MEDICAL SCIENCE MONITOR

LAB/IN VITRO RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2016; 22: 1946-1952
DOI: 10.12659/MSM.896551

Received: 2015.11.04
Accepted: 2015.11.26
Published: 2016.06.08

MicroRNA-200b Impacts Breast Cancer Cell Migration and Invasion by Regulating Ezrin-Radixin-Moesin

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BC 1 Hong Hong*
CD 1 Haizhong Yu*
DE 1 Jianfen Yuan
EF 1 Chunyan Guo
FG 1 Hongyan Cao
CF 1 Weibing Li
ABCD 2 Chunhong Xiao

1 Department of Clinical Laboratory, Nantong Traditional Chinese Medicine Hospital, Nantong, Jiangsu, P.R. China
2 Department of Clinical Laboratory, Nantong Tumor Hospital, Nantong, Jiangsu, P.R. China

* These authors contributed equally to this work

Corresponding Author: Chunhong Xiao, e-mail: chunhognxiaooo@yeah.net

Source of support: The present study was supported by the Youth Fund of Nantong, Jiangsu Province (grant no. WQ2014059)

Background: Ezrin-radixin-moesin (ERM) plays an important role in multiple links of tumors. It also involved in breast cancer invasion and metastasis, and might be a potential biomarker of breast cancer. Another study suggested that ERM expression was regulated directly by miR-200c, and had a critical role in miR-200c suppressing cell migration. This study aimed to investigate the effect of miR-200b on ERM expression in a breast cancer cell line and its influence on invasion and metastasis ability *in vitro*.

Material/Methods: Breast cancer cell lines MCF-7 and MDA-MB-231 with different metastatic potentials were selected as a model. MiR-200b overexpression or inhibition was achieved by Lipofectamine™ 2000-mediated miRNA transfection. RT-PCR was used to test miR-200b level, while Western blot was selected to detect ERM protein expression. Wound healing assay and Transwell assay were performed to determine cell migration and invasion ability.

Results: RT-PCR revealed that miR-200b level in MDA-MB-231 was obviously lower than that in MCF-7, while Western blot analysis showed that ERM expression was significantly higher. MiR-200b inhibition by transfection in MCF-7 markedly decreased miR-200b level, elevated ERM expression, and enhanced cell migration and invasion. MiR-200b overexpression in MDA-MB-231 obviously increased miR-200b level, reduced ERM expression, and weakened cell migration and invasion.

Conclusions: MiR-200b participates in breast cancer cell migration and invasion through regulating ERM in MCF-7 and MDA-MB-231.

MeSH Keywords: Amlodipine • Neuroectodermal Tumors, Primitive, Peripheral • Nuclear Receptor Coactivator 3

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/896551

1952 — 4 24

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Background

Breast cancer is a common malignant tumor in clinical practice and its incidence is increasing. Early detection and treatment is the only way to improve the survival rate. Breast cancer cells have strong invasive ability and are prone to distant metastases [1], which is a major cause of treatment failure and high mortality [2]. Breast cancer migration and invasion involves the interaction between cancer cell movement ability and host environment. It is a complex non-random process with multiple steps and links [3–5].

MicroRNAs are a popular focus in tumor research [6] and provide a new direction for investigating the mechanism of breast cancer invasion and metastasis. They are common in eukaryotic organisms, with lengths of 17 to 25 nucleotides. MicroRNAs can specifically identify target mRNA and match with 3'-UTR to regulate at the posttranscriptional level. They are involved in various physiological and pathological processes, such as cell proliferation, invasion, and metastasis [7]. It was found that miRNAs are abnormally expressed in a variety of solid cancers and can promote or inhibit tumorigenesis [8]. Recently, the role of the miR-200 family in cancer has been a research focus. It was found to be closely related to pancreatic cancer, ovarian cancer, cervical cancer, and breast cancer [9]. Research shows that miR-200 can upregulate cadherin to reduce tumor migration and invasion through inhibiting transcription factor ZEB1 and ZEB2 expression in epithelial cells [10,11].

Ezrin-radixin-moesin (ERM) plays important role in the maintenance of cytoskeleton structure and cells movement [12]. As a member of the ERM family, ERM is the connexin between the epithelial cell membrane and cytoskeleton, and has critical effects on cell division, deformation, and movement [13]. Research shows that ERM plays an important role in multiple links of tumor development. It also involved in breast cancer invasion and metastasis and may be a potential biomarker [14]. Previous research showed that ERM expression is directly regulated by miR-200c and plays an important role in miR-200c inhibiting cell migration [15].

Breast cancer has strong invasive ability, which is the major cause of treatment failure and high mortality [2]. Breast cancer metastasis is a non-random, complex process with multi-steps and multi-links, of which cancer cell migration ability is the main influencing factor. Cell actin cytoskeleton plays a key role in migration, and the ERM family has received the most extensive research attention [16]. As a member of the ERM family, ERM is the connection between the epithelial cell membrane and cytoskeleton protein, and is also an important part of the special membrane structure (apical microvilli, plate, or filopodia) [17]. A clinical study suggested that ERM is overexpressed in breast cancer and other tumor cells [18]. Research showed that ERM plays a key role in tumor invasion and metastasis, and a high level of ERM facilitates tumor invasion and metastasis. Ni et al. found that ERM expression level was positively correlated with breast cancer invasion and metastasis ability [19]. Its invasive ability may be enhanced through epithelial-mesenchymal transformation to promote MMP-9 secretion.

MicroRNAs are a popular topic in tumor research. As a type of small non-coding single-stranded RNA, it exists widely in eukaryotes, with a length of 17 to 25 nucleotides. It is involved in various physiological and pathological processes, such as cell proliferation, invasion, and metastasis [6]. miRNAs are abnormally expressed in a variety of solid cancers and can promote or inhibit tumorigenesis. Recently, the role of the miR-200 family in cancer had been a research focus. Jun discovered that miR-200c expression was positively correlated with E-cadherin in pancreatic cancer specimens, and patients with high miR-200 level had higher survival rates [20]. Bendoraite et al. found similar results in ovarian cancer [21]. Mnay and Sun's studies revealed that miR-200 expression in epithelial cells can upregulate E-cadherin to reduce tumor migration and invasion through inhibiting transcription factor ZEB1 and ZEB2 expression in epithelial cells [10,11]. In addition, Burk reported that miR-200 had target gene TGF-β2, miR-200 can downregulate ZEB1/2 and TGF-β2, and ZEB1/2 and TGF-β2 also can downregulate miR-200 [22]. These 3 factors provide feedback to each other to regulate cancer cell migration and invasion. This study used breast cancer cell lines MCF-7 and MDA-MB-231 as a model to observe the effect of miR-200b on ERM expression, migration, and invasion in MCF-7 and MDA-MB-231.

Material and Methods

Experimental materials

Breast cancer cell lines MCF-7 and MDA-MB-231 were purchased from Bio-Rad. RPMI 1640, high-glucose DMEM, and fetal bovine serum were purchased from Hyclone. Penicillin-streptomycin and trypsin were obtained from Gibco. Lipofectamine™2000 was obtained from Invitrogen. MiR-200b RT-PCR primers, miR-200b mimic and inhibitor, and negative control were from RiboBio. RT-PCR-related reagents (RNA extraction reagent, reverse transcription kit, and SYBR kit) were purchased from TAKARA. Rabbit antihuman ERM primary antibody was from Cell Signaling. β-actin was from Santa Cruz. Matrigel was from BD and Transwell chamber was from Corning Costar.

Cell culture

MCF-7 cells were cultured in RPMI 1640 medium, and MDA-MB-231 cells were maintained in DMEM containing 10% FBS and 1% penicillin-streptomycin. The cells were passaged at 1:2

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

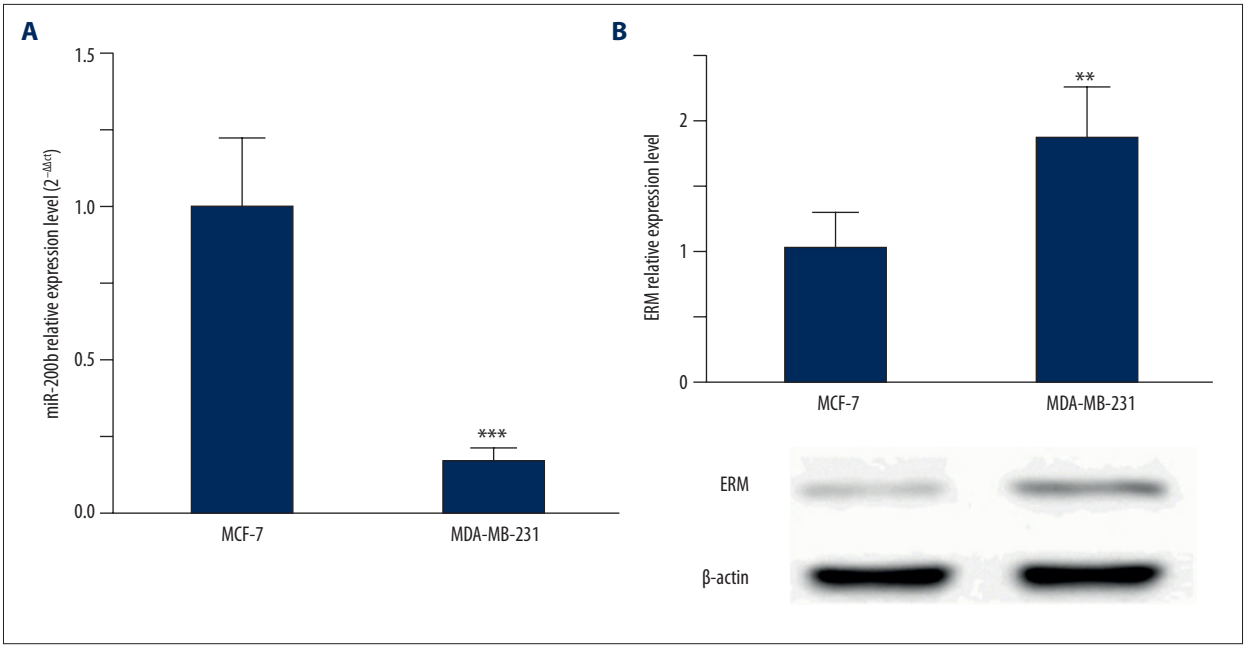

Figure 1. MiR-200b was overexpressed and ERM was decreased in MDA-MB-231 cells compared with MCF-7 cells. (**A**) miR-200b relative expression level. (**B**) ERM protein relative expression. ** P<0.01, *** P<0.001, compared with MCF-7.

or 1:3 using trypsin when the fusion reached 90%. The cells in logarithm phase were used for experimentation.

Cell transfection

The cells were seeded in 6-well plates and transfected with miR-200b mimic or inhibitor using lipofectamine™2000 according to the manufacturer's instructions. RNA was extracted at 24 h after transfection. RT-PCR was used to verify miR-200b expression. Wound healing test and Transwell assay were also performed simultaneously. Protein was collected to test ERM expression at 48 h after transfection.

RT-PCR

The cells were collected at 24 h after transfection by Trizol. Total RNA was extracted by phenol chloroform method. The purity and concentration were determined by ultraviolet spectrometry. RNA was reverse transcribed to cDNA to perform RT-PCR. The primers sequences were as follows: miR-200b, forward 5' UAAUACUGCCUGGUAAUGAUGA3', reverse 5' AUCAUUACCAGGCAGUAUAAAU3'; U6, forward 5' CTCGCTTCGGCAGCACA3', reverse 5' AACGCTTCACGAATTTGCGT3'. RT-PCR was performed on Step One real-time PCR amplifier to calculate Ct value and copy number. RT-PCR condition included 95°C for 20 s, followed by 45 cycles of 95°C for 5 s and 60°C for 30 s. U6 was selected as a reference. The results are presented as $2^{-\Delta\Delta Ct}$.

Western blot

Total protein was extracted using RIPA at 48 h after transfection. Its concentration was determined by BCA. Using SDS-PAGE, 20 μg of protein was separated and transferred to an NC membrane. After blocking with 5% skim milk for 2 h, the membrane was incubated with ERM (1:1000) and β-actin (1:2000) primary antibodies at 4°C overnight. Then the membrane was incubated in HRP-tagged secondary antibody (1:5000) at room temperature for 2 h after washing in TBST. ECL was used to develop the protein band. Gel imaging system software was used to analyze band density to calculate ERM relative expression. β-actin was selected as reference.

Cell migration assay

Wound healing assay was used to test cell migration. The cells were seeded in 12-well plates and transfected. A 200-μl tip was used to scratch the well. At 0, 12, or 24 h after scratching, cell healing state was observed under an inverted phase contrast microscope (×100). Three random fields of view were selected to test cell migration by measuring the width of scratches by using Image J software. The experiment was repeated 3 times.

Cell invasion assay

FBS-free medium was mixed with Matrigel at 3:1. We added 40 μl of mixture to the upper chamber at 37°C for 1 h. We added 200 μl of cells in FBS-free medium containing 0.1% BSA at 1×10^5/mL to the upper chamber, while medium containing

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

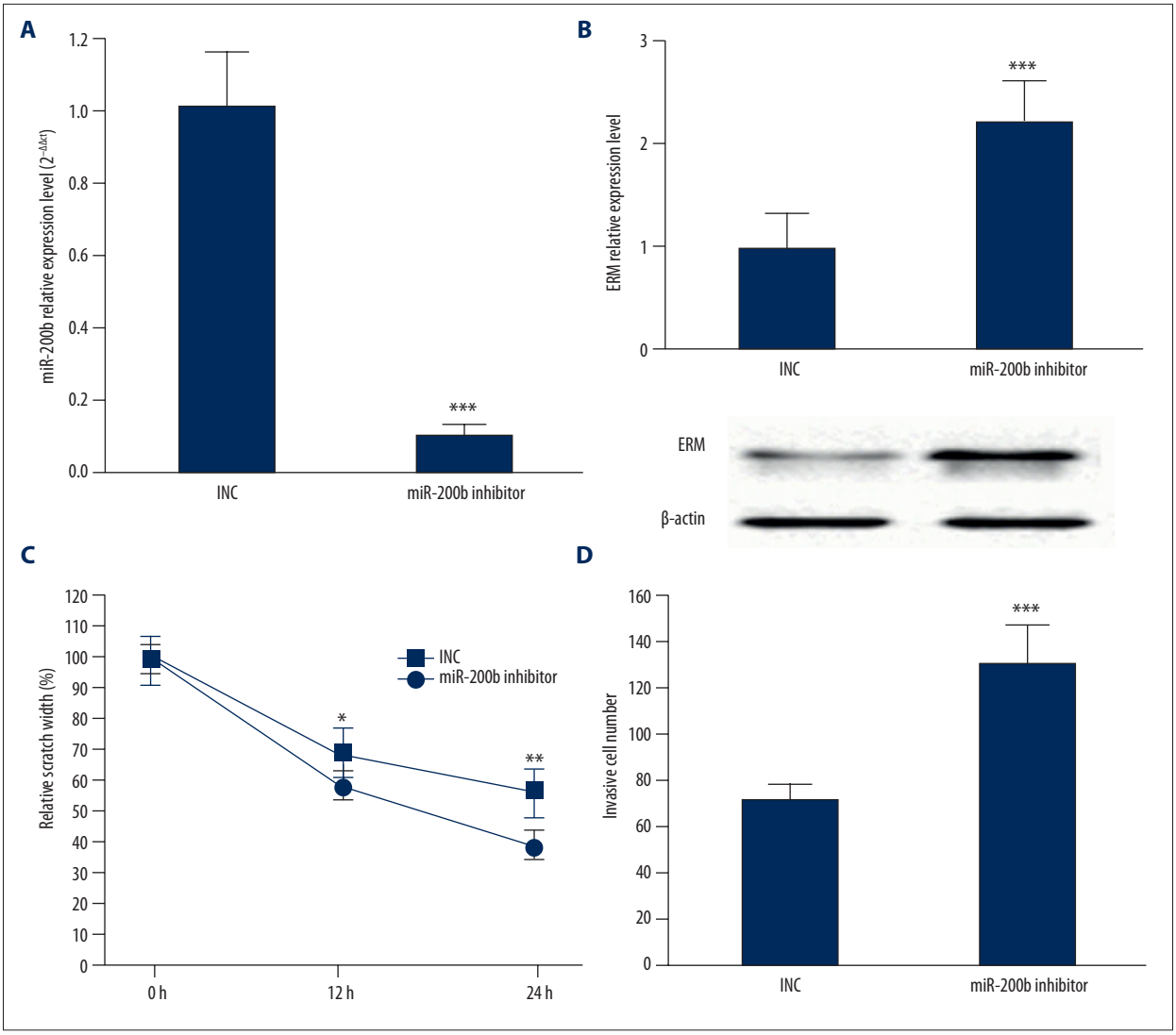

Figure 2. MiR-200b inhibition enhanced MCF-7 cells invasion. (**A**) miR-200b relative expression level. (**B**) ERM protein expression level. (**C**) Relative scratch width. (**D**) Invasive cell number. * P<0.05, ** P<0.01, *** P<0.001, compared with INC.

10% FBS was added to the lower chamber. After 4-h incubation, the cells were fixed with 70% paraformaldehyde for 15 min and stained by 0.1% crystal violet for 30 min. Six random fields of view were selected under an optical microscope to calculate the cell number. The experiment was repeated 3 times.

Statistical analysis

All statistical analysis was performed on SPSS19.0. The data are presented as mean±standard deviation ($\bar{\chi}\pm s$). The *t* test was used for comparison. P<0.05 was considered as statistical significance.

Results

MiR-200b and ERM expression in breast cancer cell line

RT-PCR revealed that the miR-200b expression level in MCF-7 (1.01±0.21) was significantly higher than that in MDA-MB-231 (0.17±0.05) (P<0.05, Figure 1A). Western blot analysis showed that the ERM level in MDA-MB-231 (1.87±0.39) was obviously higher than that in MCF-7 (1.03±0.27) (Figure 1B).

MiR-200b suppression effect in MCF-7

RT-PCR confirmed that the miR-200b level in MCF-7 was obviously lower after transfection (Figure 2A). Western blot analysis showed that compared with negative control (0.99±0.37), ERM increased markedly after miR-200b inhibition (2.21±0.42)

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

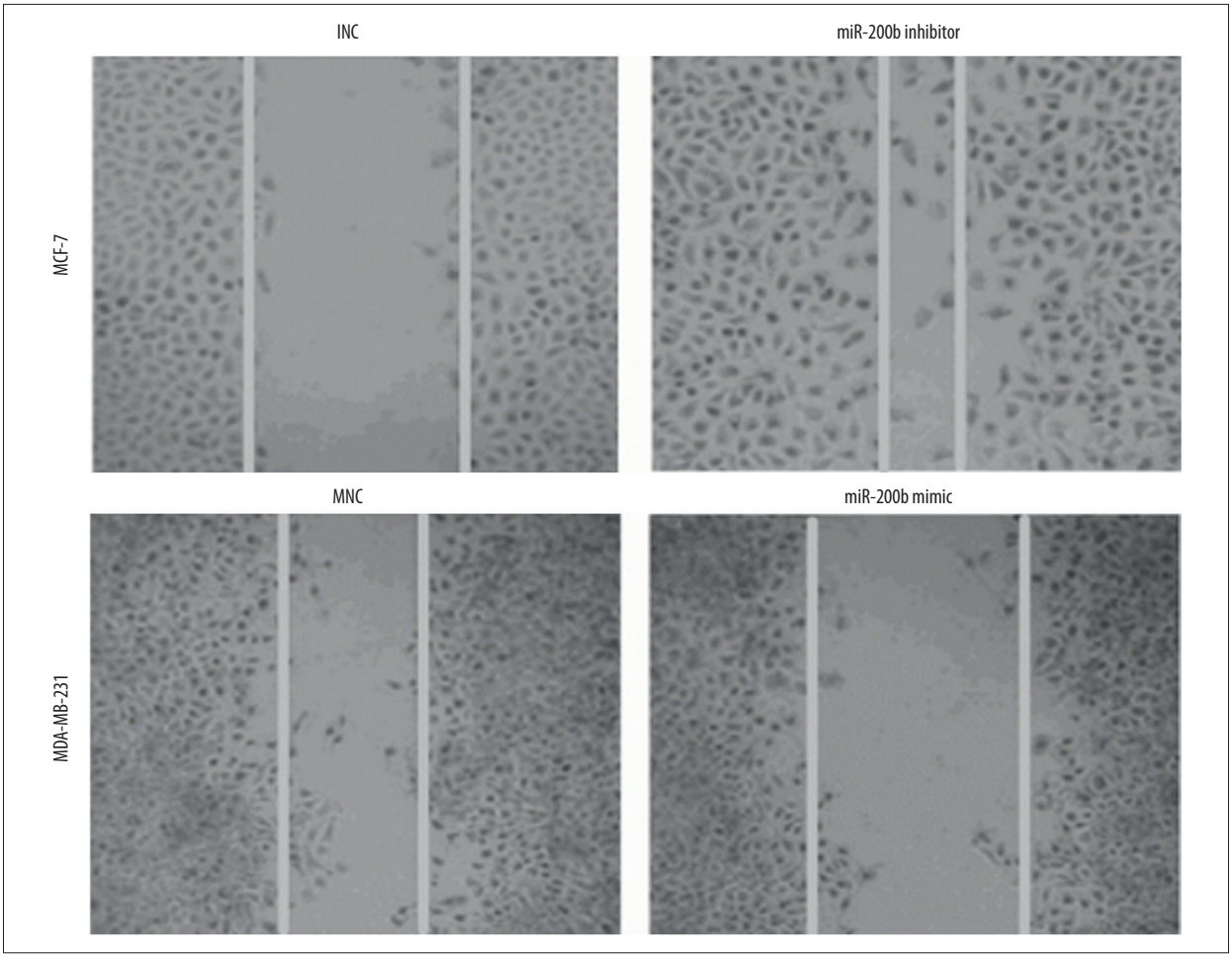

Figure 3. MiR-200b effect on MCF-7 and MDA-MB-231 migration ability (24-h).

(Figure 2B). MCF-7 was further cultured for 12 h and 24 h after miR-200b inhibition. Relative scratch width results showed that it obviously decreased in the miR-200b inhibition group (59.17±5.31, 39.50±6.83)% compared with control (68.33±7.23, 57.18±6.04)%, suggesting that migration ability was enhanced after miR-200b inhibition (Figures 2C, 3). Transwell assay also revealed that after miR-200b inhibition, MCF-7 cell number passing through the membrane at 24 h (131.67±17.41) was significantly higher than that in the negative control (73.01±6.72), indicating that miR-200b inhibition enhanced invasive ability (Figure 2D).

MiR-200b overexpression impact in MDA-MB-231

RT-PCR confirmed that miR-200b level in MDA-MB-231 was significantly elevated after transfection (Figure 4A). Western blot analysis revealed that compared with negative control (1.07±0.21), ERM declined markedly after miR-200b overexpression (0.62±0.23) (Figure 4B). MDA-MB-231 was further cultured for 12 h and 24 h after miR-200b overexpression. Relative scratch width results showed that it was obviously increased in the miR-200b overexpression group (70.17±6.51, 59.50±5.24)% compared with control (56.33±8.10, 47.32±6.22)%, indicating that migration ability weakened after miR-200b overexpression (Figures 4C, 3). Transwell assay also showed that after miR-200b overexpression, the number of MDA-MB-231 cells passing through the membrane at 24 h (114.32±7.98) was significantly lower than that in the negative control (114.32±7.98), indicating that miR-200b inhibition enhanced invasive ability (Figure 4D).

Discussion

Breast cancer is the most common malignant tumor in females and its incidence rate is rising. We confirmed that the MDA-MB-231 cell line has significantly stronger migration and invasion ability than the MCF-7 cell line by wound healing assay and Transwell assay. Western blot analysis showed that ERM expression in MCF-7 and MDA-MB-231 have different metastasis potentials. ERM expression in highly aggressive MDA-MB-231 was obviously higher than that in MCF-7 with low aggressiveness, consistent with results reported by Carmeci et al. [23]. We also discovered that miR-200b inhibition in MCF-7 led to

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

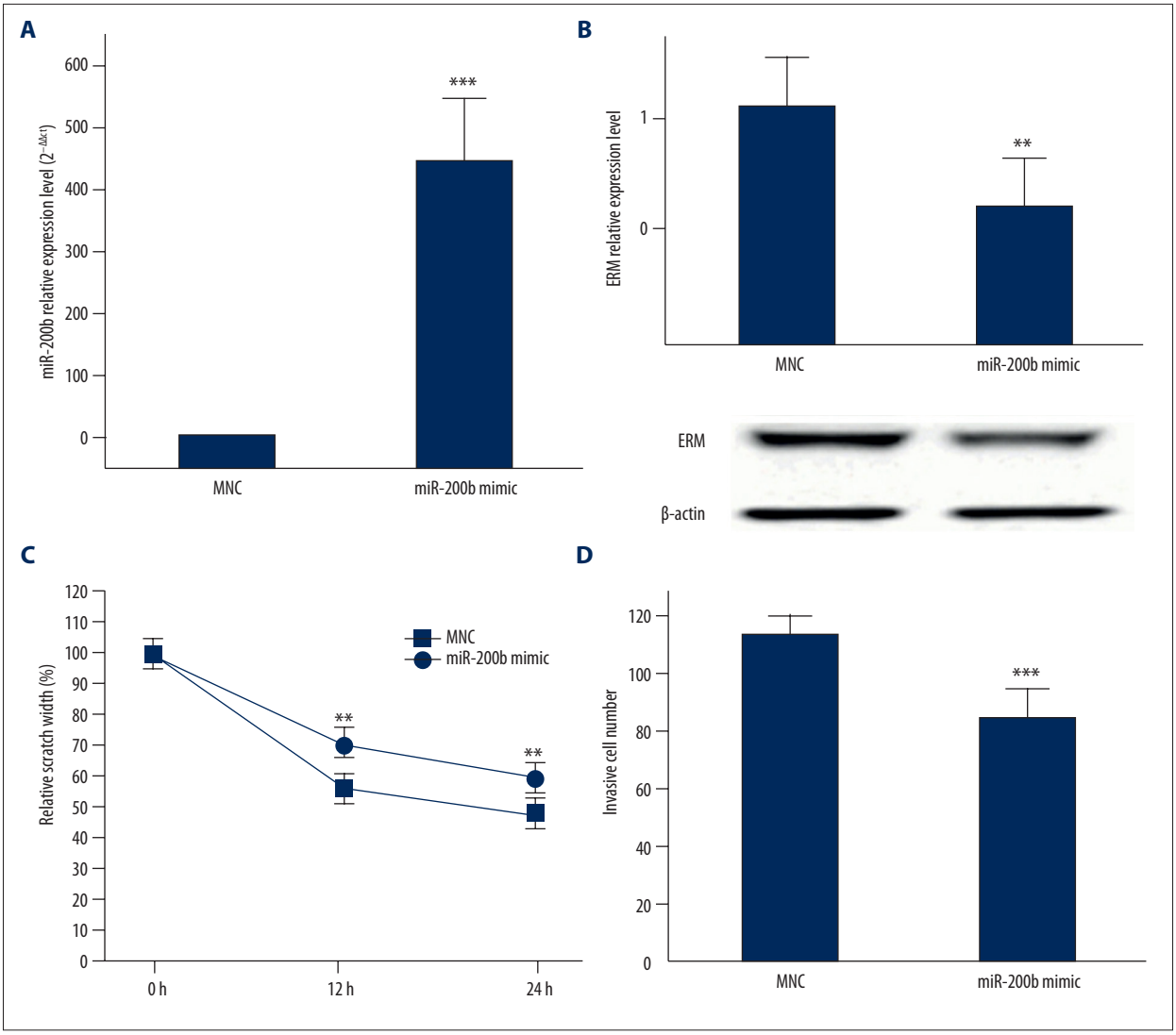

Figure 4. MiR-200b overexpression suppressed MDA-MB-231 invasion. (**A**) miR-200b relative expression level. (B) ERM protein expression level. (**C**) Relative scratch width. (**D**) Invasive cell number. * P<0.01,*** P<0.001, compared with INC.

ERM overexpression and promoted cancer cell migration and invasion, further verifying the role of ERM in breast cancer cell migration and invasion. Detecting ERM expression can help to study the biological behavior of breast cancer. We tested miR-200b in MCF-7 and MDA-MB-231 cell lines with different metastatic potentials, and found that its level in MCF-7 was obviously higher than that in MDA-MB-231, showing that miR-200b plays an important role in tumor cell migration and invasion.

We observed the effects of miR-200b inhibition in MCF-7 and miR-200b overexpression in MDA-MB-231. Western blot analysis revealed that miR-200b inhibition in MCF-7 significantly upregulated ERM protein expression, while miR-200b overexpression in MDA-MB-231 clearly reduced ERM level. Wound healing assay and Transwell assay showed that miR-200b inhibition enhanced breast cancer cell migration and invasion, whereas miR-200b overexpression weakened this ability. Our results are similar to those of Li et al. [24], who confirmed that ERM is a target gene of miR-200b on mRNA 3'-UTR. MiR-200b can directly regulate ERM expression and participates in breast cancer metastasis.

Conclusions

This study confirms that miR-200b might be an upstream regulatory factor of ERM. It can regulate ERM expression in breast cancer, thus influencing the biological behavior of breast cancer cells. We hope that our results will provide important assistance in exploring the mechanism of breast cancer migration and invasion, and in developing new treatment methods and improving prognosis.

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

References:

1. Cole M, Parajuli S, Laske D et al: Peripheral primitive neuroectodermal tumor of the dura in a 51-year-old woman following intensive treatment for breast cancer. Am J Case Rep, 2014; 15: 294–99
2. Jemal A, Bray F, Center MM et al: Global cancer statistics. Cancer J Clin, 2011; 61: 69–90
3. Mego M, Mani SA, Cristofanilli M: Molecular mechanisms of metastasis in breast cancer – clinical applications. Nat Rev Clin Oncol, 2010; 7: 693–701
4. Han J, Gao B, Jin X et al: Small interfering RNA-mediated downregulation of beta-catenin inhibits invasion and migration of colon cancer cells *in vitro*. Med Sci Monit, 2012; 18: BR273–80
5. Han DP, Zhu QL, Cui JT et al: Polo-like kinase 1 is overexpressed in colorectal cancer and participates in the migration and invasion of colorectal cancer cells. Med Sci Monit, 2012; 18(6): BR237–46
6. Cheng Q, Yi B, Wang A, Jiang X: Exploring and exploiting the fundamental role of microRNAs in tumor pathogenesis. Onco Targets Ther, 2013; 6: 1675–84
7. Gaur A, Jewell DA, Liang Y et al: Characterization of microRNA expression levels and their biological correlates in human cancer cell lines. Cancer Res, 2007; 67: 2456–68
8. Iorio MV, Croce CM: microRNA involvement in human cancer. Carcinogenesis, 2012; 33: 1126–33
9. Bojmar L, Karlsson E, Ellegard S et al: The role of microRNA-200 in progression of human colorectal and breast cancer. PLoS One, 2013; 8: e84815
10. Korpal M, Lee ES, Hu G, Kang Y: The miR-200 family inhibits epithelial-mesenchymal transition and cancer cell migration by direct targeting of E-cadherin transcriptional repressors ZEB1 and ZEB2. J Biol Chem, 2008; 283: 14910–14
11. Park SM, Gaur AB, Lengyel E, Peter ME: The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. Genes Dev, 2008; 22: 894–907
12. Kobayashi H, Sagara J, Kurita H et al: Clinical significance of cellular distribution of moesin in patients with oral squamous cell carcinoma. Clin Cancer Res, 2004; 10: 572–80
13. Fehon RG, McClatchey AI, Bretscher A: Organizing the cell cortex: the role of ERM proteins. Nat Rev Mol Cell Biol, 2010; 11: 276–87
14. Charafe-Jauffret E1, Monville F, Bertucci F et al: Moesin expression is a marker of basal breast carcinomas. Int J Cancer, 2007; 121: 1779–85
15. Howe EN, Cochrane DR, Richer JK: Targets of miR-200c mediate suppression of cell motility and anoikis resistance. Breast Cancer Res, 2011; 13: R45
16. Lallemand D, Arpin M: Moesin/ezrin: a specific role in cell metastasis? Pigment Cell Melanoma Res, 2010; 23: 6–7
17. Baumgartner M, Sillman AL, Blackwood EM et al: The Nck-interacting kinase phosphorylates ERM proteins for formation of lamellipodium by growth factors. Proc Natl Acad Sci USA, 2006; 103: 13391–96
18. Wang CC, Liau JY, Lu YS et al: Differential expression of moesin in breast cancers and its implication in epithelial-mesenchymal transition. Histopathology, 2012; 61: 78–87
19. NI Xiang SZ-m: Relationship between moesin expression and breast cancer metastasis. Fudan University Journal of Medical Sciences, 2013; 40
20. Yu J OK, Mizumoto K: MicroRNA, hsa-miR-200c, is an independent prognostic factor in pancreatic cancer and its upregulation inhibits pancreatic cancer invasion but increases cell proliferation. Mol Cancer, 2010; 9: 1–10
21. Bendoraite A, Knouf EC, Garg KS et al: Regulation of miR-200 family microRNAs and ZEB transcription factors in ovarian cancer: evidence supporting a mesothelial-to-epithelial transition. Gynecol Oncol, 2010; 116: 117–25
22. Burk U, Schubert J, Wellner U et al: A reciprocal repression between ZEB1 and members of the miR-200 family promotes EMT and invasion in cancer cells. EMBO Rep, 2008; 9: 582–89
23. Carmeci C, Thompson DA, Kuang WW et al: Moesin expression is associated with the estrogen receptor-negative breast cancer phenotype. Surgery, 1998; 124: 211–17
24. Li X, Roslan S, Johnstone CN et al: MiR-200 can repress breast cancer metastasis through ZEB1-independent but moesin-dependent pathways. Oncogene, 2014; 33: 4077–88

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]