Am J Transl Res 2018;10(10):3224-3232
www.ajtr.org /ISSN:1943-8141/AJTR0080139

Original Article

The role and mechanism of miR-374 regulating the malignant transformation of mesenchymal stem cells

Zixuan Sun[1*], Jingyan Chen[1*], Jiao Zhang[1*], Runbi Ji[1,2], Wenrong Xu[1], Xu Zhang[1], Hui Qian[1]

[1]AoYoung Cancer Research Institute, Jiangsu Key Laboratory of Medical Science and Laboratory Medicine, School of Medicine, Jiangsu University, Zhenjiang 212013, Jiangsu, P. R. China; [2]Department of Clinical Laboratory Medicine, The Affiliated People's Hospital of Jiangsu University, Zhenjiang 212002, Jiangsu, P. R. China. [*]Equal contributors.

Received May 23, 2018; Accepted September 26, 2018; Epub October 15, 2018; Published October 30, 2018

Abstract: MicroRNAs (miRNAs) play important roles in cell transformation and carcinogenesis. We have previously established a tumor cell line K3 transformed from rat bone marrow-derived mesenchymal stem cells (rBM-MSCs). However, the underlying mechanism involved in MSC transformation remains unclear. Herein, we identified the key miRNAs that regulate the transformation of rBM-MSCs, and clarified their biological roles. Microarray and qRT-PCR results showed an increased expression of miR-374 but decreased expressions of miR-199a, miR-145, miR-34a, and miR-214 in K3 cells compared to rBM-MSCs. MiR-374 overexpression in rBM-MSCs increased the colony number and the proportion of the cells in S-phase. In addition, miR-374 overexpression reduced E-cadherin expression and increased N-cadherin expression in rBM-MSCs, promoting the migration ability of these cells. On the contrary, miR-374 knockdown in K3 cells led to impaired proliferation and migration capacities. Furthermore, *wnt5a* was identified as a target gene of miR-374. MiR-374 overexpression upregulated β-catenin expression in rBM-MSCs while miR-374 knockdown downregulated that in K3 cells. In conclusion, miR-374 promotes the proliferation and migration of transformed MSCs by regulating Wnt5a/β-catenin signaling pathway, which provides evidence for the contribution of miRNA to MSC transformation and suggests a new role of miR-374 in cancer development and progression.

Keywords: MSC, miR-374, *wnt5a*, malignant transformation

Introduction

Mesenchymal stem cells (MSCs) have been suggested as a key component of tumor microenvironment [1]. MSCs have the ability to promote tumor angiogenesis, growth, and metastasis. For instance, bone marrow-derived MSCs (BM-MSCs) recruited to the tumor stroma can promote osteosarcoma and hepatocellular carcinoma growth by activating PI3K/AKT and Ras/ERK pathways [2]. In colon cancer, BM-MSCs trigger the epithelial-to-mesenchymal transition (EMT) in tumor cells by surface-bound TGF-β, which favors the acquisition of an aggressive phenotype by colon cancer cells [3]. Hochane et al. found low-dose pesticide mixture could induce an oxidative stress-related senescence in normal MSCs and promote the tumorigenic phenotype in premalignant MSCs [4]. In prostate cancer, BM-MSCs stimulated by TNF-α and IFN-γ promoted cancer growth in mice via the NRF2-HIF-1α pathway [5]. Long-term cultured human BM-MSCs were reported to sustain spontaneous malignant transformation, showing enhanced proliferation ability and altered morphology and phenotype [6]. Furthermore, He et al. found the transformed MSCs expressed high levels of mutant p53 protein, leading to tumorigenesis [7]. In our previous study, we have cloned a tumor cell line K3 mutated from rat BM-MSCs (rBM-MSCs) in vivo, and isolated a fraction of cancer stem cells (CSCs) in K3 cell line [8]. However, the underlying mechanism responsible for MSC mutation has not been well characterized.

MicroRNAs (miRNAs), a class of non-coding small single-stranded molecule, have been confirmed as oncogenes or tumor suppressor genes in various cancers [9]. For example, Zeng et al. found that miR-199a/b-3p expression level decreased in gastric tissues and gastric

cell lines. MiR-199a/b-3p overexpression could inhibit cell proliferation by silencing PAK4 and inactivating PAK4/MEK/ERK signaling pathway [10]. In addition, miR-494 highly expressed in colorectal cancer targeted adenomatous polyposis coli to promote the cell growth through the regulation of Wnt/β-catenin signaling [11].

In this study, we performed microarray to analyze the differentially expressed miRNAs between rBM-MSCs and K3 cells. We clearly found an increased expression of miR-374 and decreased expressions of miR-199a, miR-145, miR-34a, miR-214, and miR-350 in K3 cells compared to rBM-MSCs. We further demonstrated that miR-374 overexpression could promote the proliferation and migration of rBM-MSCs while miR-374 knockdown led to impaired proliferation and migration capacities of K3 cells. We found that miR-374 targeted *wnt5a* to regulate β-catenin signaling pathway. Our results suggest that miR-374 might play an important role in MSC malignant transformation.

Materials and methods

Cell lines and culture conditions

RBM-MSCs and K3 cells were cultured in Dulbecco's modified Eagle's medium with low glucose (LG-DMEM, Gibco, USA) containing 10% fetal bovine serum (FBS, Bovogen, USA) in a humidified incubator with 5% CO_2 at 37°C.

Gene transfection

RBM-MSCs and K3 cells were seeded in 6-well plates (1.0×10^5 cells per well) and cultured to about 70% confluence before transfection. Then rBM-MSCs were transfected with miR-374 mimics (10 nM) while K3 cells were transfected with miR-374 inhibitors (100 nM) by using HiPerFect Transfection Reagent (Qiagen). After transfection for 48 h, the cells were used for the following experiment.

RNA isolation and real-time reverse transcription polymerase chain reaction

Total RNA was extracted from cells using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Total extracted RNA was reverse transcribed by using miScript II RT Kit (Qiagen) according to the manufacturer's instructions. Quantitative real-time PCR (qRT-PCR) was performed by using SYBR Green reaction mixture in a Bio-Rad CFX96 PCR system to detect the expression of target genes.

Western blot

The transfected cells were collected and lysed with RIPA buffer. Equal amounts of proteins were separated on 10% SDS-PAGE gel and then transferred onto polyvinylidene difluoride membranes (Millipore, USA). After blocking with 5% non-fat milk for 1 h, the membranes were incubated with primary antibodies to E-cadherin (1:200, Santa Cruz), Vimentin (1:500, CST), Wnt5a (1:500, CST), protein kinase C (PKC, 1:300, SAB), Calcium/calmodulin-dependent kinase II (CaMK II, 1:300, SAB), β-catenin (1:500, Bioworld) and GAPDH (1:1000, CST) at 4°C overnight, followed by incubation with the secondary antibody (1:2000, SAB). The signals were visualized in an ECL chemiluminescent detection system.

Cell counting assay

At 48 h post transfection, K3 cells were seeded into 24-well plates (1.0×10^4 cells per well). The cells were trypsinized and counted every day for 4 days. The results were plotted as cell growth curves.

Flow cytometric analyses of cell cycle

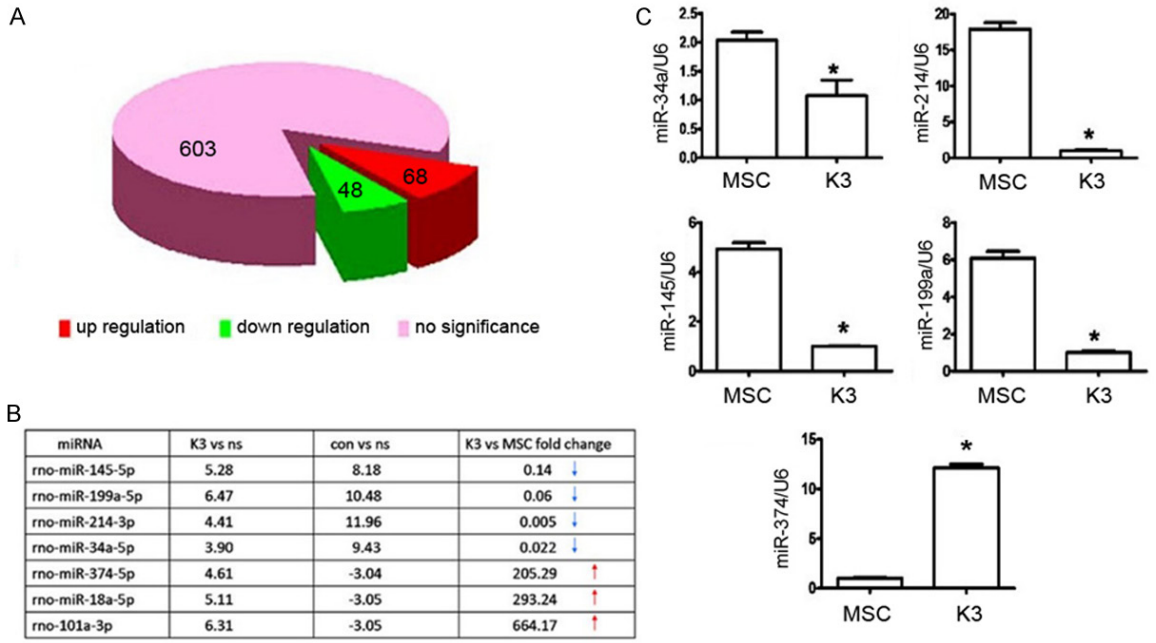
For cell cycle assay, the transfected rBM-MSCs (1.0×10^6) were harvested and washed in cold PBS twice, followed by fixation in ice-cold ethanol at 4°C overnight. The cells were stained with propidium iodide (PI) for 30 min at room temperature. The cell cycle profiles were detected by using FACS Caliber flow cytometer.

Cell colony formation assay

At 48 h post transfection, rBM-MSCs and K3 cells collected were seeded at a density of 1.0×10^3 cells/dish in a 1.5 cm cell culture dish. The medium was replaced every three days. Seven days later, the colonies were washed in cold PBS twice, fixed with 4% paraformaldehyde for 30 min, and then stained with crystal violet for 15 min. The cells were photographed and the number of colonies was counted.

Transwell migration assay

The transfected cells were trypsinized and resuspended in 200 μL serum-free medium



B

miRNA	K3 vs ns	con vs ns	K3 vs MSC fold change
rno-miR-145-5p	5.28	8.18	0.14 ↓
rno-miR-199a-5p	6.47	10.48	0.06 ↓
rno-miR-214-3p	4.41	11.96	0.005 ↓
rno-miR-34a-5p	3.90	9.43	0.022 ↓
rno-miR-374-5p	4.61	-3.04	205.29 ↑
rno-miR-18a-5p	5.11	-3.05	293.24 ↑
rno-101a-3p	6.31	-3.05	664.17 ↑

Figure 1. Differentially expressed miRNAs between rBM-MSCs and K3 cells. A. Microarray analysis of differentially expression miRNAs. B. The list of representative upregulated and downregulated miRNAs. C. QRT-PCR analysis of the expression levels of miR-34a, miR-214, miR-145, miR-199a and miR-374. Vertical bars represented SD of the mean values (n = 3). *$P < 0.05$ significantly different from MSC.

(2.0×10^4) and plated into the upper chamber (Corning, NY, USA). The lower chamber was filled with 600 μL LG-DMEM containing 10% FBS. After incubation for 10 h, the cells adhering to the lower surface membrane were fixed in 4% paraformaldehyde for 30 min, and then stained with crystal violet for 15 min. The remaining cells on the upper chamber were removed with a cotton swab. The cells were photographed and the number of migrated cells was counted.

Immunofluorescence

The transfected cells were seeded on cell slices (4.0×10^4) and cultured in 500 μL LG-DMEM containing 10% FBS. After incubation for 24 h, the cells were washed in cold PBS twice and fixed in 4% paraformaldehyde for 30 min, followed by blocking with 5% bovine serum albumin (Roche) for 30 min. The cells were incubated with antibodies against E-cadherin (1:200, Santa Cruz) and N-cadherin (1:200, SAB) at 4°C for 12 h, followed by incubation with a FITC- or PE-conjugated secondary antibody at 37°C for 30 min. The nuclei were counterstained with hoechst33342. The cells were analyzed under a fluorescent microscope.

Luciferase assay

The luciferase reporter gene vector containing wild type (WT) and mutant (MU) *wnt5a*-3'UTR were transfected into HEK293T cells along with miR-374 mimic or miR-374 negative control. At 48 h post-transfection, the luciferase activities were detected by using the Dual Luciferase Reporter Assay kit according to the manufacturer's protocol (Promega).

Statistical analysis

Data were shown as mean ± standard deviation (SD) and analyzed using GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA). Differences between measured groups were assessed using Student's *t* test. *P* values less than 0.05 were considered statistically significant.

Results

MiR-374 was upregulated in MSC-transformed tumor cell line K3

MiRNAs expression profiles of rBM-MSCs and K3 cells were determined by using Agilent Rat

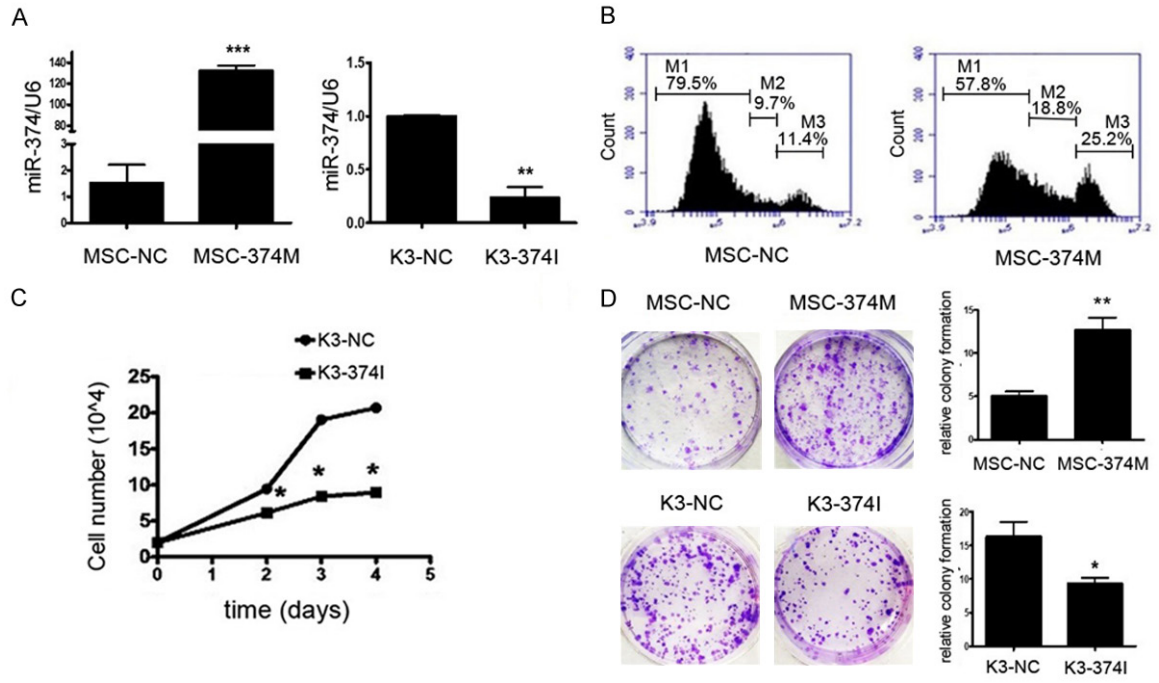


Figure 2. MiR-374 overexpression promoted the proliferation of rBM-MSCs while miR-374 knockdown inhibited that of K3 cells. A. QRT-PCR analyses of miR-374 expressions in rBM-MSCs transfected with miR-374 mimics (MSC-374M) and K3 cells transfected with miR-374 inhibitor (K3-374I), compared with the corresponding negative control (NC). B. Flow cytometric analysis of the cell cycle distribution of miR-374 mimics transfected rBM-MSCs. C. Cell counting assay of miR-374 inhibitor transfected K3 cells. D. Colony forming cell assays of the transfected rBM-MSCs and the transfected K3 cells. Vertical bars represented SD of the mean values (n = 3). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ significant difference as compared to the control group.

miRNA V19.0 microarray. The microarray results showed that a total of 116 miRNAs were differentially expressed between rBM-MSCs and K3 cells (fold change ≥ 2.0, $P < 0.05$), of which 48 genes were downregulated and 68 genes were upregulated (**Figure 1A**). We found that in K3 cells miR-145, miR-199a, miR-214 and miR-34a were significantly downregulated while miR-374, miR-18a and miR-101a were significantly upregulated (**Figure 1B**). The altered expressions of these miRNAs were further verified by using qRT-PCR. In consistence with the microarray analysis, qRT-PCR results also showed an increased expression of miR-374 in K3 cells compared to rBM-MSCs (**Figure 1C**).

MiR-374 promoted the proliferation of rBM-MSCs in vitro

We next studied the function of miR-374 in the malignant transformation of rBM-MSCs. rBM-MSCs and K3 cells were respectively transfected with miR-374 mimics and miR-374 inhibitors. The efficacy of gene transfection was verified. At 48 h post transfection, QRT-PCR results showed significant differences of miR-374 expression as compared to control groups (**Figure 2A**). The cell cycle progression of transfected cells was analyzed by using flow cytometry. We found that rBM-MSCs transfected with miR-374 mimics showed an increase in the percentage of S-phase compared to the negative control cells (**Figure 2B**). As shown in **Figure 2C**, the inhibition of miR-374 significantly retarded the growth of K3 cells compared to the negative control cells. Furthermore, the colony formation assay results showed that the transfected rBM-MSCs formed more colonies, while the transfection with miR-374 inhibitor suppressed the ability of K3 cells to form colonies (**Figure 2D**).

MiR-374 promoted the migration of rBM-MSCs and induced EMT

Then we studied the effect of miR-374 on the migration of rBM-MSCs and K3 cells. The transwell migration assay results showed that miR-374 overexpression significantly promoted the

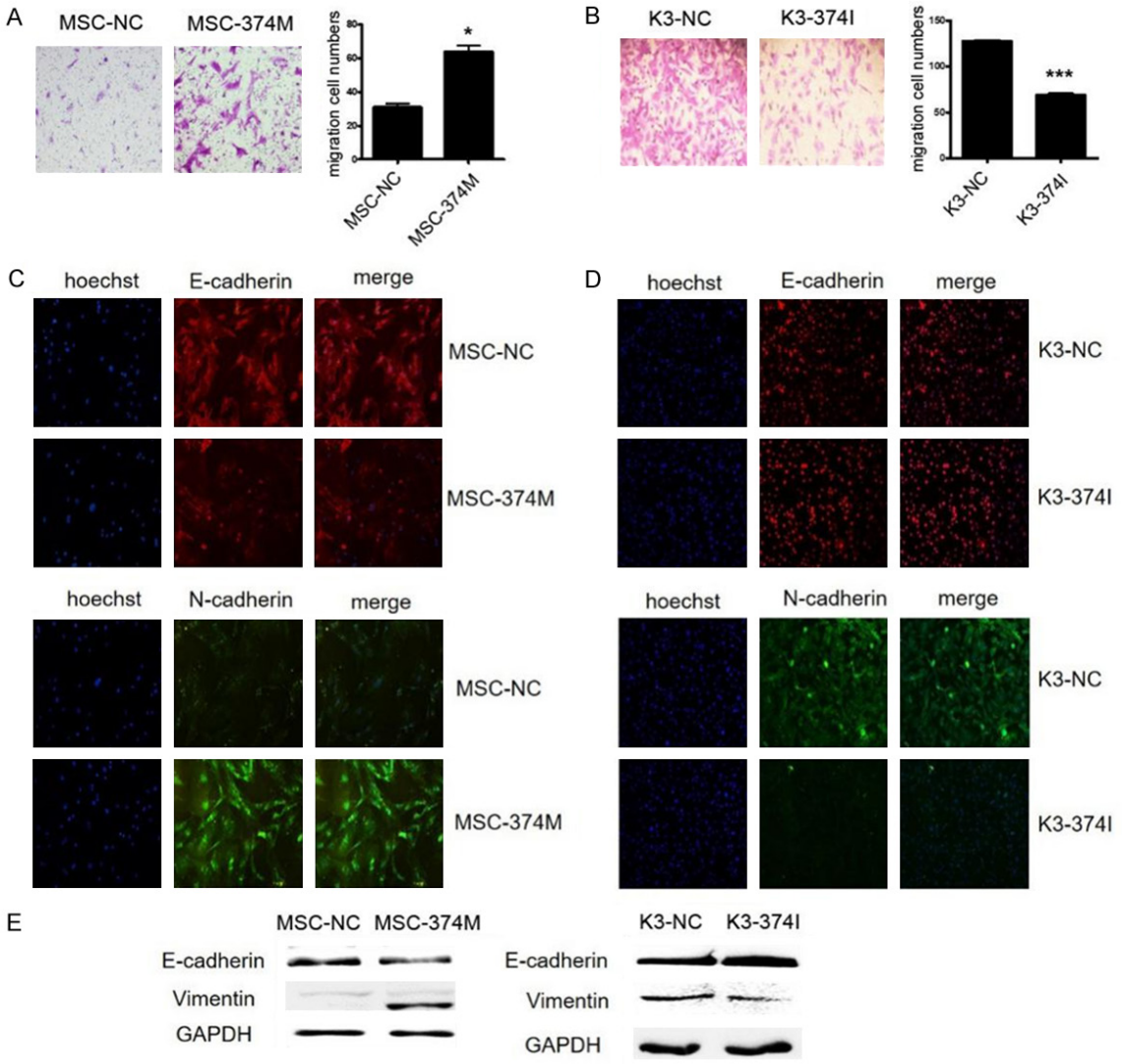


Figure 3. MiR-374 overexpression promoted the migration of rBM-MSCs while miR-374 knockdown inhibited that of K3 cells. (A, B) Transwell migration assays of miR-374 mimics transfected rBM-MSCs (A) and miR-374 inhibitor transfected K3 cells (B). (C, D) Immunofluorescent stainings of both E-cadherin and N-cadherin in the transfected rBM-MSCs (C) and the transfected K3 cells (D). (E) Western blot analyses of the expression levels of E-cadherin and vimentin in the transfected rBM-MSCs and K3 cells. Vertical bars represented SD of the mean values (n = 3). $*P < 0.05$, $***P < 0.001$ significant difference as compared to the control group.

migration of rBM-MSCs (**Figure 3A**). On the contrary, miR-374 inhibitor suppressed the migration of K3 cells (**Figure 3B**). The results of immunofluorescence staining showed that miR-374 overexpression reduced E-cadherin expression but increased N-cadherin expression in rBM-MSCs (**Figure 3C**). The transfection of miR-374 inhibitor in K3 cells showed the opposite results (**Figure 3D**). We also detected the expression of EMT-associated proteins by using western blot. We found that the expression of epithelial marker E-cadherin decreased while that of mesenchymal maker vimentin increased in miR-374 overexpressed rBM-MSCs (**Figure 3E**).

Wnt5a was a direct target of miR-374

To further study the mechanism of miR-374 regulation, we first used miRGen, TargetScan, and PicTar databases to predict the potential downstream targets for miR-374. The results showed that miR-374 could bind to the 3'-UTR of *Hivep2*, *Ap3m1*, *Ptpn12*, *Prkcd*, *Zfp423* and

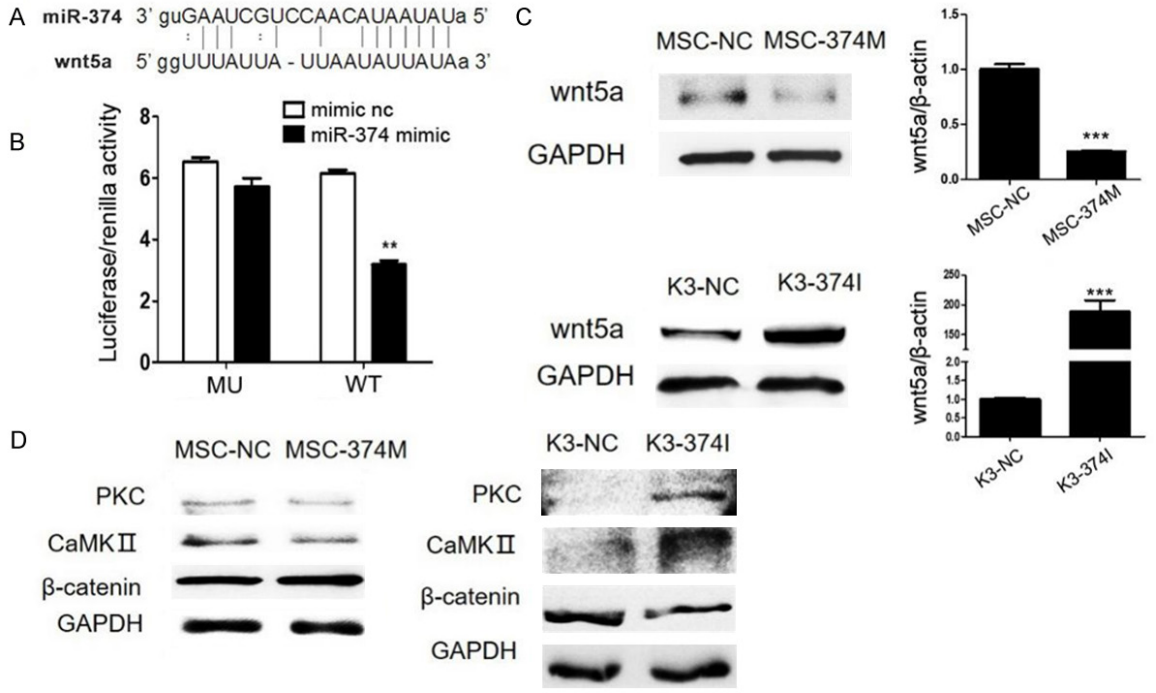


Figure 4. MiR-374 targeted *wnt5a* to regulate Wnt/Ca^{2+} cascade and Wnt/β-catenin signaling. A. Bioinformatic analysis of the binding site of the 3'-UTR of *wnt5a* mRNA for miR-374. B. Luciferase report assays of the effects of miR-374 on the activity of luciferase reporter gene vector containing wildtype (WT) and mutant (MU) 3'-UTR of *wnt5a* mRNA. C. Western blot and QRT-PCR analyses of *wnt5a* expression in miR-374 mimics transfected rBM-MSCs and miR-374 inhibitor transfected K3 cells. D. Western blot analyses of the expressions of PKC, CaMK II and β-catenin in both the transfected rBM-MSCs and the transfected K3 cells. Vertical bars represented SD of the mean values (n = 3). $^{**}P < 0.01$, $^{***}P < 0.001$ significant difference as compared to the control group.

wnt5a mRNA, all of which have been reported to be associated with cancer development and progression. Based on the previous results, we chose *wnt5a* as the research focus. The binding site of the 3'-UTR of *wnt5a* for miR-374 was shown in **Figure 4A**. The results of luciferase reporter assay showed that the luciferase activity of wild type (WT) *wnt5a* 3'UTR obviously decreased in miR-374 mimics transfected group compared to the negative control group. However, no obvious difference was observed in the luciferase activity of mutant (MU) *wnt5a* 3'UTR between miR-374 mimics transfected group and the negative control group (**Figure 4B**). Moreover, miR-374 mimics downregulated the expression of Wnt5a in rBM-MSCs, while its inhibitor upregulated that in K3 cells (**Figure 4C**).

MiR-374 might inhibit Wnt/Ca^{2+} signaling to promote cell proliferation and migration

Wnt5a, a member of noncanonical Wnt family, has been described to inhibit canonical Wnt/β-catenin signaling. Here, in our study, β-catenin expression levels increased in miR-374-overexpressing rBM-MSCs but decreased in miR-374 knockdown K3 cells (**Figure 4D**). Furthermore, in view of the ability of Wnt5a to activate Ca^{2+} signaling cascade, we chose two key proteins activated in canonical Wnt/Ca^{2+} cascade, PKC and CaMK II, to detect their expression changes. We found both of them decreased in rBM-MSCs transfected with miR-374 mimics, but increased in miR-374 inhibitor transfected K3 cells (**Figure 4D**).

Discussion

The previous studies have shown that BM-MSCs could be recruited into tumor microenvironment to promote tumor growth. In chronic myeloid leukemia, BCR-ABL1-positive microvesicles released from human leukemia cell line K562 could enhance the proliferation of BM-MSCs accompanied by TGF-β1 production and the malignant transformation of BM-MSCs [12]. There is also evidence that glioma stem-like

cells could induce BM-MSCs malignant transformation by activating TERT expression [13]. In addition, Tan et al. reported that C6 glioma-conditioned medium could induce the malignant transformation of MSCs through S100B by mediating RAGE pathway [14]. In our previous studies, we also found that a tumor cell line F6 transformed from human fetal MSCs (FMSCs) contained a population of CSCs [15], and *FHIT* gene was methylated in FMSCs-transformed F6 tumor cells [16]. Moreover, we have established a tumor cell line K3 transformed from rBM-MSCs in vivo [8]. However, the mechanism remains unclear underlying rBM-MSCs transformation and the maintenance of the malignant phenotype and function of K3 cells.

In this study, we further investigated the role of miRNAs in regulating malignant transformation of rBM-MSCs. Microarray and qRT-PCR results showed a higher expression level of miR-374 and lower expression levels of miR-199a, miR-145, miR-34a, and miR-214 in K3 cells than in rBM-MSCs. We chose miR-374 as our research focus as it had been shown to be related to cancer development and progression. Xu et al. demonstrated that miR-374a could promote cell proliferation, migration and invasion by targeting SRCIN1 in gastric cancer [17]. Li et al. suggested that miR-374a could promote hepatocellular carcinoma cell growth by targeting MIG-6 and activating AKT/ERK signaling pathway [18]. Moreover, miR-374a promoted breast cancer metastasis by activating Wnt/β-catenin signaling [19]. Here we found that miR-374 overexpression increased the proliferation of rBM-MSCs, and the inhibition of miR-374 significantly retarded the growth of K3 cells. MiR-374 overexpression also promoted the migration of rBM-MSCs and miR-374 inhibition significantly suppressed the migration of K3 cells. Consistent with the previous findings, our results suggest that miR-374 can also promote the malignant transformation of MSCs.

Wnt5a, a noncanonical Wnt signaling ligand, plays a dual role in cancer progression [20]. In breast cancer, *wnt5a* expression was upregulated by forkhead box C1, which in turn activated NF-κB signaling pathway to promote breast cell invasion [21]. Dong et al. demonstrated that R-spondin 2 suppressed *wnt5a*-regulated noncanonical Wnt signaling to inhibit colorectal cancer cell migration, invasion, and metastasis [22]. Wnt5a has also been shown to inhibit the growth, migration and invasion of CSCs. Recently, Huang et al. suggested that the dual role of Wnt5a was due to its two distinct isoforms, Wnt5a-short isoform and Wnt5a-long isoform. Knockdown of Wnt5a-short isoform, as well as reactivation of Wnt5a-long isoform, could inhibit the growth of colorectal cancer cells [23]. Wnt5a also has the ability to activate the Wnt/Ca^{2+} cascade to inhibit cell growth and migration [24]. In our study, miR-374 overexpression decreased the expression level of Wnt5a protein, as well as the expression levels of PKC and CaMK II, the two key proteins in Wnt/Ca^{2+} cascade, suggesting that miR-374 might participate in the malignant transformation of MSCs by inhibiting Wnt/Ca^{2+} canonical signaling. The tumor suppressive role of Wnt5a in cancer cell growth and migration is related to its antagonizing canonical Wnt signaling. We found that miR-374 overexpression led to an increased expression of β-catenin in rBM-MSCs while miR-374 inhibition resulted in a decreased expression of β-catenin in K3 cells, indicating that miR-374 might also be involved in MSC malignant transformation by indirectly activating Wnt/β-catenin signaling.

In conclusion, we demonstrated that the expression of miR-374 increased in the tumor cell K3 transformed from BM-MSC. MiR-374 might promote the cell growth and metastasis by inhibiting Wnt/Ca^{2+} signaling and activating Wnt/β-catenin signaling. Our findings indicated that miR-374 might play an important role in MSC malignant transformation and the development and progression of cancer.

Acknowledgements

This study was supported by National Natural Science Foundations of China (Grant No. 81572075 & 81702429), Jiangsu Province Major Projectin Research and Development (Grant No. BE2015667), Jiangsu Province Natural Science Foundation (Grant No. BK2017-0561), China Postdoctoral Science Foundation Funded Project (Grant No. 2016M591791), Jiangsu Province Postdoctoral Science Foundation Funded Project (Grant No. 1501071C), Scientific Research Foundation of Jiangsu University (Grant No. 13JDG025), and Priority Academic Programme Development of Jiangsu Higher Education Institutions.

Disclosure of conflict of interest

None.

Address correspondence to: Hui Qian and Xu Zhang, AoYoung Cancer Research Institute, Jiangsu Key Laboratory of Medical Science and Laboratory Medicine, School of Medicine, Jiangsu University, Zhenjiang 212013, Jiangsu, P. R. China. Fax: 86-511-85038483; E-mail: lstmmmlst@163.com (HQ); xuzhang@ujs.edu.cn (XZ)

References

[1] Bergfeld SA and Declerck YA. Bone marrow-derived mesenchymal stem cells and the tumor microenvironment. Cancer Metastasis Rev 2010; 29: 249-261.

[2] Fontanella R, Pelagalli A, Nardelli A, Nardelli A, D'Alterio C, Ieranò C, Cerchia L, Lucarelli E, Scala S and Zannetti A. A novel antagonist of CXCR4 prevents bone marrow-derived mesenchymal stem cell-mediated osteosarcoma and hepatocellular carcinoma cell migration and invasion. Cancer Lett 2015; 370: 100-107.

[3] Mele V, Muraro MG, Calabrese D, Pfaff D, Amatruda N, Amicarella F, Kvinlaug B, Bocelli-Tyndall C, Martin I, Resink TJ, Heberer M, Oertli D, Terracciano L, Spagnoli GC and Iezzi G. Mesenchymal stromal cells induce epithelial-to-mesenchymal transition in human colorectal cancer cells through the expression of surface-bound TGF-β. Int J Cancer 2014; 134: 2583-2594.

[4] Hochane M, Trichet V, Pecqueur C, Avril P, Oliver L, Denis J, Brion R, Amiaud J, Pineau A, Naveilhan P, Heymann D, Vallette FM and Olivier C. Low-dose pesticide mixture induces senescence in normal mesenchymal stem cells (MSC) and promotes tumorigenic phenotype in premalignant MSC. Stem Cells 2017; 35: 800-811.

[5] Yang KQ, Liu Y, Huang QH, Mo N, Zhang QY, Meng QG and Cheng JW. Bone marrow-derived mesenchymal stem cells induced by inflammatory cytokines produce angiogenetic factors and promote prostate cancer growth. BMC Cancer 2017; 17: 878-887.

[6] Røsland GV, Svendsen A, Torsvik A, Sobala E, McCormack E, Immervoll H, Mysliwietz J, Tonn JC, Goldbrunner R, Lønning PE, Bjerkvig R and Schichor C. Long-term cultures of bone marrow-derived human mesenchymal stem cells frequently undergo spontaneous. Cancer Res 2009; 69: 5331-5339.

[7] He L, Zhao F, Zheng Y, Wan Y and Song J. Loss of interactions between p53 and survivin gene in mesenchymalstem cells after spontaneous transformation in vitro. Int J Biochem Cell Biol 2016; 75: 74-84.

[8] Qian H, Ding X, Zhang J, Mao F, Sun Z, Jia H, Yin L, Wang M, Zhang X, Zhang B, Yan Y, Zhu W and Xu W. Cancer stemness and metastatic potential of the novel tumor cell line K3: an inner mutated cell of bone marrow-derived mesenchymal stem cells. Oncotarget 2017; 13: 39522-39533.

[9] Zhou K, Liu M and Cao Y. New insight into microRNA functions in cancer: oncogene-microRNA-tumor suppressor gene network. Front Mol Biosci 2017; 46: 1-7.

[10] Zeng B, Shi W and Tan G. MiR-199a/b-3p inhibits gastric cancer cell proliferation via down-regulating PAK4/MEK/ERK signaling pathway. BMC Cancer 2018; 18: 36-40.

[11] Zhang Y, Guo L, Li Y, Feng GH, Teng F, Li W and Zhou Q. MicroRNA-494 promotes cancer progression and targets adenomatous polyposis coli in colorectal cancer. Mol Cancer 2018; 17: 1-11.

[12] Fu FF, Zhu XJ, Wang HX, Zhang LM, Yuan GL, Chen ZC and Li QB. BCR-ABL1-positive microvesicles malignantly transform human bone marrow mesenchymal stem cells in vitro. Avta Pharmacol Sin 2017; 38: 1475-1485.

[13] Zhao Y, Chen J, Dai X, Cai H, Ji X, Sheng Y, Liu H, Yang L, Chen Y, Xi D, Sheng M, Xue Y, Shi J, Liu J, Li X and Dong J. Human glioma stem-like cells induce malignant transformation of bone marrow mesenchymal stem cells by activating TERT expression. Oncotarget 2017; 8: 104418-104429.

[14] Tan B, Shen L, Yang K, Huang D, Li X, Li Y, Zhao L, Chen J, Yi Q, Xu H, Tian J and Zhu J. C6 glioma-conditioned medium induces malignant transformation of mesenchymal stem cells: possible role of S100B/RAGE pathway. Biochem Biophys Res Commun 2017; 495: 78-85.

[15] Xu X, Qian H, Zhu W, Zhang X, Yan Y, Wang M and Xu W. Isolation of cancer stem cells from transformed human mesenchymal stem cell line F6. J Mol Med 2010; 88: 1181-1190.

[16] Xu XJ, Gao S, Wang M, Qian H, Gu GY, Zhang K and Xu WR. Methylation status of the FHIT gene in the transformed human mesenchymal F6 stem cell line. Oncol Lett 2015; 9: 2661-2666.

[17] Xu X, Wang W, Su N, Zhu X, Yao J, Gao W, Hu Z and Sun Y. miR-374a promotes cell proliferation, migration and invasion by targeting SRCIN1 in gastric cancer. FEBS Lett 2015; 589: 407-413.

[18] Li H, Chen H, Wang H, Dong Y, Yin M, Zhang L and Wei J. MicroRNA-374a promotes hepatocellular carcinoma cell proliferation by targeting mitogen-inducible gene-6 (MIG-6). Oncol Res 2017; [Epub ahead of print].

[19] Cai J, Guan H, Fang L, Yang Y, Zhu X, Yuan J, Wu J and Li M. MicroRNA-374a activates Wnt/β-catenin signaling to promote breast cancer metastasis. J Clin Invest 2013; 123: 566-579.

[20] Asem MS, Buechler S, Wates RB, Miller DL and Stack MS. Wnt5a signaling in cancer. Cancers (Basel) 2016; 8: 79-96.

[21] Han B, Zhou B, Qu Y, Gao B, Xu Y, Chung S, Tanaka H, Yang W, Giuliano AE and Cui X. FOXC1-induced non-canonical WNT5A-MMP7 signaling regulates invasiveness in triple-negative breast cancer. Oncogene 2018; 37: 1399-1408.

[22] Dong X, Liao W, Zhang L, Tu X, Hu J, Chen T, Dai X, Xiong Y, Liang W, Ding C, Liu R, Dai J, Wang O, Lu L and Lu X. RSPO2 suppresses colorectal cancer metastasis by counteracting the Wnt5a/Fzd7-driven noncanonical Wnt pathway. Cancer Lett 2017; 402: 153-165.

[23] Huang TC, Lee PT, Wu MH, Huang CC, Ko CY, Lee YC, Lin DY, Cheng YW and Lee KH. Distinct roles and differential expression levels of Wnt5a mRNA isoforms in colorectal cancer cells. PLoS One 2017; 12: e0181034.

[24] Cheng R, Sun B, Liu Z, Zhao X, Qi L, Li Y and Gu Q. Wnt5a suppresses colon cancer by inhibiting cell proliferation and epithelial-mesenchymal transition. J Cell Physiol 2014; 229: 1908-1917.