2000 (Thermo Fisher Scientific). The transfection mixtures contained 5 pmol of miR-186 and

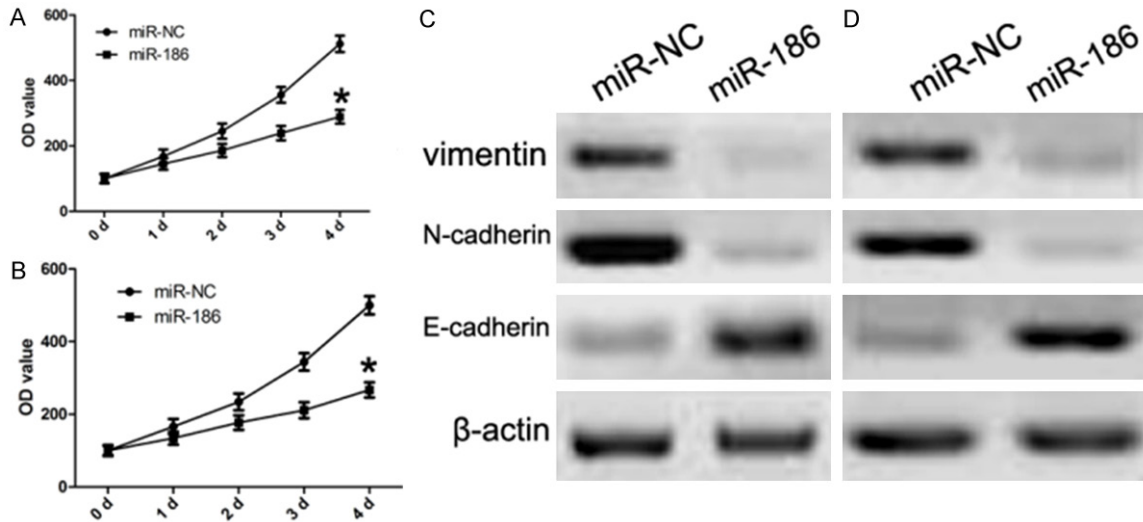
100 ng of firefly luciferase reporter plasmid, and pRL-TK (Promega Corporation, Fitchburg, WI, USA) was also transfected as normalization control. Cells were collected 48 hours post-transfection, and the luciferase activity was determined using a dual-luciferase reporter assay system (Promega Corporation).

*In vivo tumor xenograft model*

Twelve healthy female BALB/c nude mice with 4-5 weeks of age were purchased from the Center of Experimental Animal of The First Affiliated Hospital of Harbin Medical University. All animal experiments were approved by the Institutional Animal Care and Use Committee of The First Affiliated Hospital of Harbin Medical University. Twelve nude mice were randomized into two groups (ie, control group and miR-186 group). Cells (2×10<sup>6</sup> cells in 200 μL phosphate-buffered saline) transfected with miR-186 mimics or NC mimics were subcutaneously inoculated into the right dorsal flank of nude mice. The tumor size was measured weekly with slide caliper, and the tumor volume was calculated as follows: tumor volume = length × width<sup>2</sup>/2. Four weeks later, all tested nude mice were euthanized, and tumors were excised and weighed. Moreover, the expression levels of ROCK1, MMP-2, MMP-9, E-cadherin, vimentin, and N-cadherin proteins in xenografts were determined by Western blot analysis.

*Statistical analysis*

Statistical analysis was performed on GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA,



**Figure 2.** miR-186 inhibits CML cell proliferation and EMT. A. SW1353 cells transfected with miR-186 mimics had significant increases in the expression of miR-186 compared with those transfected with negative control. B. L3252 cells transfected with miR-186 mimics had obvious decreases in the proliferation rate compared with the control. C, D. A562 and KCL-22 cells transfected with miR-186 mimics had dramatic increases in E-cadherin expression and marked decreases in vimentin and N-cadherin expression compared to the controls. \*P<0.05.

USA). All values were presented as mean ± SD. The differences between the two groups were analyzed using the Student's t-test, and a P-value ≤0.05 was considered statistically significant.

**Results**

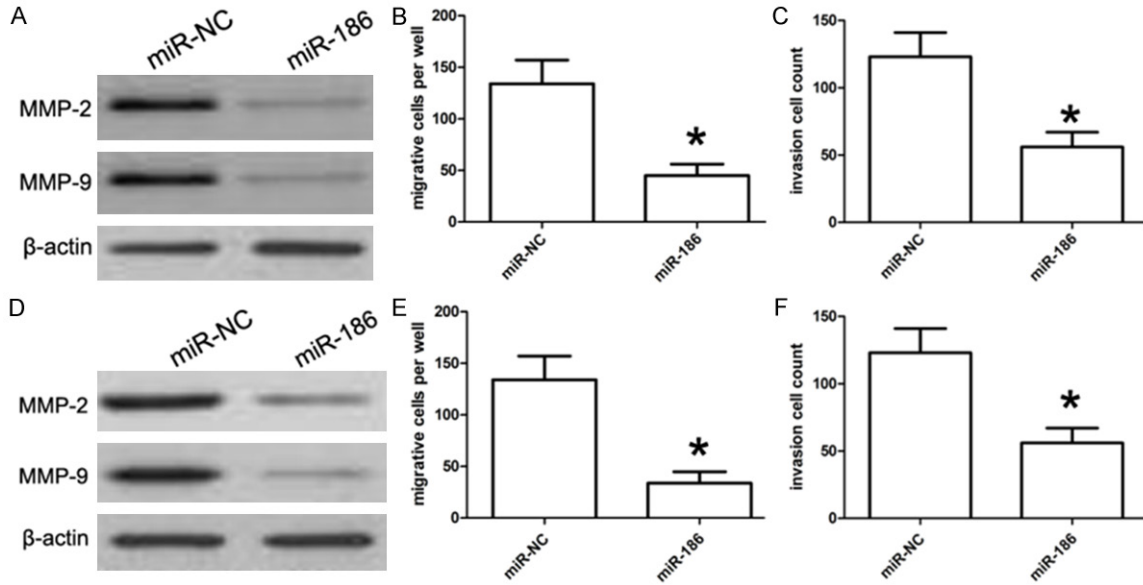
*miR-186 expression is down-regulated in CML samples and cells*

Previous studies have demonstrated that the expression of miR-186 is obviously decreased in CML samples compared to that in normal MSCs. We also tested the expression status of miR-186 in CML samples and cell lines by quantitative real-time (qRT)-PCR. Our findings revealed that the expression of miR-186 in CML cancer samples was obviously down-regulated compared with that in adjacent normal samples (Figure 1A). Similarly, the expression of miR-186 was significantly reduced in both human CML cell lines A562 and KCL-22 compared to that in normal MSCs (Figure 1B). Together with data, our data suggested that the expression of miR-186 is indeed down-regulated in CML cells.

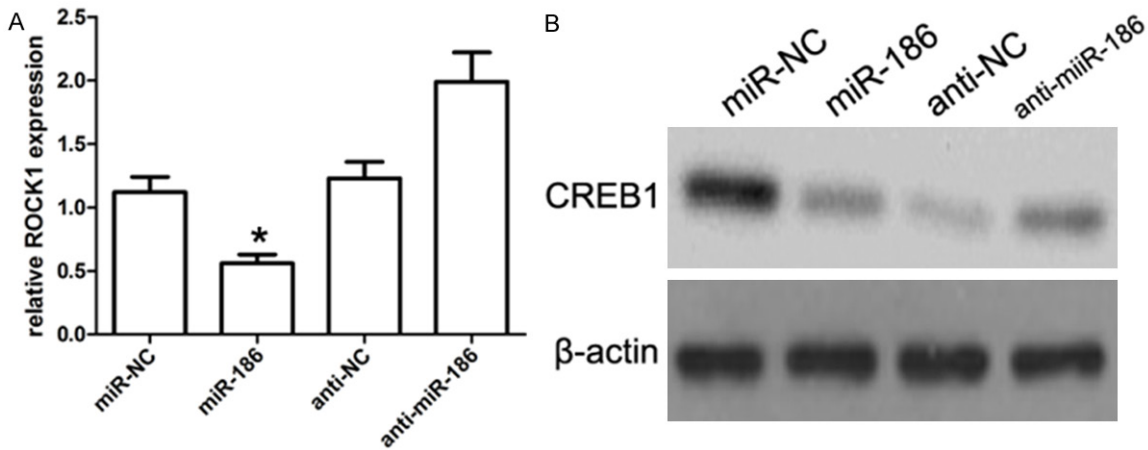
*miR-186 suppresses CML cell proliferation and EMT in vitro*

To figure out the impact of miR-186 on CML cell proliferation, A562 and KCL-22 cells were tra-

nsfected with miR-186 mimics or miR-NC, and then we detected transfection efficiency using qRT-PCR. Our findings revealed that A562 and KCL-22 cells transfected with miR-186 mimics showed a significant increase in the expression of miR-186 when compared with those cells transfected with miR-NC. Subsequently, we conducted MTT assays on A562 and KCL-22 cells transfected with miR-186 mimics or miR-NC. As shown in Figure 2A, 2B, A562 and KCL-22 cells transfected with miR-186 mimics both showed a significant reduce in cell proliferation when compared with the control miR-NC, suggesting that the over-expression of miR-186 affected in vitro cell proliferation of CML. EMT, characterized as the decrease of the epithelial marker E-cadherin and the increase of mesenchymal markers vimentin and N-cadherin, has been recommended as the major molecular mechanism of tumor metastasis. To explore whether miR-186 affects the progression of EMT of A562 and KCL-22 cells which were transfected with miR-186 mimics or miR-NC, we used Western blot assay to detect the protein expression of E-cadherin, vimentin, and N-cadherin. As shown in Figure 2C, 2D, the ectopic expression of miR-186 mimics led to a marked increase in the expression of E-cadherin and a significant decrease in the expression of vimentin and N-cadherin in A562 and KCL-22 cells, suggesting that miR-186 expression represses EMT of CML cells.



**Figure 3.** miR-186 inhibits CML cell invasion and migration. A. SW1353 cells transfected with miR-186 mimics had significant decreases in MMP-2 and MMP-9 expression. B. SW1353 cells transfected with miR-186 mimics had weak invasion capacity compared with the controls. C. Transfection with miR-186 mimics obviously reduced the migration capacity of SW1353 cells. D. L3252 cells transfected with miR-186 mimics had significant decreases in MMP-2 and MMP-9 expression. E. L3252 cells transfected with miR-186 mimics had weak invasion capacity compared with the controls. F. Transfection with miR-186 mimics obviously reduced the migration capacity of L3252 cells. \*P<0.05.

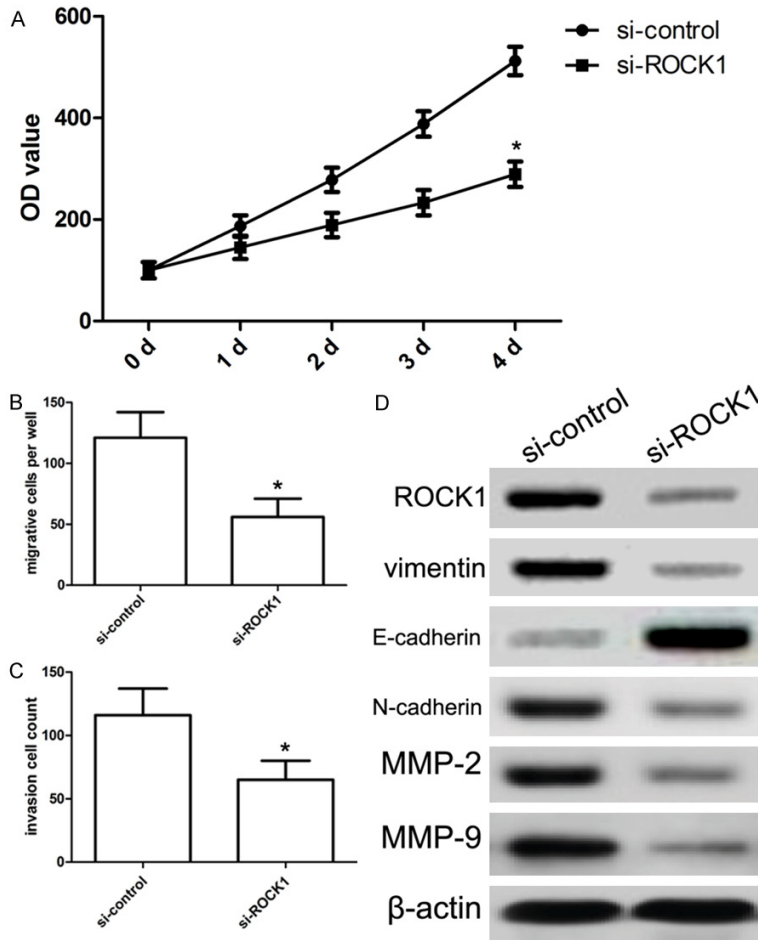


**Figure 4.** ROCK1 is a direct target of miR-186. A. Upregulation of miR-186 attenuated the expression of ROCK1 mRNA, and downregulation of miR-186 significantly enhanced the expression of ROCK1 mRNA. B. Upregulation of miR-186 repressed ROCK1 protein expression, and downregulation of miR-186 obviously increased ROCK1 protein expression. \*P<0.001.

*miR-186 affects migration and invasion of CML cells*

In order to figure out the role of miR-186 in migration and invasion of CML cells, we first detected the corresponding proteins, and found that the expression of proteins related to

cancer invasion including MMP-2 and MMP-9 in A562 and KCL-22 cells transfected with miR-186 mimics were significantly reduced than those in the controls (**Figure 3A, 3B**). To further elucidate whether miR-186 affects the migration and invasion of CML cells, both A562 and KCL-22 cells were transfected with miR-186



**Figure 5.** ROCK1 affects proliferation, migration and invasion. A. MTT assay shows that the siROCK1 group had a lower proliferation rate than the control group. B. Invasion assay shows that the number of invaded cells in the siROCK1 group remarkably decreased compared to that in the control. C. Migration assay shows that the number of migrated cells in the siROCK1 group markedly decreased compared with that in the control. D. The si-ROCK1 obviously decreased the expression of ROCK1, MMP-2, MMP-9, vimentin, and N-cadherin and significantly increased the expression of E-cadherin compared to the control. \*P<0.001.

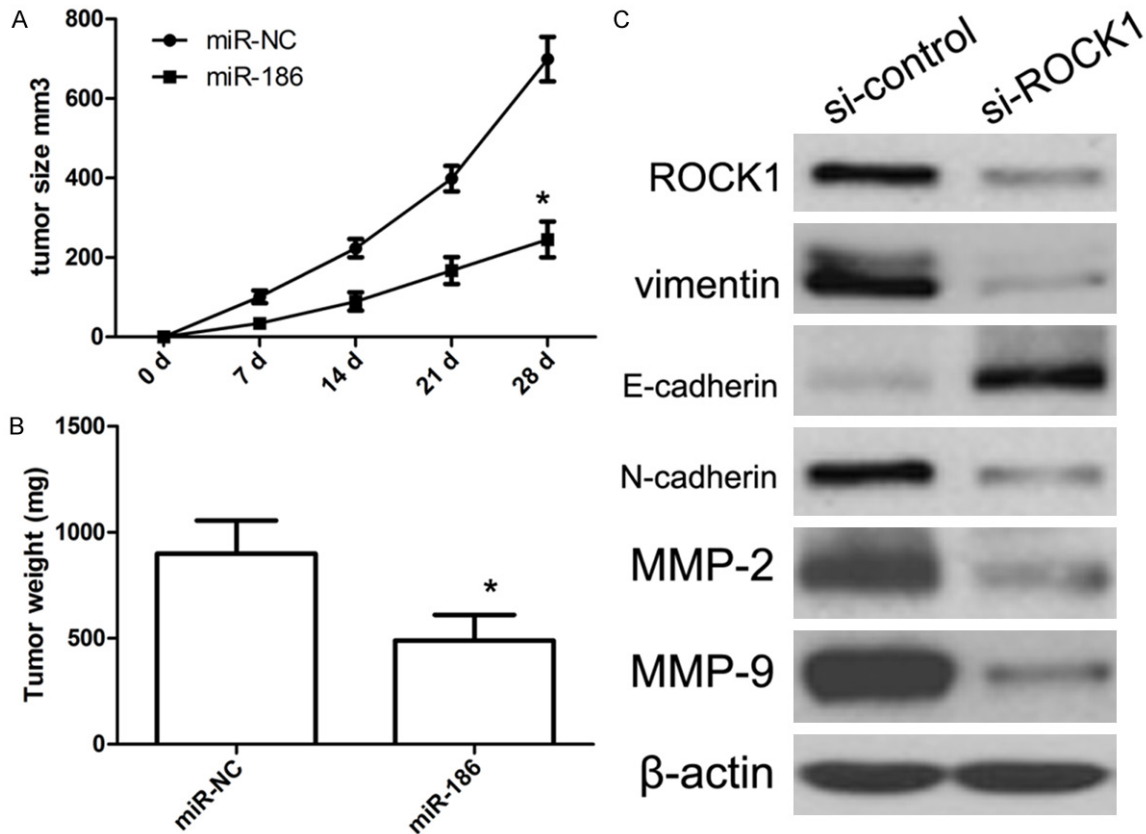
mimics or miR-NC, and then the Transwell assay was applied to conduct invasion and migration assays. As shown in **Figure 3B, 3E**, the migration capacity of A562 and KCL-22 cells transfected with miR-186 mimics was obviously decreased when compared with the A562 and KCL-22 cells transfected with miR-NC. These findings suggested that miR-186 expression repressed the migration of CML cells. Accordingly, overexpression of miR-186 also resulted in decreased invasion in A562 and KCL-22 cells (**Figure 3C, 3F**). These results suggested that the expression of miR-186 affects migration and invasion of CML cells.

*ROCK1 acts as a direct target of miR-186*

To figure out the mechanisms underlying miR-186 affects cell proliferation and EMT of CML, our team applied three bioinformatic softwares, including TargetsScan, microrna.org and miRanda, to hunt for the target gene of miR-186, and then we chose ROCK1 as a potential target. To figure out whether ROCK1 is controlled by miR-186 by directly binding to 3'UTR of ROCK1, we conducted luciferase reporter assays to clarify these questions. Our findings identified that cotransfection with miR-186 mimics significantly decreased the luciferase activity of the ROCK1 wild-type 3'UTR reporter. However, miR-186 mimics did not reduce the luciferase activity of the ROCK1 mutation 3'UTR reporter. These findings indicated that ROCK1 is indeed a direct target of miR-186. Besides, in order to illustrate the regulatory impact of miR-186 on ROCK1, our team conducted qRT-PCR and Western blot assays. We found that up-regulation of miR-186 led to a significant decrease in the expression of ROCK1 based on the mRNA and protein levels. Conversely, down-regulation of miR-186 resulted in a significant increase in the mRNA and protein of ROCK1 (**Figure 4A, 4B**). These contents suggested that the expression of ROCK1 is managed by miR-186 in the mRNA and protein levels.

Conversely, down-regulation of miR-186 resulted in a significant increase in the mRNA and protein of ROCK1 (**Figure 4A, 4B**). These contents suggested that the expression of ROCK1 is managed by miR-186 in the mRNA and protein levels.

To figure out whether the expression of miR-186 inhibits cell proliferation, migration and invasion by the regulation of ROCK1, our team transfected ROCK1 siRNAs (siROCK1) into SW1353 cells to knockdown the expression of ROCK1, and we determined transfection efficiency using Western blot. Our findings revealed that there was a significant decrease in ROCK1



**Figure 6.** The antitumor effect of miR-186 in vivo. A. The tumor volume in the miR-186 group was obviously lower than that in the control. B. After 4 weeks, the tumor weight in the miR-186 group was significantly less than that in the control. C. Compared with the control group, the miR-186 group had dramatic decreases in ROCK1, MMP-2, MMP-9, vimentin, and N-cadherin expression and an obvious increase in E-cadherin expression. \* $P < 0.001$ ;  $n = 6$ .

expression of SW1353 cells transfected with siROCK1 when compared with SW1353 cells transfected with si-control. After that, MTT, invasion, migration, and Western blot assays were carried out on SW1353 cells transfected with si-ROCK1 or si-control. MTT assay revealed that SW1353 cells transfected with si-ROCK1 led to an obvious decrease in cell proliferation (**Figure 5A**). Invasion and migration assays also revealed that invasive and migration cells were significantly reduced in the si-ROCK1 group compared with si-control (**Figure 5B, 5C**). Western blot results revealed that transfection with si-ROCK1 resulted in a remarkable reduce in the expression of MMP-2, MMP-9, vimentin and N-cadherin, and a dramatic increase in the expression of E-cadherin (**Figure 5D**). These findings suggested that si-ROCK1 repressed cell proliferation, invasion and migration of SW1353 cells. Consistent with SW1353 cells, similar effects of si-ROCK1 was observed in L3252

cells, indicating that si-ROCK1 can simulate the anti-tumor effect of miR-186.

#### *Overexpression of miR-186 affects growth of CML tumors in vivo*

Based on the previous studies, we investigated the impact of miR-186 on tumorigenicity of CML cells. In this work, SW1353 cells were transfected with miR-186 mimics or miR-NC, and then SW1353 cells were subcutaneously inoculated into the right dorsal flank of nude mice. After that, we measured and recorded the tumor size every week, and then calculated the tumor volume. We found that the tumor volume was obviously reduced in the miR-186 group compared with that in the control group (**Figure 6A**). Following 4 weeks treatment, the nude mice tumors were excised and weighed. The tumor weight in the miR-186 group was markedly reduced than that in the control group (**Figure 6B**). Additionally, the expression of

ROCK1, MMP-2, MMP-9, vimentin, and N-cadherin showed a marked decrease in the miR-186 group, while the expression of E-cadherin showed a remarkable increase compared with the control group (Figure 6C). Overall, these findings inferred that miR-186 affects cell growth of CML tumor.

### Discussion

MiRNA acts as a kind of small and non-coding RNAs, and regulates the expression of genes by binding to complementary sequences of 3'UTR of mRNA, leading to the inhibition of miRNA translation or degradation of target mRNA [6]. It has been reported that miRNA regulates the expression of about 90% of all human genes, and plays an essential role in different biological and pathological processes, involving cell proliferation, apoptosis, invasion and migration [7, 8]. In this study, we detected the expression of miR-186 in CML samples and cell lines by qRT-PCR and confirmed that the expression level of miR-186 was down-regulated in CML tissues and cell lines. Furthermore, overexpression of miR-186 significantly repressed cell proliferation, EMT, migration and invasion of CML in vitro. We next explored the molecular mechanisms by which miR-186 inhibits CML cell proliferation, EMT, migration and invasion, and observed that the effect of miR-186 on CML cell lines, at least in part depending on ROCK1 as one of direct targets. Animal experiments also confirmed that overexpression of miR-186 affected cell growth of CML tumors. These results indicate that miR-186 serves as a tumor suppressor and suppresses proliferation, EMT, and metastasis in CML.

Many studies reported that EMT plays an essential role in the initiation and development of progression of cancers. During the EMT process, epithelial cells lose their characteristics, and gain mesenchymal features, and then become more motile and invasive. Loss of epithelial marker E-cadherin and gain of mesenchymal markers N-cadherin and vimentin are regarded as the most important molecular markers of EMT. Mounting evidence showed that many miRNAs play a crucial role in the EMT of cancers, for example, miR-23a regulated TGF- $\beta$ -induced EMT in lung cancer; and miR-194 affected EMT of endometrial cancer; and miR-134 inhibits EMT in CML cells [16-18]. Our findings demonstrated that miR-186 reversed the expression level of epithelial marker and

mesenchymal markers in A562 and KCL-22 cells, by which miR-186 affected EMT. Additionally, our team also explored the impact of miR-186 on the proliferation, migration, and invasion of CML cells, and observed that miR-186 repressed A562 and KCL-22 cell proliferation, migration and invasion.

In view of previous data, we investigated the molecular mechanisms underlying miR-186-regulated CML cell proliferation, EMT. Using bioinformatics analysis, our team identified many conserved targets of miR-186, and selected ROCK1 as a potential target for further analysis. ROCK1 acts as the cAMP responsive element binding protein 1, and is a proto-oncogenic transcription factor, and involves in oncogenesis. As a potent oncogene, ROCK1 promotes tumorigenesis by affecting cell proliferation, metastasis and invasion of tumor cells [19, 20]. For example, ROCK1 was reported to be highly expressed in glioma samples, and ectopic expression of ROCK1 attenuated the miR-200b-induced growth inhibition of glioma cells [21]. Kong et al identified that oncogenic ROCK1 was increased in gastric cancer, and miR-182 targeted the ROCK1 gene to suppress the cell growth of gastric adenocarcinoma [22]. Yang et al reported that miR-433-inhibited ROCK1 expression repressed cell migration of hepatocellular cancer [23]. In this work, we performed luciferase assay and confirmed that miR-186 directly targeted ROCK1. The expression of ROCK1 at both mRNA and protein levels was regulated by miR-186, and knockdown of ROCK1 by siRNA reversed CML cell proliferation, EMT, and metastasis. These findings implied that miR-186 suppresses ROCK1 protein expression by directly binding on the 3'UTR of ROCK1 mRNA to negatively regulate CML cell proliferation, metastasis, and EMT. Furthermore, in vivo study also confirmed that overexpression of miR-186 suppressed the growth of CML tumors.

In conclusion, our findings showed that miR-186 is down-regulated in CML samples and cells; miR-186 affects cell proliferation, migration and invasion of CML cells directly by inhibiting the expression of ROCK1 by targeting the 3'UTR. Our data suggest that miR-186 is an important tumor suppressor in CML, and miR-186/ROCK1 pathway might be investigated by a therapeutic strategy for CML patients in the future.



**Disclosure of conflict of interest**

None.

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**References**

- [1] Cai J, Wu G, Tan X, Han Y, Chen C, Li C, Wang N, Zou X, Chen X, Zhou F, He D, Zhou L, Jose PA, Zeng C. Transferred BCR/ABL DNA from K562 extracellular vesicles causes chronic myeloid leukemia in immunodeficient mice. *PLoS One* 2014; 9: e105200.
- [2] Joshi D, Chandrakala S, Korgaonkar S, Ghosh K, Vundinti BR. Down-regulation of miR-199b associated with imatinib drug resistance in 9q34.1 deleted BCR/ABL positive CML patients. *Gene* 2014; 542: 109-112.
- [3] Nishioka C, Ikezoe T, Yang J, Nobumoto A, Tsuda M, Yokoyama A. Downregulation of miR-217 correlates with resistance of Ph(+) leukemia cells to ABL tyrosine kinase inhibitors. *Cancer Sci* 2014; 105: 297-307.
- [4] Karimiani EG, Marriage F, Merritt AJ, Burthem J, Byers RJ, Day PJ. Single-cell analysis of K562 cells: an imatinib-resistant subpopulation is adherent and has upregulated expression of BCR-ABL mRNA and protein. *Exp Hematol* 2014; 42: 183-191, e185.
- [5] Shibuta T, Honda E, Shiotsu H, Tanaka Y, Velasamy S, Shiratsuchi M, Umemura T. Imatinib induces demethylation of miR-203 gene: an epigenetic mechanism of anti-tumor effect of imatinib. *Leuk Res* 2013; 37: 1278-1286.
- [6] Caporali A, Emanuelli C. MicroRNA regulation in angiogenesis. *Vascul Pharmacol* 2011; 55: 79-86.
- [7] Baranwal S, Alahari SK. miRNA control of tumor cell invasion and metastasis. *Int J Cancer* 2010; 126: 1283-1290.
- [8] Hwang H, Mendell J. MicroRNAs in cell proliferation, cell death, and tumorigenesis. *Br J Cancer* 2006; 94: 776-780.
- [9] Kumar MS, Lu J, Mercer KL, Golub TR, Jacks T. Impaired microRNA processing enhances cellular transformation and tumorigenesis. *Nat Genet* 2007; 39: 673-677.
- [10] Calin GA, Croce CM. MicroRNA signatures in human cancers. *Nat Rev Cancer* 2006; 6: 857-866.
- [11] Cao M, Seike M, Soeno C, Mizutani H, Kitamura K, Minegishi Y, Noro R, Yoshimura A, Cai L, Gemma A. MiR-23a regulates TGF- $\beta$ -induced epithelial-mesenchymal transition by targeting E-cadherin in lung cancer cells. *Int J Oncol* 2012; 41: 869-875.
- [12] Li HL, Zhang BG, Shi LH, Liu NB, Lu SJ, Liu YQ, Zhang J. PTTG1 promotes migration and invasion of human non-small cell lung cancer cells and is modulated by miR-186. *Carcinogenesis* 2013; 34: 2144-2145.
- [13] Zhu X, Shen H, Yin X, Long L, Xie C. miR-186 regulation of Twist1 and ovarian cancer sensitivity to cisplatin. *Oncogene* 2015; 84: 1-10.
- [14] Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M, Stephens RM, Okamoto A, Yokota J, Tanaka T, Calin GA, Liu CG, Croce CM, Harris CC. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer Cell* 2006; 9: 189-198.
- [15] Dacic S, Kelly L, Shuai Y, Nikiforova MN. miRNA expression profiling of lung adenocarcinomas: correlation with mutational status. *Mod Pathol* 2010; 23: 1577-1582.
- [16] Cui G, Cui M, Li Y, Liang Y, Li W, Guo H, Zhao S. MiR-186 targets ROCK1 to suppress the growth and metastasis of NSCLC cells. *Tumour Biol* 2014; 35: 8933-8937.
- [17] Dong P, Kaneuchi M, Watari H, Hamada J, Sudo S, Ju J, Sakuragi N. MicroRNA-194 inhibits epithelial to mesenchymal transition of endometrial cancer cells by targeting oncogene BMI-1. *Mol Cancer* 2011; 10: 99.
- [18] Li J, Wang Y, Luo J, Fu Z, Ying J, Yu Y, Yu W. miR-134 inhibits epithelial to mesenchymal transition by targeting FOXM1 in non-small cell lung cancer cells. *FEBS Lett* 2012; 586: 3761-3765.
- [19] Conkright MD, Montminy M. CREB: the undicted cancer co-conspirator. *Trends Cell Biol* 2005; 15: 457-459.
- [20] Klemm DJ, Watson PA, Frid MG, Dempsey EC, Schaack J, Colton LA, Nesterova A, Stenmark KR, Reusch JE. cAMP response element-binding protein content is a molecular determinant of smooth muscle cell proliferation and migration. *J Biol Chem* 2001; 276: 46132-46141.
- [21] Peng B, Hu S, Jun Q, Luo D, Zhang X, Zhao H, Li D. MicroRNA-200b targets ROCK1 and suppresses cell growth in human malignant glioma. *Mol Cell Biochem* 2013; 379: 51-58.
- [22] Kong WQ, Bai R, Liu T, Cai CL, Liu M, Li X, Tang H. MicroRNA-182 targets cAMP-responsive element-binding protein 1 and suppresses cell growth in human gastric adenocarcinoma. *FEBS J* 2012; 279: 1252-1260.
- [23] Yang Z, Tsuchiya H, Zhang Y, Hartnett ME, Wang L. MicroRNA-433 inhibits liver cancer cell migration by repressing the protein expression and function of cAMP response element-binding protein. *J Biol Chem* 2013; 288: 28893-28899.