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miR-888 regulates side population properties and cancer metastasis in breast cancer cells

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ABSTRACT

Cancer stem cells (CSCs) have recently been reported to possess properties related to cancer metastasis. However, the mechanism by which microRNAs (miRNAs) regulate these properties remains unclear. This study aims to investigate a correlation between miRNAs and the side population (SP) of human breast cancer cell line MCF-7 with CSC properties. miR-888 was found in our previous study to be up-regulated in SP cells and predicted to target E-Cadherin directly, indicating a potential role in maintaining SP properties and regulating the epithelial-mesenchymal transition (EMT) and cancer metastasis. After the over-expression of miR-888 in MCF-7 cells and knock-down of its expression in SP cells, we found that miR-888 played a role in maintaining CSC-related properties. Next, miR-888 was found to regulate the EMT process by targeting related gene expression. Lastly, MCF-7 cells over-expressing miR-888 exhibited a significant reduction in their ability to adhere to the extracellular matrix and an increased potential for migration and invasion, whereas knock-down of miR-888 expression in SP cells reversed these trends. In conclusion, miR-888 maintains SP properties and regulates EMT and metastasis in MCF-7 cells, potentially by targeting E-Cadherin expression.

1. Introduction

Breast cancer is one of the most common types of cancer in women, and it typically causes mortality through metastasis from the primary tumor to other organs [1]. Therefore, metastasis plays a crucial role in breast cancer aggressiveness and malignancy. Recently, cancer stem cells (CSCs) were hypothesized to exist and found to be a cause of cancer metastasis [2,3]. The first and most important step of metastasis is local invasion, during which adherent epithelial cells are converted into migratory cells that invade into the extracellular matrix (ECM), a process known as epithelial–mesenchymal transition (EMT) [4]. Moreover, CSCs also display an EMT phenotype, such as the loss of E-Cadherin expression, which promotes tumor growth and metastasis [5].

In recent years, microRNAs (miRNAs) have been implicated in CSCs and cancer metastasis [2,6–8]. As EMT is strongly associated with CSCs and metastasis, the regulation of EMT by miRNAs should shed light on cancer progression and be informative for therapeutics [9]. The side population of MCF-7 cells was found to possess CSC characteristics in a previous study [10], though little is known about its relationship with cancer metastasis and regulation by miRNA. In this study, a novel miRNA, miR-888, was found to be up-regulated in MCF-7 SP cells; miR-888 is predicted to directly target E-Cadherin expression [11]. Due to the crucial role of E-Cadherin in EMT and metastasis, it is essential to uncover the mechanism by which miR-888 regulates these processes.

2. Materials and methods

2.1. Cell culture and SP sorting

The human breast cell line, MCF-7 was purchased from the Cell Bank of the Chinese Academy of Sciences and cultured in DMEM medium (with 10% FBS, 100 U/ml penicillin, and 1000 U/ml streptomycin). Side population sorting was conducted as previously described [12]. In brief, 10^6 MCF-7 cells/ml were counted and re-suspended in DMEM supplemented with 10% FBS. All samples were divided into two groups: in the experimental group, Hoechst 33342 (Sigma) was added to a final concentration of 5 μg/ml; in addition, 50 μmol/ml verapamil (Sigma) was added to the negative control group. The cells were then placed in a 37 °C water bath for 90 min, and the staining process was terminated in an ice-bath for 10 min. The cells were re-suspended in 200 μl HBSS prior to flow

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cytometry. The SP cells were cultured in serum-free complete medium (DMEM/F-12 medium supplemented with 20 ng/ml EGF, 20 ng/ml bFGF, and 5 mg/ml insulin) and maintained as spheres in ultra-low-adherent plates.

2.2. Vector construction and cell transfection

miR-888 (sequence: 5′UACUCAAAAAGCUGUCAGUCA3′) was cloned into the miRNA expression vector pcDNA6.2-GW/EmGFP-miR (Invitrogen) according to the manufacturer's instructions. The vector without a target site for any gene (from 1519 to 1578 bp) was used as a negative control (mock). A locked nucleic acid (LNA) was designed as an antisense oligonucleotide of miR-888 (LNA-miR-888) and synthesized by Sangon Biotech. The sequence of LNA-miR-888 is as follows: 5′TGA + CTG + ACA + GCT + TTT + TGA + GTA + 3′ (the superscripted '+' represents LNA-modified nucleotides); a LNA lacking a specific target was used as a negative control (LNA-mock). Both miRNA expression vectors and the antisense oligonucleotides were transfected into MCF-7 cells using Entranster™-D (Engreen, China) according to the manufacturer's instructions.

2.3. Animal model and tumorigenicity

Four-week-old NOD/SCID mice were purchased from the SLAC Laboratory Animal Center (Shanghai, China). Adherent MCF-7 cells (2×10^5) transfected with miR-888 and the mock vector and 2×10^4 MCF-7 SP sphere cells transfected with LNA-miR-888 and LNA-mock were injected subcutaneously into each mouse ($n = 6$). Tumorigenicity was evaluated at 4 weeks after the injection. All procedures followed the institutional guidelines for animal welfare.

2.4. RNA purification and mRNA quantification

Total RNA was extracted using the Trizol total RNA isolation reagent (Invitrogen) according to the manufacturer's instructions. cDNA was synthesized from total RNA using TransScript II One-Step gDNA Removal and cDNA Synthesis SuperMix Kit (Beijing TransGen Biotech, China) following the manufacturer's instructions. Real-time PCR was performed using SYBR-Green Master PCR Mix (Roche) with a BIO-RAD CFX96™ real-time PCR system and mRNA-specific primers (Table 1). All data were normalized to *GAPDH* expression.

Table 1
Sequences of primers used in the real-time PCR amplifications.

Gene name	Primer sequences (from 5′ to 3′)
ABCB1	F TGCTGCTTACATTCAGGTTTCA
	R AGCCTATCTCCTGTCGCATTA
ABCC1	F CTCTATCTCTCCCGACATGACC
	R AGCAGACGATCCACAGCAAAA
CDH1	F TGGGCAGTGTAGGATGTGATT
	R CGTAGCAGTGACGAATGTGG
OCLN	F ACAAGCGGTTTTATCCAGAGTC
	R GTCATCCACAGGCGAAGTTAAT
CDH2	F TCAGGCGTCTGTAGAGGCTT
	R RTGCACATCCTTCGATAAGACTG
VIM	F GACGCCATCAACACCGAGTT
	R CTTTGTCGTTGGTTAGCTGGT
ZEB1	F GATGATGAATGCGAGTCAGATGC
	R ACAGCAGTGTCTTGTTGTTGT
GAPDH	F CTTCGCTCTCTGCTCCTCCT
	R ATCCGTTGACTCCGACCTTC

2.5. Cell adhesion assay

The 96-well plates used in the assay were coated with 10 μg/ml fibronectin at 37 °C for 1 h and washed twice with washing buffer (0.1% BSA in DMEM). BSA (0.5%, blocking buffer) solution was added to the wells and incubated at 37 °C in a 5% CO_2 incubator for 45–60 min. A 50-μl aliquot of cells at a density of 4×10^5 -cells/ml was added to each well and incubated at 37 °C for 30 min. The plate was shaken on a horizontal platform at 2000 rpm for 10–15 s, and then washed 3 times with washing buffer. The cells were then fixed with 4% paraformaldehyde by incubation at RT for 10–15 min. Crystal Violet dye (1%, Sigma) was added for 10 min to stain the cells, and the cells were then washed with distilled water. SDS solution (2%) was added to the cells and incubated at RT for 30 min before measuring the OD values at 540 μm.

2.6. In vitro cell migration and invasion assay

In vitro cell migration assays were performed using Transwell chambers (8 μm pore size; Costar). The undersides of the filter inserts were coated with 20 μg/ml of fibronectin overnight at 4 °C and then air dried before adding them to the cells. The cultured cells were trypsinized, re-suspended, and then washed twice with FBS-free medium. A 0.5-ml aliquot of cells (4×10^5 cells/ml) in FBS-free medium was applied to the top of the insert, and 1 ml of medium (containing 10% FBS and 10 μg/ml collagen I) was added to the lower chamber. The filter insert was then placed into the lower chamber. The entire apparatus was incubated at 37 °C for 24 h. The cells on the top side of the filter were removed by scrubbing twice with a cotton-tipped swab moistened with culture medium-lacking FBS. The cells were stained with 0.1% Crystal Violet for 10 min. The membranes were separated and photographed under a microscope, and the number of migrated cells was counted in 10 fields. Additionally, the separated membranes were de-stained with 33% acetic acid. The de-staining fluid was then transferred into a 96-well-plate, and the OD value was determined at 540 μm. The *in vitro* cell invasion assay was similar to the migration assay, but the Transwell used in the migration assay was first covered with Matrigel before seeding the cells on it to serve as a basement membrane. The invasive activity of the cells was measured by counting the cells in the Matrigel after incubation at 37 °C overnight.

2.7. Statistical analysis

The data are presented as the means ± SEM, and Student's *t*-test (two-tailed) was used to compare two groups ($P < 0.05$ was considered significant) for independent samples, assuming equal variances for all experimental data sets.

3. Results

3.1. miR-888 expression was increased in MCF-7 SP cells and played a role in maintaining SP properties

In a previous study, we found that miR-888 was approximately 20-fold increased in MCF-7 SP cells compared with non-SP cells [11], indicating a potential role for miR-888 in maintaining SP properties. As SP cells are characterized with CSC properties, it is postulated that miR-888 may also maintain stem cell properties. To uncover the regulating role of miR-888 regarding CSC properties, we conducted several assays in MCF-7 cells using different treatments.

In a sphere-forming assay, MCF-7 cells were transfected with a plasmid over-expressing miR-888 and a mock vector and cultured in serum-free medium. MCF-7 SP sphere cells (cultured for one

week) were transfected with LNA-miR-888 and LNA-mock and maintained in serum-free medium. After one week of culture, sphere-forming ability was increased in the miR-888-transfected MCF-7 cells compared with the mock-treated cells and was decreased in the LNA-miR-888-treated SP sphere cells compared with those treated with LNA-mock (Fig. 1A and B). Moreover, the LNA-miR-888-treated SP sphere cells showed a differentiation phenotype, even when cultured in serum-free medium (Fig. 1B).

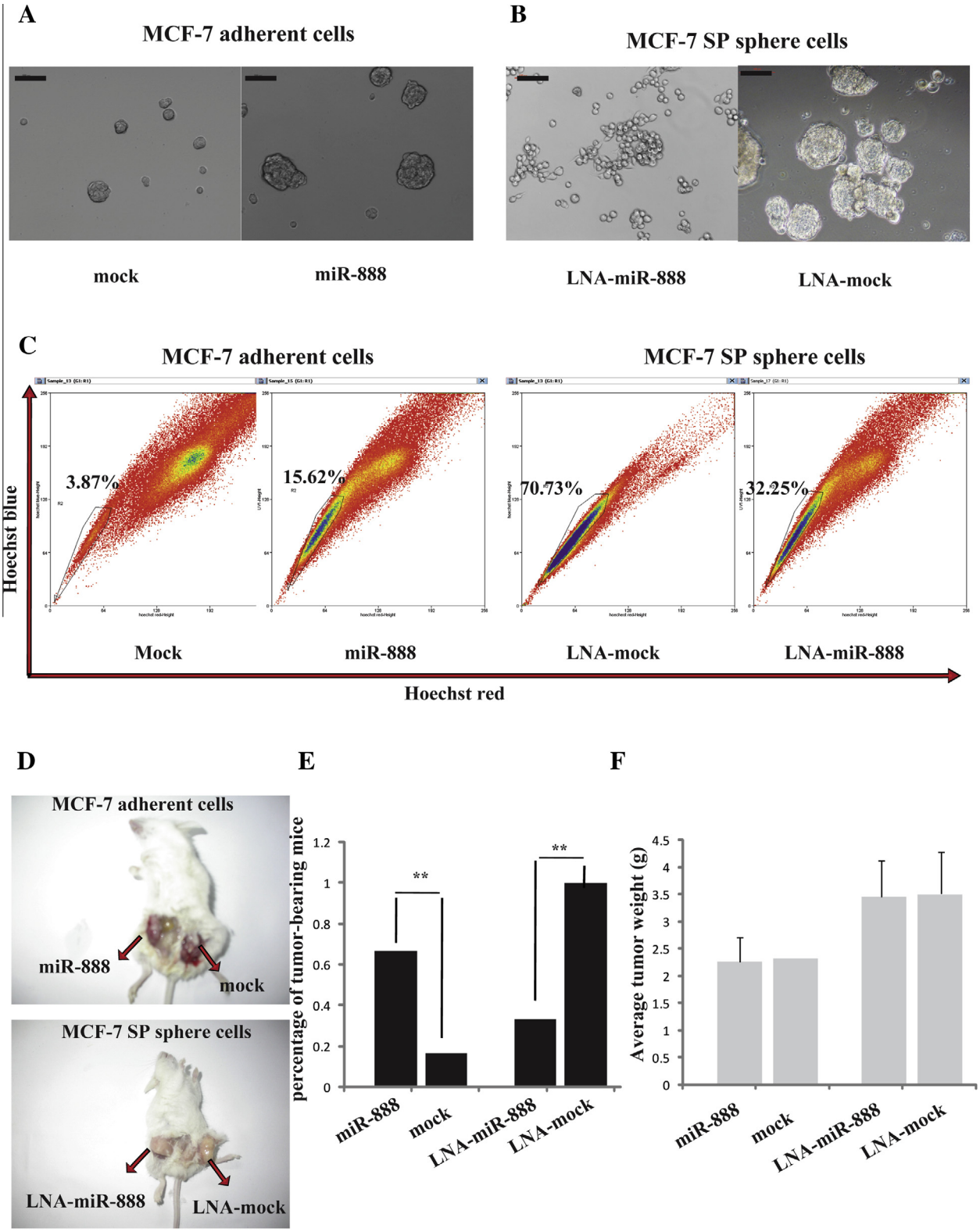

Fig. 1. miR-888 maintains SP properties in MCF-7 cells. (A) Over-expression of miR-888 in MCF-7 cells indicates the up-regulation of sphere-forming ability in miR-888-transfected cells. (B) Knock-down of miR-888 by LNA in MCF-7 SP cells indicates the down-regulation of sphere-forming ability in LNA-miR-888-transfected cells. (C) Over-expression of miR-888 increases the SP proportion in MCF-7 cells, and knock-down of miR-888 expression in SP sphere cells decreases the SP proportion. (D) Over-expression of miR-888 increases the tumorigenicity of MCF-7 cells, and knock-down of miR-888 expression in SP sphere cells decreases tumorigenicity. (E) Tumorigenicity and (F) tumor weight. *Represents $p < 0.05$; **represents $p < 0.01$.

In an SP sorting assay, SP cells were counted after different treatments of MCF-7 cells. The results showed that the over-expression of miR-888 increased the SP proportion of MCF-7 adherent cells (from 3.87% to 15.62%), whereas the knock-out of its expression in SP sphere cells decreased the SP proportion (from 70.73% to 32.25%) (Fig. 1C).

In a tumorigenicity assay, the over-expression of miR-888 increased tumorigenicity in MCF-7 adherent cells (from 1/6 to 4/6); miR-888 knock-out decreased tumorigenicity in SP sphere cells (from 5/5 to 2/6) (Fig. 1D and E). However, the miR-888-transfected MCF-7 cells and LNA-miR-888-treated SP sphere cells did not exhibit a significant effect with regard to tumor weight compared with their controls (Fig. 1F).

3.2. miR-888 played a potential role in regulating EMT properties in both MCF-7 adherent cells and SP sphere cells

In our previous publication, we found that miR-888 directly targeted E-Cadherin expression [11], which suggested a potential role of miR-888 in regulating EMT. We therefore detected the expression of EMT-related genes (three up-regulated genes and two down-regulated) between MCF-7 SP and non-SP cells by real-time RT-PCR. The results showed that SP cells had a high level expression of genes that are up-regulated during EMT (*CDH2*, *VIM*, and *ZEB1*) and a low expression of EMT down-regulated genes (*OCLN* and *CDH1*, Fig. 2A and B).

To validate the function of miR-888 in EMT, we over-expressed miR-888 in MCF-7 and knocked down its expression in SP sphere cells. The results showed that increased miR-888 in MCF-7 cells up-regulated EMT-high expression genes and down-regulated EMT-low expression genes (Fig. 2C); miR-888 knock-down in SP sphere cells had the opposite effect (Fig. 2D).

3.3. miR-888 played a potential role in regulating MCF-7 cell adhesion

As E-Cadherin (coded by *CDH1*) is vital for cell adhesion and metastasis and is directly targeted by miR-888, we predicted that miR-888 would play a role in regulating these properties. To characterize the role of miR-888 in regulating cell adhesion, we over-expressed miR-888 in MCF-7 adherent cells and down-regulated its expression in SP sphere cells. As shown in Fig. 3A and B, the over-expression of miR-888 in MCF-7 adherent cells decreased adhesion in comparison with the negative control. Moreover, the knock-down of miR-888 expression in SP sphere cells increased adhesion.

3.4. miR-888 played a potential role in regulating MCF-7 cell migration and invasion

During the EMT process, cancer cells become less adhesive and are prone to migrate and invade other parts of the body. Therefore, cancer cells become more migratory and invasive when cell adhe-

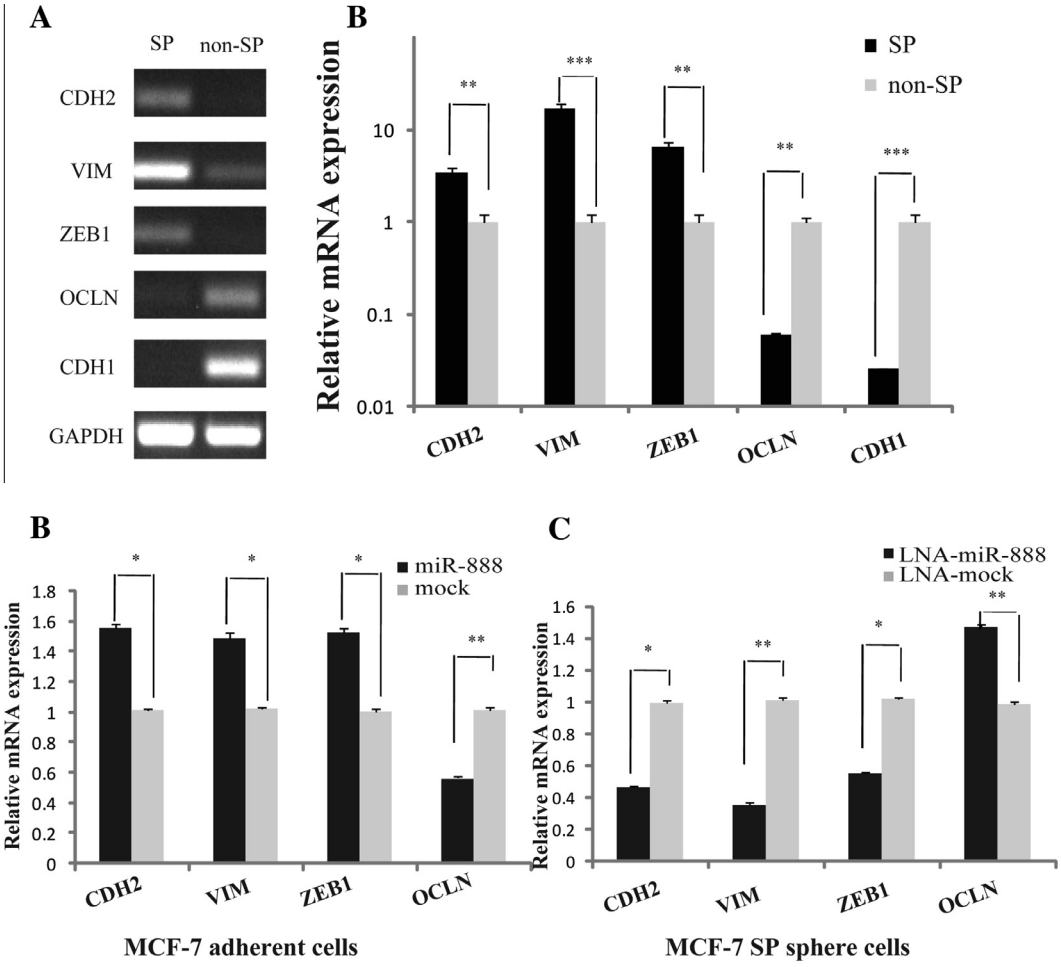

Fig. 2. miR-888 regulates EMT properties in MCF-7 cells. (A) and (B) Analysis of the relative expression of EMT-related genes by real-time RT-PCR between MCF-7 non-SP and SP cells. (C) Over-expression of miR-888 increases mesenchymal marker genes and decreases epithelial marker genes in MCF-7 cells. (D) knock-down of miR-888 expression in MCF-7 SP sphere cells decreases the expression of mesenchymal marker genes and increases the expression of epithelial marker genes by real-time RT-PCR. Bar, 50 μm. *Represents $p < 0.05$; **represents $p < 0.01$; ***represents $p < 0.001$.

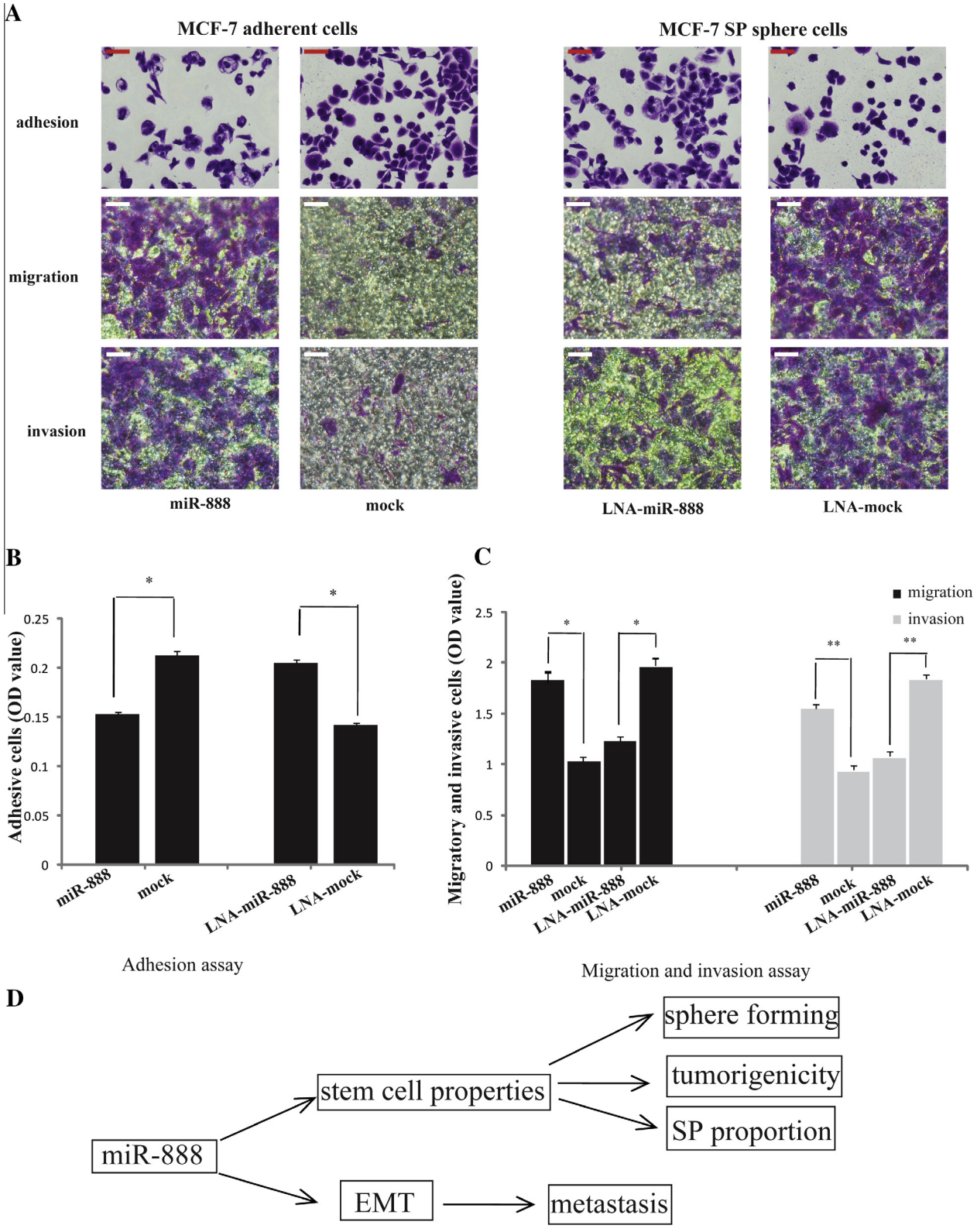

Fig. 3. miR-888 regulates metastasis properties in MCF-7 cells. (A) and (B) Over-expression of miR-888 in MCF-7 cells decreases cell adhesion, and knock-down of miR-888 expression in MCF-7 SP sphere cells increases cell adhesion by an adhesion assay. (A) and (C) Over-expression of miR-888 in MCF-7 cells increases cell migration and invasion, and knock-down miR-888 expression in MCF-7 SP sphere cells decreases cell migration and invasion by a migration and invasion assay. (D) Schematic depicting the potential functions of miR-888 in the SP properties and cancer metastasis of MCF-7 cells. Bar, 50 μm. *Represents $p < 0.05$; **represents $p < 0.01$.

sion becomes weaker. To characterize the role of miR-888 in regulating cell migration and invasion, we over-expressed miR-888 in MCF-7 adherent cells and down-regulated its expression in SP sphere cells. As shown in Fig. 3A and C, the miR-888 over-expressing cells exhibited an enhanced ability to cross the Transwell membrane (migration) and to pass through Matrigel (invasion), whereas the reduction in miR-888 expression in SP sphere cells induced the opposite effects. These results indicate that miR-888

is an important factor regulating cell adhesion, migration, and invasion in the breast cancer cell line MCF-7.

Fig. 3D outlines the molecular pathway by which miR-888 regulates SP properties and EMT, which leads to changes in cancer cell metastasis properties (including cell adhesion, migration, and invasion).

4. Discussion

The side population (SP) is characterized as having stem cell properties in both normal and malignant tissues [10,13] due to the over-expression of ABC transporter proteins. Therefore, knowledge of the expression regulation of these genes and related genes sheds light on SP properties. In recent years, miRNAs have been shown to play increasingly important roles in regulating SP characteristics [14–16]. For example, both miR-520 h and miR-328 directly target ABCG2 expression, though in different cancers [14,15]. In addition, the over-expression of miR-520 h reduces the SP cell number and inhibits cell migration and invasion [15]. In our work, miR-888 was found to be up-regulated in MCF-7 SP cells, which were characterized with CSC properties in a previous study [11], indicating a potential role of miR-888 in maintaining SP properties. To validate the function of miR-888 in SP, we over-expressed miR-888 in MCF-7 cells and down-regulated its expression in SP sphere cells. miR-888 over-expression in MCF-7 cells increased the sphere-forming ability, SP proportion, and tumorigenicity, whereas miR-888 knock-down in MCF-7 SP sphere cells decreased these properties (Fig. 1). Thus, miR-888 plays a potential role in maintaining MCF-7 SP properties. However, we searched all the predicted target genes of miR-888 and found none associated with ABC transporters. Rescreening all the predicted targets, we selected five tumor suppressor genes and validated expression with that of miR-888. One of the selected tumor suppressor genes, LATS2, was down-regulated when miR-888 was over-expressed (data not shown). LATS2 is associated with P53, which is down-regulated in CSCs and plays an important role in regulating CSC proliferation and differentiation [17]. Therefore, miR-888 may regulate SP properties in MCF-7 by regulating LATS2 expression, though this needs further study in future work.

As mentioned above, we previously found that miR-888 directly targets cell adhesion molecule E-Cadherin and plays an important role in regulating the AJ pathway [11]. Both E-Cadherin and the AJ pathway are involved in EMT, a process in which the expression of E-Cadherin is lost and cells acquire a mesenchymal phenotype [5]. As a correlation between CSCs and EMT, differentiated cancer cells undergo transformation during EMT and acquire a CSC-like phenotype. Therefore, EMT is a CSC property, and CSCs also express EMT markers [18]. In our study, three mesenchymal markers and two epithelial markers (including *CDH1*) were selected, and their expression was tested between MCF-7 SP and non-SP cells. As expected, the SP cells highly expressed mesenchymal markers and expressed epithelial markers at low levels compared with non-SP cells (Fig. 2A and B). This result suggests that MCF-7 SP cells acquired an EMT phenotype after cell sorting by Hoechst. Due to the crucial role of EMT in CSCs, knowledge of its regulation will shed light on stem cell-like properties. In recent years, EMT-related genes such as ZEB, SNAIL, and TWIST have been reported to be regulated by miRNAs in cancer progression [19]. For example, miR-203 expression is repressed in the stem cell-enriched fraction. The over-expression of miR-203 in mesenchymal cells compromises the tumor initiation and metastasis induced by EMT [19,20]. In our study, miR-888 was up-regulated in MCF-7 SP cells and played an important role in maintaining SP properties. However, it has remained unclear whether this newly studied miRNA functions in EMT. To address this, we forced the expression of miR-888 in MCF-7 cells and down-regulated its expression in SP sphere cells to unveil its function in EMT. In miR-888-over-expressing MCF-7 cells, the expression of mesenchymal markers and epithelial markers was up-regulated and down-regulated, respectively (Fig. 2C). In LNA-miR-888-transfected SP cells, the expression of mesenchymal markers and epithelial markers was decreased and increased, respectively (Fig. 2D). Altogether, miR-888 plays an important role in regulating the EMT phenotype in MCF-7 cells, possibly by targeting E-Cadherin. However, further study is necessary to support this idea.

During cancer metastasis, cells from primary tumors acquire the EMT phenotype, lose cell adhesion, migrate to blood vessels, and invade different organs [4,9,21]. Therefore, EMT is an essential step of cancer metastasis and determining its regulation at the genetic and epigenetic levels will help to uncover the mechanisms of cancer progression and malignancy. As a dual crucial role of E-Cadherin in EMT and metastasis, knowledge of the regulation of E-Cadherin expression will also shed light on cancer metastasis properties. In miRNA research with regard to E-Cadherin, both miR-9 and miR-495 are been revealed to directly target expression of this protein and to regulate metastasis-related properties in breast cancer [6,22]. miR-9 is up-regulated in breast cancer and activated by MYC and MYCN. Over-expression of miR-9 increases cell migration and invasiveness by targeting E-Cadherin expression and activating β-catenin signaling [6]. miR-495 is up-regulated in two breast CSC sub-populations, $CD44^{+}/CD24^{-/low}$ and $PROCR^{+}/ESA^{+}$, and is modulated by the transcription factor E12/E47. Ectopic expression of miR-495 maintains CSC properties, such as *in vitro* colony formation and tumorigenesis, in mice and promotes cell invasion and proliferation [22]. In our study, miR-888 was found to be up-regulated in MCF-7 SP cells and also to target E-Cadherin expression, which suggests that miR-888 plays an important role in cancer metastasis. Therefore, we ectopically expressed miR-888 and LNA-miR-888 in MCF-7 and SP cells. As expected, was found to function in reducing cell adhesion and promoting cell migration and invasion (Fig. 3A–C). These results indicate that miR-888 plays potential roles in cancer metastasis.

In conclusion, up-regulated miR-888 in MCF-7 SP cells has dual roles: maintaining SP properties and regulating cancer metastasis (Fig. 3D). miR-888 was first found to be up-regulated in aggressive endometrial tumors and was suggested to have an important function in cancer development and progression [23]. miR-888 was recently reported to be up-regulated in the human metastatic prostate cancer cell line PC3-ML [24]. Over-expression of miR-888 increases cell migration and proliferation by targeting the tumor suppressor genes RBL1 and SMAD4, the latter of which is in the AJ pathway and is also a predicted target in our study. Therefore, miR-888 is up-regulated in several tumors and plays a potential role in cancer metastasis, which is consistent with the findings of our study. Most importantly, the inhibition of miR-888 expression will shed light on the regulation of cancer metastasis and provide evidence for cancer therapy in the future.

Acknowledgments

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References

[1] M. Montagner, E. Enzo, M. Forcato, F. Zanconato, A. Parenti, E. Rampazzo, G. Basso, G. Leo, A. Rosato, S. Bicciato, SHARP1 suppresses breast cancer metastasis by promoting degradation of hypoxia-inducible factors, Nature 48 (2012) 380–384.

[2] C. Liu, K. Kelnar, B. Liu, X. Chen, T. Calhoun-Davis, H. Li, L. Patrawala, H. Yan, C. Jeter, S. Honorio, The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44, Nat. Med. 17 (2011) 211–215.
[3] M.A. Velasco-Velázquez, V.M. Popov, M.P. Lisanti, R.G. Pestell, The role of breast cancer stem cells in metastasis and therapeutic implications, Am. J. Pathol. 179 (2011) 2–11.
[4] A. Singh, J. Settleman, EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer, Oncogene 29 (2010) 4741–4751.
[5] M.S. Schieber, N.S. Chandel, ROS links glucose metabolism to breast cancer stem cell and EMT phenotype, Cancer Cell 23 (2013) 265–267.
[6] L. Ma, J. Young, H. Prabhala, E. Pan, P. Mestdagh, D. Muth, J. Teruya-Feldstein, F. Reinhardt, T.T. Onder, S. Valastyan, miR-9, a MYC/MYCN-activated microRNA, regulates E-cadherin and cancer metastasis, Nat. Cell Biol. 12 (2010) 247–256.
[7] P. Wong, M. Iwasaki, T.C. Somervaille, F. Ficara, C. Carico, C. Arnold, C.-Z. Chen, M.L. Cleary, The miR-17-92 microRNA polycistron regulates MLL leukemia stem cell potential by modulating p21 expression, Cancer Res. 70 (2010) 3833–3842.
[8] W.W. Du, L. Fang, M. Li, X. Yang, Y. Liang, C. Peng, W. Qian, Y.Q. O'Malley, R.W. Askeland, S.L. Sugg, MicroRNA miR-24 enhances tumor invasion and metastasis by targeting PTPN9 and PTPRF to promote EGF signaling, J. Cell Sci. 126 (2013) 1440–1453.
[9] C.S. Tellez, D.E. Juri, K. Do, A.M. Bernauer, C.L. Thomas, L.A. Damiani, M. Tessema, S. Leng, S.A. Belinsky, EMT and stem cell-like properties associated with miR-205 and miR-200 epigenetic silencing are early manifestations during carcinogen-induced transformation of human lung epithelial cells, Cancer Res. 71 (2011) 3087–3097.
[10] K. Engelmann, H. Shen, O.J. Finn, MCF7 side population cells with characteristics of cancer stem/progenitor cells express the tumor antigen MUC1, Cancer Res. 68 (2008) 2419–2426.
[11] S. Huang, M. Cai, Y. Zheng, L. Zhou, Q. Wang, L. Chen, Increased expression of miR-888 in MCF-7 side population sphere cells directly targets E-Cadherin expression, J. Genet. Genomics 41 (2014) 35–42.
[12] M.A. Goodell, K. Brose, G. Paradis, A.S. Conner, R.C. Mulligan, Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo, J. Exp. Med. 183 (1996) 1797–1806.
[13] F. Behbod, W. Xian, C.A. Shaw, S.G. Hilsenbeck, A. Tsimelzon, J.M. Rosen, Transcriptional profiling of mammary gland side population cells, Stem Cells 24 (2006) 1065–1074.
[14] W.-Q. Li, Y.-M. Li, B.-B. Tao, Y.-C. Lu, G.-H. Hu, H.-M. Liu, J. He, Y. Xu, H.-Y. Yu, Downregulation of ABCG2 expression in glioblastoma cancer stem cells with miRNA-328 may decrease their chemoresistance, Med. Sci. Monit. 16 (2010) 27–30.
[15] F. Wang, X. Xue, J. Wei, Y. An, J. Yao, H. Cai, J. Wu, C. Dai, Z. Qian, Z. Xu, Hsa-miR-520h downregulates ABCG2 in pancreatic cancer cells to inhibit migration, invasion, and side populations, Br. J. Cancer 103 (2010) 567–574.
[16] H.J. Wang, Y.Q. Guo, G. Tan, L. Dong, L. Cheng, K.J. Li, Z.Y. Wang, H.F. Luo, miR-125b regulates side population in breast cancer and confers a chemoresistant phenotype, J. Cell. Biochem. 114 (2013) 2248–2257.
[17] Y. Aylon, N. Yabuta, H. Besserglick, Y. Buganim, V. Rotter, H. Nojima, M. Oren, Silencing of the Lats2 tumor suppressor overrides a p53-dependent oncogenic stress checkpoint and enables mutant H-Ras-driven cell transformation, Oncogene 28 (2009) 4469–4479.
[18] S.A. Mani, W. Guo, M.-J. Liao, E.N. Eaton, A. Ayyanan, A.Y. Zhou, M. Brooks, F. Reinhard, C.C. Zhang, M. Shipitsin, The epithelial–mesenchymal transition generates cells with properties of stem cells, Cell 133 (2008) 704–715.
[19] J.H. Taube, G.G. Malouf, E. Lu, N. Sphyris, V. Vijay, P.P. Ramachandran, K.R. Ueno, S. Gaur, M.S. Nicoloso, S. Rossi, Epigenetic silencing of microRNA-203 is required for EMT and cancer stem cell properties, Sci. Rep. 3 (2013) 2687–2696.
[20] M.D. Bullock, A.E. Sayan, G.K. Packham, A.H. Mirnezami, MicroRNAs: critical regulators of epithelial to mesenchymal (EMT) and mesenchymal to epithelial transition (MET) in cancer progression, Biol. Cell 104 (2012) 3–12.
[21] J.P. Thiery, Epithelial–mesenchymal transitions in tumour progression, Nat. Rev. Cancer 2 (2002) 442–454.
[22] W. Hwang-Verslues, P. Chang, P. Wei, C. Yang, C. Huang, W. Kuo, J. Shew, K. Chang, E.Y.P. Lee, W. Lee, miR-495 is upregulated by E12/E47 in breast cancer stem cells, and promotes oncogenesis and hypoxia resistance via downregulation of E-cadherin and REDD1, Oncogene 30 (2011) 2463–2474.
[23] A.M. Hovey, E.J. Devor, K.K. Leslie, MiR-888: a newly identified miRNA significantly over-expressed in endometrial cancers, Proc. Obstet. Gynecol. 2 (2013). 1-1.
[24] H. Lewis, R. Lance, D. Troyer, H. Beydoun, M. Hadley, J. Orians, T. Benzine, K. Madric, O.J. Semmes, R. Drake, miR-888 is an expressed prostatic secretions-derived microRNA that promotes prostate cell growth and migration, Cell Cycle 13 (2013) 227–239.