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Original Article

MicroRNA-138 inhibits proliferation and migration of breast cancer cells by targeting c-Met

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Abstract: MicroRNAs (miRNAs) have been considered as critical post-transcriptional regulators involved in multiple biological processes. Previous studies demonstrated that microRNA-138 (miR-138) plays an important role in the pathogenesis of cancers, but its role in breast cancers has not been elucidated. In the present study, the expression of miR-138 was determined by real time PCR. MTT assay, wound healing assay, and colony formation assay were used to measure cell proliferation, migration and colony formation, respectively. The expression of c-Met was assessed by real time PCR and western blot. Luciferase assay was performed to validate the potential targets of miR-138. Results showed that the expression of miR-138 was significantly down-regulated in both breast cancer tissues and cell lines. Moreover, over-expression of miR-138 inhibited cell proliferation and migration in breast cancer cells. In addition, miR-138 up-regulation resulted in a reduction in c-Met expression, whereas inhibition of miR-138 enhanced c-Met level in breast cancer cells. The luciferase reporter assay further suggested that c-Met is a direct target of miR-138 in breast cancer cells. Taken together, our study demonstrated that miR-138 suppressed cell proliferation and migration via c-Met, providing a novel target for the molecular treatment of breast cancer.

Keywords: Breast cancer, cell proliferation, miR-138, c-Met

Introduction

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among females, accounting for 23% of the total cancer cases and 14% of the cancer deaths [1, 2]. Although genetic and epigenetic regulation contributes breast cancer, the molecular mechanism underlying carcinogenesis is not fully elucidated [3]. Thus, further understanding of the molecular mechanisms underlying breast cancer initiation and progression is urgently needed.

In the past few years, small regulatory RNAs had drawn enormous attention in cancer research. MicroRNAs (miRNAs) are a class of 18 to 25 nucleotides single-stranded non-coding RNA, and can regulate genes expression at the post-transcriptional level [4]. Accumulating evidences suggest that miRNAs play a diverse role in regulation of tumor initiation, progression and molecular targeted therapy by directly targeting oncogenes or tumor suppressor genes [5, 6]. Among many miRNAs, microRNA-138 (miR-138) stands out as an important entity. miR-138 has been reported to be down-regulated in different cancers, such as aggressive papillary thyroid carcinoma, lung cancer, and head and neck squamous cell carcinoma [7, 8]. Functional study suggested that miR-138 could inhibit cell migration and invasion by targeting RhoC and ROCK2 in tongue squamous cell carcinoma cell lines [9]. In addition, a recent study demonstrated that ectopic expression of miR-138 attenuated the FAK, Src and Erk 1/2 signaling pathways via down-regulation of RhoC in head and neck squamous cell carcinoma [10]. However, the biological role of miR-138 in breast cancer has not been reported. Thus, our current study aimed to elucidate the role of miR-138 in breast cancer as well as its underlying mechanism.

Material and methods

Clinical samples

A total of 23 human breast cancer tissues and matched normal adjacent breast tissues were

obtained during the surgery in Zaozhuang Municipal Hospital. None of the patients received chemotherapy or radiotherapy before the surgery. Written informed consent was obtained from all participants. This study was performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the local hospitals.

Cell culture and transfection

Human breast cancer cell lines (MCF-7, MDA-MB-231, SKBR3 and Bcap-37) and normal human mammary epithelial cell line MCF10A were obtained from American Type Culture Collection and cultured in DMEM (Thermo Scientific HyClone, Beijing, China) supplemented with 10% fetal calf serum, 100 IU/ml penicillin and 100 mg/ml streptomycin (Gibco, Beijing, China). Cells were incubated at 37°C and 5% CO_2 in a humidified incubator. Oligonucleotide siRNA duplex was synthesized by Shanghai Gene Pharma Corporation (Shanghai, China). The siRNA transfection was carried out with Lipofectamine 2,000 (Invitrogen), according to the manufacturer's instructions.

RNA extraction and real time PCR

Total RNA was isolated according to the standard procedure using TRIzol reagent (Invitrogen, Carlsbad, CA). The expression of miR-138 was determined using a miRNA-specific TaqMan MiRNA Assay Kit (Applied Biosystems). PCR reactions were performed on ABI 7,500 Real-Time PCR System (Applied Biosystems) with the following conditions: 95°C, 10 min for 1 cycle, then 95°C, 15 sec, 60°C, 1 min for 40 cycles. The U6 small nuclear RNA was used as a loading control. The mRNA expression of c-Met was measured by real time PCR on ABI 7,500 Real-Time PCR System with GAPDH used as control.

Cell proliferation assay

Cell proliferation was measured by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay. To be brief, cultured cells were seeded into 96-well plates at the density of 4×10^4 (cells/well). Then 10 ml of 5 mg/ml MTT was added and incubated in dark at 37°C for another 2 h. The absorbance was determined with the wavelength of 490 nm.

Cell migration assay

Cell migration was measured using an *in vitro* wound healing assay. To be brief, cells were cultured in six-well plates and experimental wounds were made by dragging a rubber Policeman™ (Fisher Scientific, Hampton, NH) across the cell culture. The cultures were rinsed with PBS and placed in fresh quiescence medium. Three wounds were created for each sample and the relative distance traveled by the cells was measured.

Colony formation assay

For colony formation assays, 5.0×10^2 cells were plated onto 6-well plates and transfected with miR-138 or negative control. When cells grew to visible colonies, the colonies were washed with PBS and fixed with 4% paraformaldehyde for 20 min. Finally, cells were stained with crystal violet and the colony numbers per well were counted.

Western blot

Total cell lysate was extracted and then separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). After transferring the proteins onto nitrocellulose membranes, the membranes were blocked with 5% non-fat dry milk at room temperature. Then the membranes were incubated overnight with mouse anti-human c-Met monoclonal antibody (Abcam, USA). After incubation with primary antibody, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody (Abcam, USA). Finally, protein bands were detected with enhanced chemiluminescence (ECL) kit (Thermo Scientific, Waltham, MA, USA).

Luciferase activity assay

Luciferase reporters were generated based on the firefly luciferase expressing vector pMIR-REPORT (Ambion, USA). Cells were seeded in 24-well plates at the density of 5×10^4 cells per well the day before transfection. Luciferase reporter (500 ng), 50 pmol (miRNA-138 mimic, inhibitor or negative control) and 40 ng of pRL-TK were added in each well. Cells were collected 48 h after transfection and analyzed using the Dual-Luciferase Reporter Assay System (Promega, USA).

Statistical analysis

All data are presented as mean ± SD. For comparisons between two groups, statistical significance was determined using the Student's

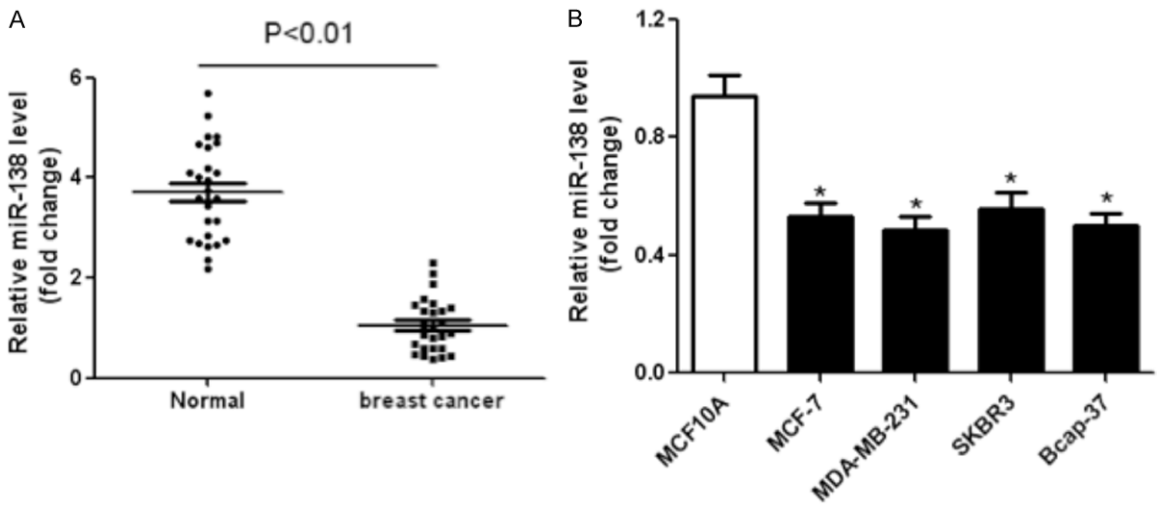

Figure 1. Down-regulation of miR-138 in breast cancer tissues and cell lines. A. Real time PCR was used to determine the expression of miR-138 in human breast cancer tissues and adjacent non-tumor tissues. *P<0.05; **P<0.01. B. The normal mammary epithelial cell line MCF10A and four breast cancer cells lines including MCF-7, MDA-MB-231, SKBR3 and Bcap-37 were analyzed by real time-PCR to measure the miR-138 level. *P<0.05; **P<0.01.

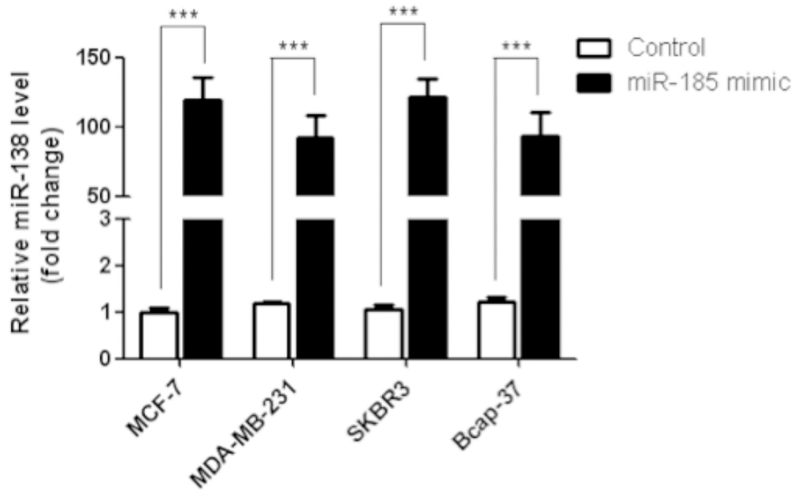

Figure 2. Overexpression of miR-138 in breast cancer cells. Breast cancer cell lines including MCF-7, MDA-MB-231, SKBR3 and Bcap-37 were transfected with miR-138 mimic or negative control. Then, the expression of miR-138 was determined by real time PCR. *P<0.05; **P<0.01.

Figure 1A, miR-138 expression was markedly decreased in cancer tissues compared with the matched non-tumor samples. In addition, the expression levels of miR-138 in breast cancer cell lines including MCF-7, MDA-MB-231, SKBR3 and Bcap-37 were examined. Compared with normal mammary epithelial cell line MCF10A, miR-138 exhibited reduced expression in four breast cancer cells lines (**Figure 1B**). These data indicate that miR-138 is down-regulated in breast cancer tissues and cell lines.

t-test. Comparisons among three or more groups were performed using analysis of variance (ANOVA). A value of $P<0.05$ was considered significant.

Results

Down-regulation of miR-138 in breast cancer tissue

Firstly, we assessed the expression of miR-138 in 23 paired breast cancer and adjacent non-tumor tissues by real-time PCR. As shown in

MiR-138 inhibits cell proliferation and migration in breast cancer cells

To elucidate the function of miR-138 in breast cancer, we transfected miR-138 mimic into breast cancer cell lines (MCF-7, MDA-MB-231, SKBR3 and Bcap-37) and then determined the effects on cell growth and migration. As shown in **Figure 2**, transfection of miR-138 oligonucleotides into tumor cells significantly enhanced the expression of miR-138 in four breast cancer cell lines. In addition, we found that ecto-

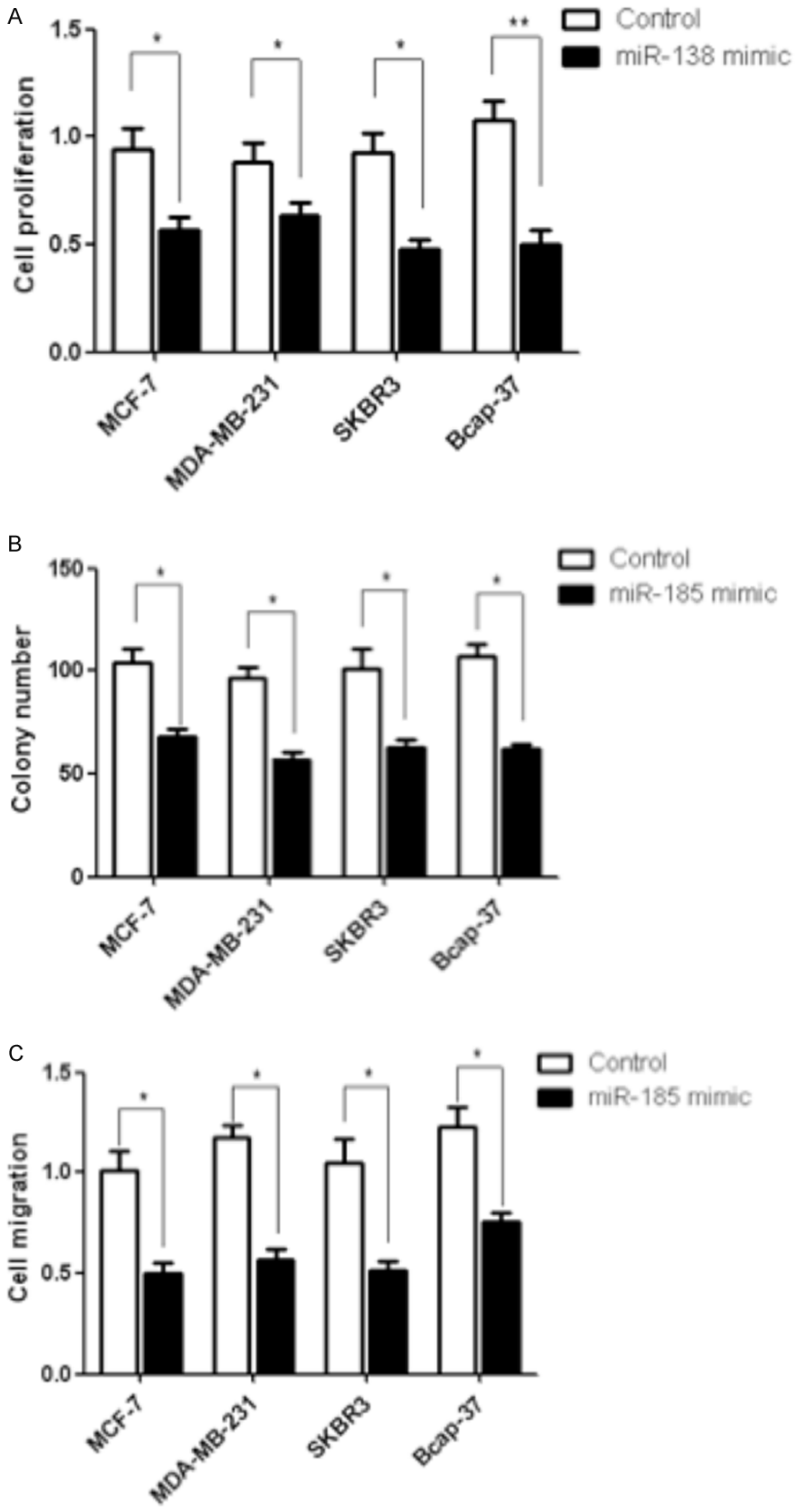

Figure 3. MiR-138 inhibits cell proliferation and migration in breast cancer cells. Four breast cancer cells lines including MCF-7, MDA-MB-231, SKBR3 and Bcap-37 were transfected with miR-138 mimic or negative control. 48 h after transfection, MTT assay, colony formation assay, and wound healing assay were performed to measure cell proliferation (A), colony formation (B) and migration (C). *P<0.05; **P<0.01.

pic expression of miR-138 obviously suppressed cell prolieration and colony formation in breast cancer cells as shown by MTT assay (**Figure 3A**) and colony formation assay (**Figure 3B**). Furthermore, up-regulation of miR-138 significantly inhibited the metastatic potential of breast cancer cells (**Figure 3C**). Taken together, these results demonstrate that miR-138 serves as a tumor suppressive miRNA in breast cancer cells.

c-Met is a target of miRNA-138 in breast cancer cells

To explore the molecular mechanism by which miRNA-138 suppressed breast cancer cells growth and migration, we searched for putative miRNA-138 targets using the web-based programs and found 3' UTR of c-Met containing the conserved putative miRNA-138 binding sites. To determine whether miR-138 targeted c-Met *in vitro*, we transfected MCF-7 and MDA-MB-231 cells with miR-138 mimic or inhibitor and assessed c-Met mRNA expression with real time PCR. Our results showed that ectopic expression of miR-138 led to a dramatic reduction in c-Met mRNA expression, whereas inhibition of miR-138 significantly promoted the mRNA expression of c-Met in MCF-7 (**Figure 4A**) and MDA-MB-231 cells (**Figure 4B**). Consistently, western blot analysis indicated that transfection with miR-138 mimic significantly reduced c-Met protein expression, while inhibition of miR-

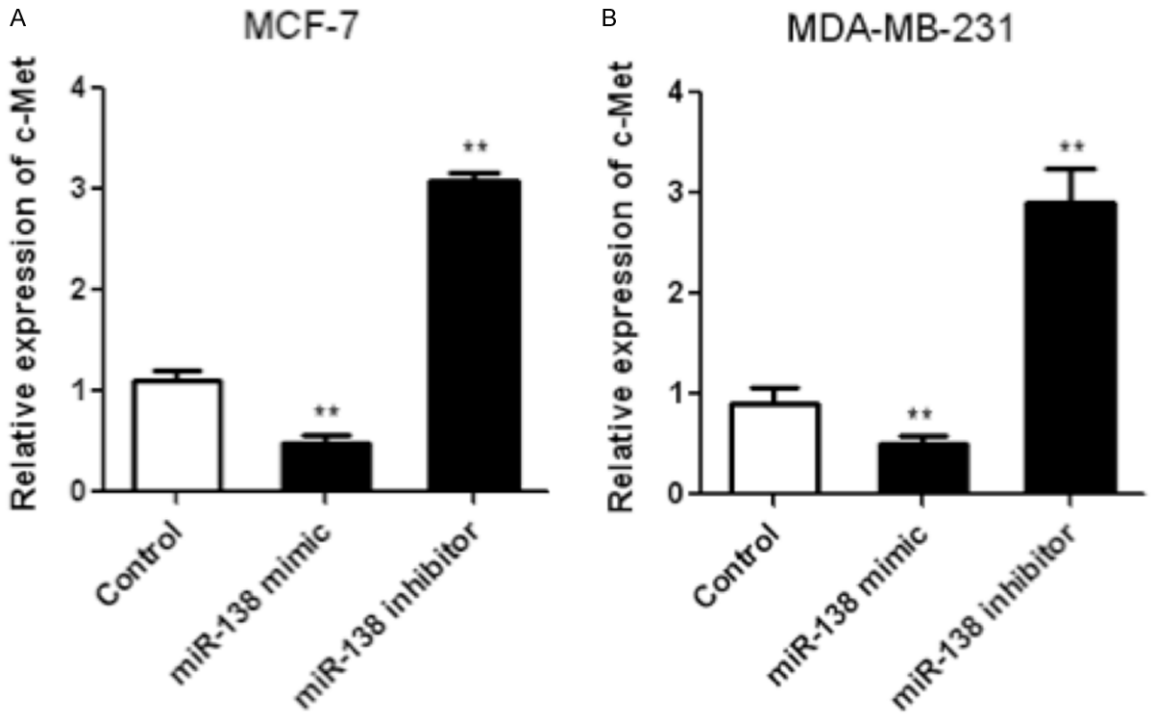

Figure 4. MiR-138 suppresses the mRNA expression of c-Met in breast cancer cells. MCF-7 (A) and MDA-MB-231 (B) cells were transfected with miR-138 mimic or inhibitor. Then the mRNA expression of c-Met was determined by real time PCR. *P<0.05; **P<0.01.

138 increased the protein level of c-Met in MCF-7 (**Figure 5A**) and MDA-MB-231 (**Figure 5B**) cells. In addition, the firefly luciferase activity was significantly reduced in MCF-7 (**Figure 6A**) and MDA-MB-231 (**Figure 6B**) cells after transfection with miR-138 mimic. However, down-regulation of miR-138 caused a significant increase in luciferase activity, suggesting that c-Met is a direct target of miR-138 in breast cancer cells.

Discussion

Emerging evidences indicate that miRNAs play a significant role in the progression of carcinogenesis [11], but the mode of action of miR-138 in breast cancer has yet to be elucidated. In the current study, our results demonstrated that ectopic expression of miRNA-138 suppressed cell growth and metastatic potential by targeting c-Met in breast cancer.

Breast cancer progression is a complex process in which multiple oncogenes and tumor suppressors were dysregulated [12]. Up to now, many miRNAs have been identified to modulate tumor suppressor or oncogenic pathways involved in the pathogenesis of breast cancer [13-15]. For example, the miR-10b is unregulated in breast cancer cells and correlates with increased cell growth and metastasis [16]. Several studies indicate that miR-155 serves as an oncogenic miRNA in breast cancer by targeting Suppressor of cytokine expression 1 (SOCS1) both *in vitro* and *in vivo* [17]. On the other hand, various studies demonstrate that let-7 is down-regulated in breast cancer cells and inhibits the metastasis and invasion of malignant cells by suppressing oncogenes RAS and High-Mobility Group AT-hook 2 (HMGA2) [18]. Several research groups have suggested that miR-138 is down-regulated in different cancers and up-regulation of miR-138 inhibits cancer cell proliferation, migration and invasion [19, 20]. However, a recent study in colorectal cancer indicated that down-regulation of miR-138 enhanced the metastasis capacity via regulating TWIST2 [21], suggesting the diverse role of miR-138 in different types of cancers. In our study, we found that miR-138 was down-regulated in breast cancer tissues and cell lines. Furthermore, ectopic expression of miR-138 inhibited the proliferation, colony formation and metastatic potential of breast cancer cells. Taken together, these results

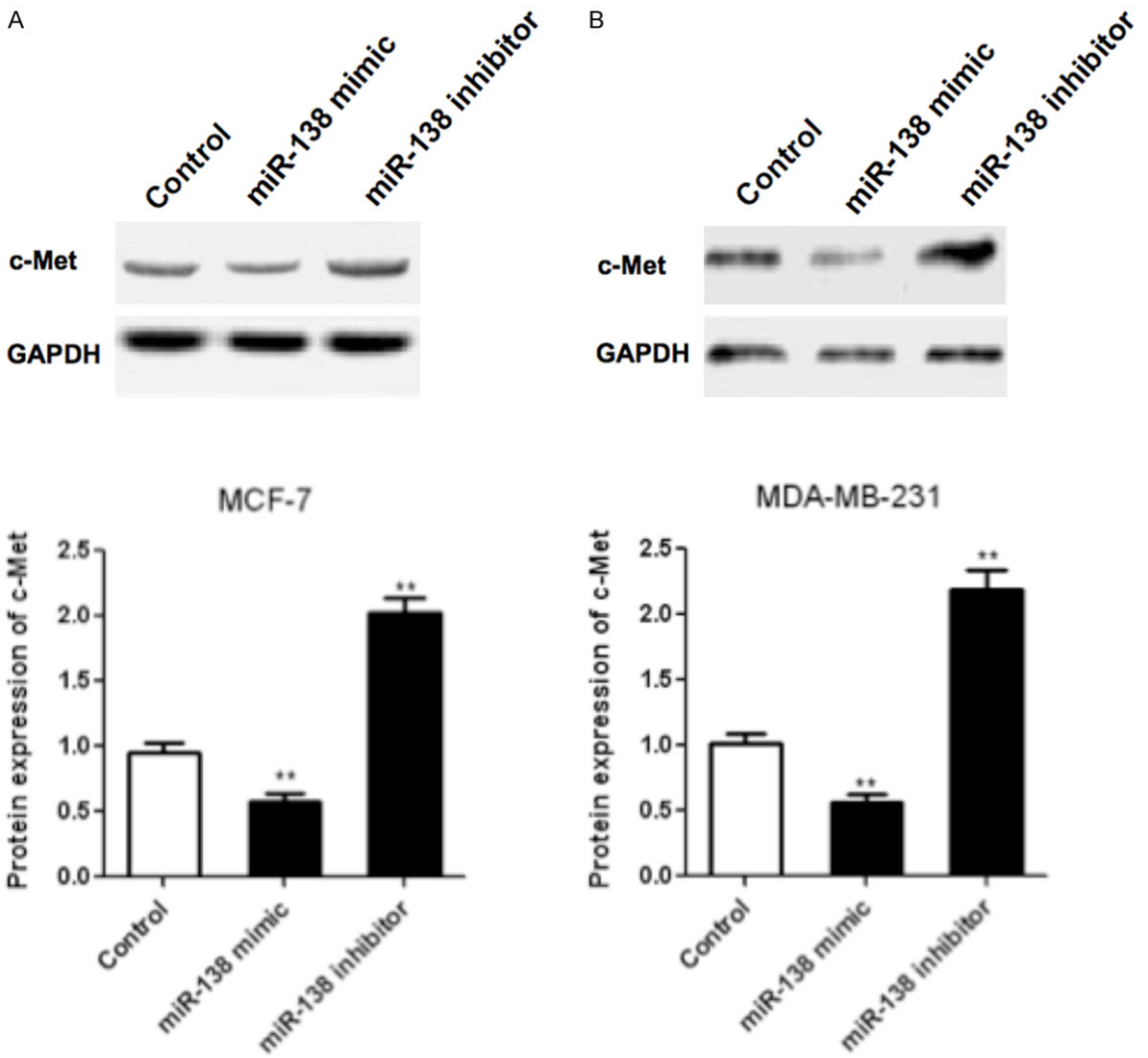

Figure 5. MiR-138 inhibits c-Met protein expression in breast cancer cells. Western blot analysis was performed to measure c-Met protein expression in MCF-7 (A) and MDA-MB-231 (B) cells after transfection with miR-138 mimic or inhibitor. *P<0.05; **P<0.01.

demonstrate that miR-138 serves as a tumor suppressor miRNA in breast cancer.

It is well known that miRNAs regulate tumor initiation and progression by targeting various oncogenes and tumor suppressors [22]. Thus, in order to elucidate the molecular mechanism by which miR-138 regulated various cellular processes, we searched its putative target using bioinformatics analysis. Previous studies have reported several targets regulated by miR-138 such as FOXC1 [23], cyclin D3 [24], and PDK1 [25] in different cancers. In the current study, we found that c-Met was a potential target of miR-138 in breast cancer cells. It has been reported that c-Met is up-regulated in a variety of malignancies and that increased c-Met signaling potentiates the growth and migration of tumor cells via several pathways such as the phosphatidyl inositol 3-kinase (PI3K), extracellular signal-regulated kinase (ERK) pathways, and focal adhesion kinase (FAK) [26, 27]. Our results showed that miR-138 up-regulation resulted in a reduction in c-Met expression, whereas inhibition of miR-138 enhanced c-Met level in breast cancer cells. Moreover, the firefly luciferase activities were remarkably reduced following transfection with miR-138 mimic. While cells transfected with miR-138 inhibitor exhibited enhanced luciferase activity, suggesting that c-Met is a direct target of miR-138 in breast cancer cells.

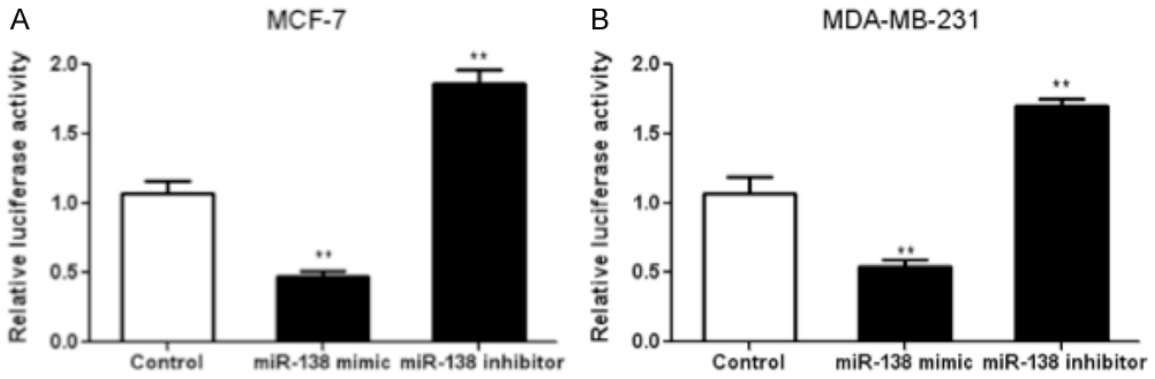

Figure 6. c-Met was a target of miRNA-138 in breast cancer cells. MCF-7 (A) and MDA-MB-231 (B) cells were transfected with miR-138 mimic or inhibitor, and the luciferase activities were determined by dual-luciferase reporter assay. *P<0.05; **P<0.01.

In summary, our study shows that miR-138 is down-regulated in breast cancer, and up-regulation of miR-138 suppresses the proliferation and migration of breast cancer cells by targeting c-Met. The current study implied that miR-138 may represent a valuable therapeutic tool for breast cancer treatment.

Disclosure of conflict of interest

None.

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References

[1] DeSantis C, Ma J, Bryan L, Jemal A. Breast cancer statistics, 2013. CA Cancer J Clin 2014; 64: 52-62.

[2] Thun MJ, DeLancey JO, Center MM, Jemal A, Ward EM. The global burden of cancer: Priorities for prevention. Carcinogenesis 2010; 31: 100-110.

[3] Lorusso G, Ruegg C. New insights into the mechanisms of organ-specific breast cancer metastasis. Semin Cancer Biol 2012; 22: 226-233.

[4] He L, Hannon GJ. MicroRNAs: Small RNAs with a big role in gene regulation. Nat Rev Genet 2004; 5: 522-531.

[5] Esquela-Kerscher A, Slack FJ. Oncomirs-microRNAs with a role in cancer. Nat Rev Cancer 2006; 6: 259-269.

[6] Meltzer PS. Cancer genomics: Small RNAs with big impacts. Nature 2005; 435: 745-746.

[7] Liu X, Jiang L, Wang A, Yu J, Shi F, Zhou X. MicroRNA-138 suppresses invasion and promotes apoptosis in head and neck squamous cell carcinoma cell lines. Cancer Lett 2009; 286: 217-222.

[8] Seike M, Goto A, Okano T, Bowman ED, Schetter AJ, Horikawa I, Mathe EA, Jen J, Yang P, Sugimura H, Gemma A, Kudoh S, Croce CM, Harris CC. MiR-21 is an EGFR-regulated anti-apoptotic factor in lung cancer in never-smokers. Proc Natl Acad Sci U S A 2009; 106: 12085-12090.

[9] Jiang L, Liu X, Kolokythas A, Yu J, Wang A, Heidbreder CE, Shi F, Zhou X. Downregulation of the Rho GTPase signaling pathway is involved in the microRNA-138-mediated inhibition of cell migration and invasion in tongue squamous cell carcinoma. Int J Cancer 2010; 127: 505-512.

[10] Islam M, Datta J, Lang JC, Teknos TN. Down regulation of RhoC by microRNA-138 results in de-activation of FAK, Src and Erk 1/2 signaling pathway in head and neck squamous cell carcinoma. Oral Oncol 2014; 50: 448-456.

[11] Gregory PA, Bert AG, Paterson EL, Barry SC, Tsykin A, Farshid G, Vadas MA, Khew-Goodall Y, Goodall GJ. The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. Nat Cell Biol 2008; 10: 593-601.

[12] Hutti JE, Shen RR, Abbott DW, Zhou AY, Sprott KM, Asara JM, Hahn WC, Cantley LC. Phosphorylation of the tumor suppressor CYLD by the breast cancer oncogene IKKepsilon promotes cell transformation. Mol Cell 2009; 34: 461-472.

[13] Shah NR, Chen H. MicroRNAs in pathogenesis of breast cancer: Implications in diagnosis and treatment. World J Clin Oncol 2014; 5: 48-60.

[14] Gotte M. MicroRNAs in breast cancer pathogenesis. Minerva Ginecol 2010; 62: 559-571.

[15] Chou J, Shahi P, Werb Z. MicroRNA-mediated regulation of the tumor microenvironment. Cell Cycle 2013; 12: 3262-2371.
[16] Tang J, Ahmad A, Sarkar FH. The role of microRNAs in breast cancer migration, invasion and metastasis. Int J Mol Sci 2012; 13: 13414-13437.
[17] Jiang S, Zhang HW, Lu MH, He XH, Li Y, Gu H, Liu MF, Wang ED. MicroRNA-155 functions as an OncomiR in breast cancer by targeting the suppressor of cytokine signaling 1 gene. Cancer Res 2010; 70: 3119-3127.
[18] Yu F, Yao H, Zhu P, Zhang X, Pan Q, Gong C, Huang Y, Hu X, Su F, Lieberman J, Song E. Let-7 regulates self renewal and tumorigenicity of breast cancer cells. Cell 2007; 131: 1109-1123.
[19] Yeh YM, Chuang CM, Chao KC, Wang LH. MicroRNA-138 suppresses ovarian cancer cell invasion and metastasis by targeting SOX4 and HIF-1alpha. Int J Cancer 2013; 133: 867-878.
[20] Mitomo S, Maesawa C, Ogasawara S, Iwaya T, Shibazaki M, Yashima-Abo A, Kotani K, Oikawa H, Sakurai E, Izutsu N, Kato K, Komatsu H, Ikeda K, Wakabayashi G, Masuda T. Downregulation of miR-138 is associated with overexpression of human telomerase reverse transcriptase protein in human anaplastic thyroid carcinoma cell lines. Cancer Sci 2008; 99: 280-286.
[21] Long L, Huang G, Zhu H, Guo Y, Liu Y, Huo J. Down-regulation of miR-138 promotes colorectal cancer metastasis via directly targeting TWIST2. J Transl Med 2013; 11: 275.
[22] Hannafon BN, Sebastiani P, de Las MA, Lu J, Rosenberg CL. Expression of microRNA and their gene targets are dysregulated in preinvasive breast cancer. Breast Cancer Res 2011; 13: R24.
[23] Yu C, Wang M, Li Z, Xiao J, Peng F, Guo X, Deng Y, Jiang J, Sun C. MicroRNA-138-5p regulates pancreatic cancer cell growth through targeting FOXC1. Cell Oncol (Dordr) 2015; 38: 173-181.
[24] Huang B, Li H, Huang L, Luo C, Zhang Y. Clinical significance of microRNA 138 and cyclin D3 in hepatocellular carcinoma. J Surg Res 2015; 193: 718-723.
[25] Han L, Zhang G, Zhang N, Li H, Liu Y, Fu A, Zheng Y. Prognostic potential of microRNA-138 and its target mRNA PDK1 in sera for patients with non-small cell lung cancer. Med Oncol 2014; 31: 129.
[26] Tang MK, Zhou HY, Yam JW, Wong AS. C-Met overexpression contributes to the acquired apoptotic resistance of nonadherent ovarian cancer cells through a cross talk mediated by phosphatidylinositol 3-kinase and extracellular signal-regulated kinase 1/2. Neoplasia 2010; 12: 128-138.
[27] Zhang W, Mendoza MC, Pei X, Ilter D, Mahoney SJ, Zhang Y, Ma D, Blenis J, Wang Y. Down-regulation of CMTM8 induces epithelial-to-mesenchymal transition-like changes via c-MET/extracellular signal-regulated kinase (ERK) signaling. J Biol Chem 2012; 287: 11850-11858.