Original Article

Asian Pacific Journal of Tropical Biomedicine

apjtb.org

doi: 10.4103/2221-1691.391158

Impact Factor® 1.7

Macrophage-secreted exosomes inhibit breast cancer cell migration *via* the miR-101-3p/DLG5 axis

Yu Liu[1,2#], Chao-Qun Wang[3#], Yong-Kang Zhu[1✉], Jia-Fang Xu[4✉], Si-Qi Yin[2], Qing-Jie Hu[2], Rui-Qi Yang[3]

[1]*First Clinical Medical College, Nanjing University of Chinese Medicine, Nanjing 210001, China*

[2]*Department of Breast Surgery, The First Affiliated Hospital of Hainan Medical University, Haikou 570102, China*

[3]*Department of Nuclear Medicine, Hainan General Hospital, Hainan Affiliated Hospital of Hainan Medical University, Haikou 570311, China*

[4]*Reproductive Medicine Center, The First Affiliated Hospital of Hainan Medical University, Haikou 570102, China*

ABSTRACT

Objective: To investigate the role of macrophages in regulating breast cancer cell migration and its related mechanisms.

Methods: Human leukemia monocytic cell line THP-1-secreted exosomes were isolated using multi-step ultracentrifugation and verified using nanoparticle tracking analysis. Differentially expressed miRNAs were identified using RNA sequencing. Overexpression of inhibitors of hsa-miR-101-3p in breast cancer MDA-MB-231 cells was performed by infecting their lentiviral constructs. The luciferase reporter assay was used to evaluate the interaction of DLG5 and miR-101. DGL5 expression was detected using qRT-PCR and Western blot analyses.

Results: The migration of breast cancer cells was significantly inhibited after addition of exosomes. RNA sequencing results showed that miR-101-3p expression was significantly upregulated. Targetscan analysis predicted that miR-101-3p could target DLG5, and this prediction was verified using the luciferase assay. The addition of the miR-101-3p precursor significantly increased the expression of miR-101-3p, and the mRNA and protein levels of DLG5 were suppressed. In contrast, inhibiting the expression of miR-101-3p increased the mRNA and protein levels of DLG5. Furthermore, the scratch assay showed that inhibiting miR-101-3p could promote the migration of MDA-MB-231 cells.

Conclusions: Macrophage exosomes can inhibit the migration of breast cancer cells, and increasing the expression of miR-101-3p to inhibit DLG5 expression may play an important role in this process, which needs further investigation.

KEYWORDS: Micro-RNA; Tumor-associated macrophages; Exosomes; Breast cancer; DLG5

1. Introduction

Breast cancer is a common malignancy primarily affecting women, with more than two million new cases diagnosed annually[1]. Breast cancer has become the leading cancer worldwide, with lung cancer the second most common. In cases of advanced breast cancer, there is no effective therapy[2]. Cancer recurrence and metastasis are the main causes of mortality[3-5]. Non-coding RNAs [such as microRNA (miR)] have been found to promote or inhibit tumor progression[6]. The mature hsa-miR-101 is 21 nucleotides (nt) long and is generated *via* the Dicer enzyme from its precursor, which is a stem-loop structure of about 75 nt pre-miR-101-1 and 79 nt pre-miR-101-2 in length, respectively. Both miR-101-1 and miR-101-2 are highly conserved between different species[7]. miR-101

Significance

Exosomes, as an important component of tumor microenvironment, participate in intercellular signal transduction and tumor formation and deterioration. The present study shows that M2 macrophage-secreted exosomes can inhibit the migration of MDA-MB-231 cells by regulating the miR-101-3p/DLG5 axis.

✉To whom correspondence may be addressed. E-mail: feipeterpan2@sina.com (YK. Zhu); xujiafangyuyu@163.com (JF. Xu)

#These authors contributed equally to this study.

How to cite this article: Liu Y, Wang CQ, Zhu YK, Xu JF, Yin SQ, Hu QJ, et al. Macrophage-secreted exosomes inhibit breast cancer cell migration *via* the miR-101-3p/DLG5 axis. Asian Pac J Trop Biomed 2023; 13(12): 532-538.

Article history: Received 4 April 2023; Revision 21 June 2023; Accepted 16 November 2023; Available online 28 December 2023

expression is downregulated in several cancers, such as liver cancer, osteosarcoma, lung cancer, ovarian cancer, colorectal cancer, and other malignant tumors, suggesting the importance of miR-101 in tumorigenesis and cancer development[8].

Tumor-associated macrophages (TAMs) are important cells that interact with tumor cells and surrounding stroma cells in the tumor microenvironment (TME)[9]. The TME is closely related to the initiation and progression of cancer, especially cancer invasion, and metastasis. The most abundant infiltrating immune cells in the TME are macrophages that are polarized and classified as two primary phenotypes: classically activated macrophages (or M1) that respond to interferon-γ (IFN-γ) and alternately activated macrophages (or M2) that are further classified into several subgroups based on their responses to different stimuli[10].

TAMs that mainly consist of M2 macrophages are abundant in the TME, accounting for approximately 30%-50% of stromal cells. Other important factors in the TME include exosomes that belong to the smallest extracellular vesicles released from cells (~30-200 nm) and are composed of a lipid bilayer, including transmembrane proteins and different nucleic acids (including miRs). These exosomes can affect TME remodeling and tumor metastasis[11]. Lipid-free proteins and lipid-based carriers of extracellular miRs are additional TME factors that are involved in the regulation of gene expression and cell-to-cell transfer. The exosome-carrying miRs can be absorbed by adjacent or distant cells, thereby taking part in the metabolism or functions of recipient cells[12]. An imbalance of exosome-mediated miR could affect interactions between cancer cells and the TME[13].

Discs large homolog 5 (DLG5) belongs to the membrane-associated guanylate kinase family and plays an important role in the formation of epithelial tubes and cell polarity. DLG5 expression is dysregulated in a variety of malignancies and correlated with malignant behaviors of subpopulations of breast cancer cells, such as proliferation and migration. However, it is not clear whether TAMs affect mammary tumorigenesis and breast cancer development[14,15]. This study aimed to investigate the effect of M2 macrophage-secreted exosomes to identify new targets for treating triple-negative breast cancer.

2. Materials and methods

2.1. Cell lines

The human leukemia monocytic cell line THP-1 and human breast cancer cell line MDA-MB-231 were provided by American Type Culture Collection (ATCC). THP-1 and MDA-MB-231 cells were maintained in RPMI 1640 medium (Corning) and Dulbecco's Modified Eagle's Medium (Corning), respectively, and supplemented with 10% fetal bovine serum (FBS; non-heated from Invitrogen or heated from Ausbian) and 1% penicillin/streptomycin, with/without 4 500 mg/L glucose, 2 mM *L*-glutamine, 10 mM 4-(2-hydroxyerhyl) piperazine-1-erhanesulfonic acid (HEPES), and 0.05 mM β-mercaptoethanol. Cells were maintained at 37 ℃ and 5% CO_2.

2.2. Isolation and identification of THP–1 cell–released exosomes

The THP-1 cells were stimulated with phorbol-12-myristate-13-acetate (PMA, 500 ng/mL) for 48 h and then the culture medium was collected and centrifuged at 120 000 ×*g* at 4 ℃ for 2 h to obtain the pellet, which was dissolved in chilled phosphate buffered saline (PBS). After ultracentrifugation under the same conditions as above, the pellet containing exosomes was resuspended in 200 μL pre-chilled PBS and used for nanoparticle tracking analysis.

2.3. Construction of stable cell lines

According to an established lentivirus transfection protocol, cells with good growth status were selected 24 h prior to transfection. The cell density was adjusted to approximately 5×10^6 cells/15 mL in the medium containing 10% serum under normal culture conditions. After 24 h, when the cell fusion rate reached 70%-90%, the old culture medium was removed and 0.5 mL of lentivirus (5-8 mg/L) was added to each well. To establish cell lines stably expressing hsa-miR-101, we purchased an hsa-miR-101-1 overexpression-lentiviral vector (GV309-hU6-MCS-Ubiquitin-EGFP-IRES-puromycin) and hsa-miR-101-3p-interfering lentiviral vector (GV280-hU6-MCS-Ubiquitin-EGFP-IRES-puromycin) from Shanghai Genechem Co., Ltd. MDA-MB-231 cells were co-transfected with miR-101-1 or miR-101-3p vector and the helper plasmids (Helper 1.0 and Helper 2.0) for 6 h. The old culture media were replaced with fresh media and cells were incubated for 72 h post-transfection. The collected supernatant was used to isolate viral particles by centrifugation at 25 000 ×*g* at 4 ℃ for 2 h. The pellet was preserved and dissolved in a virus-preservation solution. After centrifugation at 10 000 rpm at 4 ℃ for 5 min, the supernatant was aliquoted for further construction of stable cell lines. Parts of the aliquoted viruses were used to determine virus titers using fluorescence.

2.4. Western blot analysis

Cells in different treatment groups were collected and then lysed in RIPA buffer with 1 mM phenylmethanesulfonyl fluoride (PMSF) on ice for 10-15 min, followed by sonication as follows: 20 times at 40 W, 1 s each at 2 s apart, and centrifugation at 12 000 ×*g* for 15 min at 4 ℃. The protein concentration of the collected supernatant was measured using the BCA Protein Assay Kit (Biyuntian). The protein concentration of all samples was adjusted to 2 μg/μL, and then 1/5

volume of 6× loading buffer was added, mixed well, heated in a 100 ℃ metal bath for 10 min, and centrifuged briefly for further use. After separation on a 10% SDS-PAGE gel, proteins were transferred onto a PVDF membrane, followed by incubation with 5% non-fat milk/Tween-Tris buffered saline for 1 h and incubation with primary antibodies (DLG5, 15687-1-AP, 1∶500; GAPDH, sc-32233, 1∶2 000) at 4 ℃ overnight. On the following day, the membranes were washed and further incubated with horseradish peroxidase-labeled secondary antibodies: anti-rabbit IgG (1∶3 000) or anti-mouse IgG (1∶3 000) at room temperature for 1 h. The target bands were detected using an ECL agent and visualized using X-ray film, with GAPDH as an internal reference. All antibodies were purchased from Abcam Inc. (Cambridge, USA).

2.5. Quantitative real-time PCR (qRT-PCR)

Trizol reagent (Shanghai Pufei) was used to isolate total RNA. The M-MLV Reverse Transcription Kit (Promega) was used for synthesis of cDNA from the isolated RNA. An miRNA Reverse PCR kit (Guangzhou RiboBio) was used for reverse transcription of miRNAs. qRT-PCR was used to quantify both mRNA and miRNA expression using a SYBR Green kit (Takara) and LightCycler 480 II (ROCHE). The $2^{-\Delta\Delta Ct}$ method was used to quantify the relative expression levels of mRNA and miRNA, in which target mRNA was normalized to *GAPDH* and target miRNA was normalized to *U6*. The primer sequences were as follows: *miR-101-1*: forward 5'-CGATGAAGCTGAGCGTAGA-3', reverse 5'-TGCGAAGCTGAAGCGTGAG-3'; *DLG5*: forward 5'-ACGGAAGTTGTAGAGTTCGA-3', reverse 5'-ATTCTCAGCAGCCAGTCATT-3'; *GAPDH*: forward 5'-TGACTTCAACAGCGACACCCA-3', reverse 5'-CACCC TGTTGCTGTAGCCAAA-3'; *U6*: forward 5'-GAATCGAACGCTGATGCCA-3', reverse 5'-ATCGAGCCGATGAGGCTA-3'.

2.6. Detection of differentially expressed miRNAs

NanoDrop 2000 was used to measure RNA concentration and the Agilent Bioanalyzer 2100 was used to determine the quality of the purified RNA. The RNA samples were used in the GeneChip™ miRNA 4.0 Array, and the results were utilized in bioinformatics analysis of miR expression between the control group and the PMA-treated group to identify differentially expressed target genes.

2.7. Scratch assay

Cells were split, placed in a 96-well plate, and grown overnight. A scratch was created by gently disrupting the cell layer along a line in the central part of a 96-well plate using a scratch instrument. After washing twice and adding fresh culture medium containing 1% FBS, cells were continually cultured for 24 h. The migrated cells within the scratch area were observed and calculated at 0 h and 24 h.

2.8. Exosome endocytosis assay

The exosome endocytosis assay was performed in a 96-well plate, in which 2 000 cells were plated per well. The exosomes secreted by TAMs were collected and added to the working solution containing 2 μM of PKH26, a lipophilic dye that stably integrates into the cell membrane without disturbing the expression of surface markers. The exosomes and dye were mixed for 5 min at room temperature. The mixed dye solution was filtered through a 0.22 μm filter, and the filtrate was added to the cultured cells (20 μL/well). Finally, the stained cells were counted at 0 h, 3 h, and 6 h.

2.9. Transwell migration assay

The migration ability of the cancer cells in the different treatment groups was compared. A drug-free medium was added to the control group, while a medium containing 400 μg/mL of the PKH26 cell linker kit labeled exosomes was added to the experimental group. After 48 h, the MDA-MB-231 cells were split into a Transwell chamber (Corning) at a density of 10^5 cells per well, and 600 μL of 30% FBS medium was added to the lower chamber. The cells were cultured for 16 h and the cells that migrated into the lower chamber were fixed with 4% paraformaldehyde (Sinopharm Group) at room temperature for 30 min and then stained with 0.5% crystal violet (Shanghai source). The number of stained migrated cells was determined under a microscope (XDS-100 Leaf organisms, R20755).

2.10. Dual luciferase reporter assay

Bioinformatics software was used to predict the binding sites of miR-101 and *DLG5*. The 3'UTR sequences of *DLG5* and its mutants were cloned into the luciferase reporter plasmid GV272 vector (Genechem) to construct wild-type and mutant recombinant dual luciferase reporter plasmids, respectively. PCR and gene sequence analysis were used to verify the construction of dual luciferase reporter plasmid. Cells were randomly divided into 6 groups and transfected with 1) DLG5-3'UTR-NC plus miR-101-1-NC, 2) DLG5-3'UTR-NC plus miR-101-1, 3) DLG5-3'UTR plus miR-101-1-NC, 4) DLG5-3'UTR plus miR-101-1, 5) DLG5-3'UTR-M plus miR-101-1-NC (mutant), 6) DLG5-3'UTR-M plus miR-101-1 (mutant). The Dual-Luciferase® Reporter Assay System (PROMEGA) was used to determine luciferase reporter activity in the transfected cells.

2.11. Statistical analysis

All data are expressed as mean ± standard deviation. SPSS 17.0

software (SPSS, Inc., USA) was used for statistical analysis. Differences between groups were analyzed using one-way analysis of variance, and $P<0.05$ was considered significantly different.

3. Results

3.1. Inhibitory effect of M2 macrophage–secreted exosomes on breast cancer cell migration

For sequential experiments, stimulation of THP-1 cells with PMA (500 ng/mL) for 48 h resulted in M2 macrophage-secreted exosomes that were identified using nanoparticle tracking analysis (Figure 1A). The M2 macrophage-secreted exosomes were labeled with PKH26, a lipophilic dye to detect endocytosis in MDA-MB-231 cells. Exosomes entered the cell cytoplasm 3 h after incubation with the labeled exosomes and reached a significant enrichment at 6 h (Figure 1B). The concentration of exosomes used for wound healing and Transwell assays (24 h) in MDA-MB-231 cells was 400 μg/mL, and the untreated cells were used as controls. As shown in Figure 2, M2 macrophage-secreted exosomes significantly inhibited the migration of MDA-MB-231 cells in the scratch assay and Transwell chamber assay, compared with the control group.

3.2. RNA sequencing of exosomes secreted by PMA–treated THP–1 cells

We next used miRNA sequencing to identify RNAs that play a major role in exosomes. RNA sequencing data of the M2 macrophage-secreted exosomes were analyzed using R software.

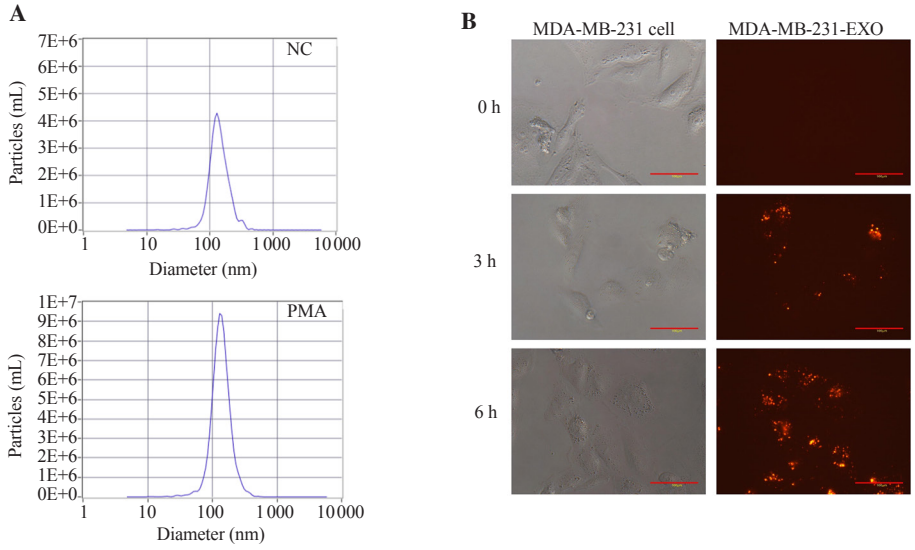

Figure 1. (A) Normal THP-1 cells and M2 macrophages are induced by 500 ng/mL PMA for 48 h. M2 macrophage-secreted exosomes are identified using nanoparticle tracking analysis. (B) Observation of endocytic exosomes in MDA-MB-231 cells (magnification: 100×). PMA: phorbol-12-myristate-13-acetate; EXO: exosome; NC: negative control.

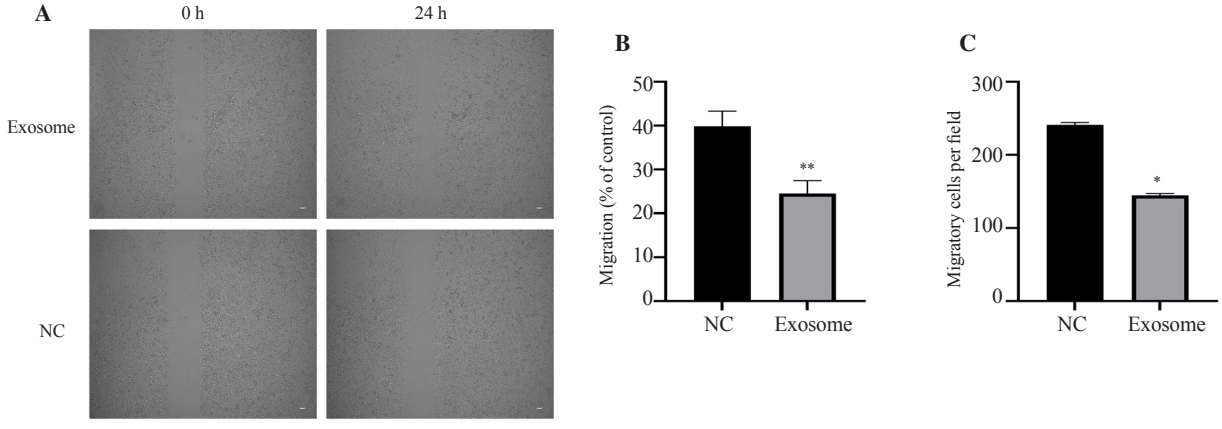

Figure 2. Effect of M2 macrophage-secreted exosomes on the migration of MDA-MB-231 cells using (A-B) scratch assay and (C) Transwell assay (magnification: 100×). $^{*}P<0.05$ and $^{**}P<0.01$ *vs.* the negative control (NC) group.

Sequencing identified 741 differentially expressed miRNAs, including 35 upregulated miRNAs and 706 downregulated miRNAs. Upregulated or downregulated differentially expressed genes based on padj < 0.05 AND $\log_2$FC > 0.5 or $\log_2$FC < −0.5 are shown in Figure 3. RNA sequencing results showed that miR-101-3p expression was significantly upregulated. Targetscan predicted that miR-101-3p could target DLG5 (Figure 4A). The dual luciferase reporter assay showed that reporter activity was significantly reduced in the DLG5 wild type group compared to the DLG5 mutant group (P<0.001), indicating that miR-101-3p and DLG5 expression are correlated (Figure 4B) and miR-101-3p could bind to the 3'UTR of DLG5 mRNA to suppress its expression.

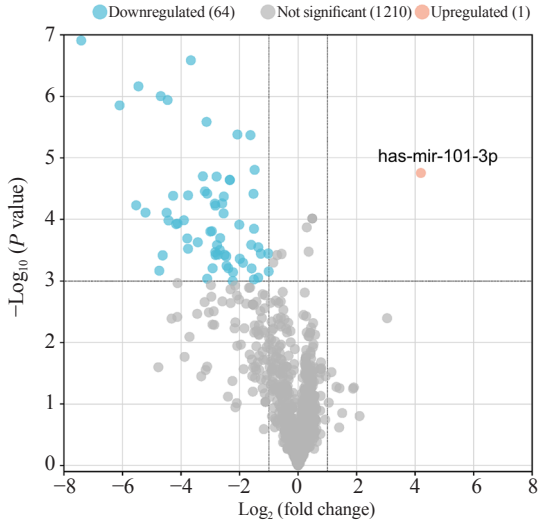

Figure 3. GeneChip™ miRNA 4.0 Array analysis of significant differentially expressed genes after treatment with exosomes. Volcano map shows the differential expression of microRNA. The threshold of screening is > 2 fold change and P<0.001. microRNA-101 is screened out and its expression is upregulated. Downregulated microRNAs are shown in blue color, and upregulated microRNA is demonstrated in orange color.

3.3. miR-101 can regulate DLG5 expression in breast cancer cells

The miR-101-3p was stably expressed in MDA-MB-231 cells, and cells with miR-101-3p knockdown were established by infecting the corresponding lentiviral constructs (Figure 5A). The mRNA level of DLG5 was determined in the no-load vector group and the miR-101 knockdown group and untreated MDA-MB-231 cells (NC). We found that the DLG5 mRNA level was increased in the miR-101-3p knockdown group compared to the NC group (Figure 5B). The upregulating effect of miR-101-3p knockdown on DLG5 protein expression was also confirmed using Western blot analysis (Figure 5C-D). As shown in Figure 6, miR-101-3p inhibition significantly increased the migration of MDA-MB-231 cells in the scratch assay (Figure 6A-B).

4. Discussion

Breast cancer is a common female malignancy that has been associated with changes in estrogen levels, lifestyle, and environmental factors[16]. Many breast cancer patients have metastasized lesions before diagnosis, which significantly impacts treatment and prognosis. Therefore, there is an urgent need to understand the key mechanism of breast cancer development and metastasis. miRNA plays a vital important role in the development of tumor cells and endothelial-to-mesenchymal transition (EMT). miRNA can regulate the EMT process mediated by oncogenes or tumor suppressor genes, thus promoting tumor occurrence, development, and distant metastasis. miR-101-3p is a member of the miR-101 family. As an important member, miR-101-3p has anti-tumor effects in many cancers and could become a potential therapeutic target[17,18]. miR-101-3p expression was low in prostate cancer tissues, and overexpression of miR-101-3p was shown to inhibit the proliferation, metastasis, and invasion of prostate cancer cells, thus playing an anti-tumor role[19]. Wang *et al.* found that miR-101-3p was downregulated in colorectal cancer tissues, and

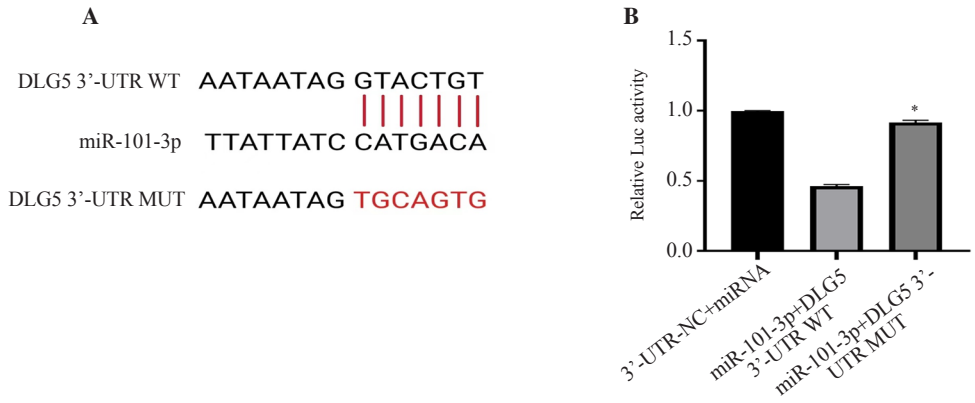

Figure 4. Bioinformatics software was applied to predict the binding sites of miR-101-3p and DLG5. Luciferase reporter activity of chimeric vectors carrying the luciferase gene and a fragment of the DLG5 3'-UTR containing the wild-type (WT) or mutated (MUT) miR-101 binding site (n=3), $^*P$<0.001 *vs.* the miR-101-3p+DLG5 3'-UTR WT group. The mutated nucleotides are shown in red color.

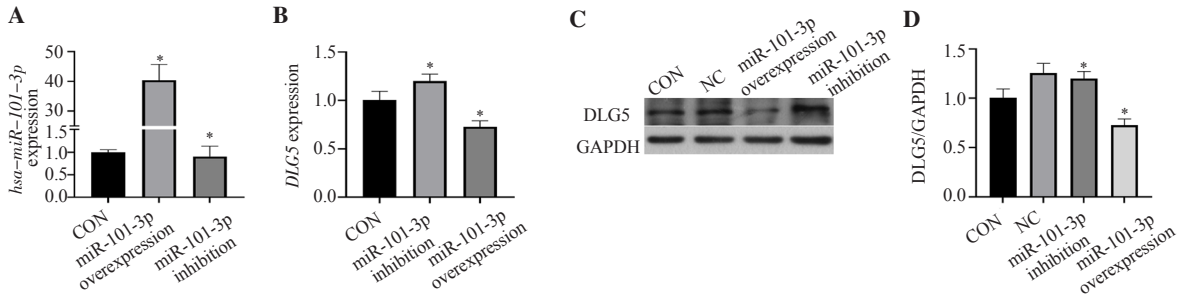

Figure 5. Effects of miR-101-3p on DLG 5 mRNA and protein expression in MDA-MB-231 cells. (A) *miR–101–3p* was measured using qRT-PCR assay. *U6* was used as an internal control (n=3), $^*P$<0.05 *vs*. the CON group. (B) *DLG5* mRNA expression in MDA-MB-231 cells with miR-101-3p overexpression or miR-101-3p inhibition. *GAPDH* was used as an internal control (n=3), $^*P$<0.05 *vs.* the CON group. (C-D) DLG5 protein expression using Western blot analysis. GAPDH was used as an internal control (n=3), $^*P$<0.05 *vs.* the NC group. CON stands for the blank cell group; NC stands for the empty virus group.

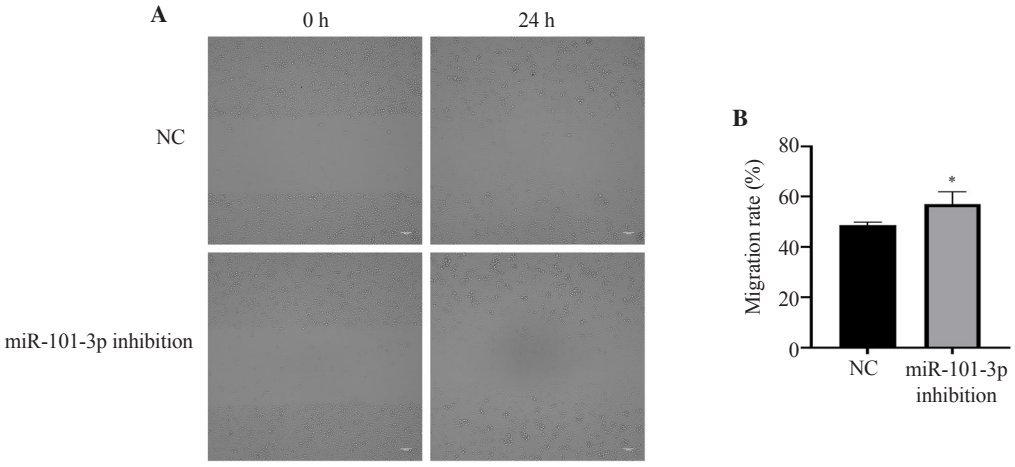

Figure 6. Effect of miR-101-3p inhibition on the migration of MDA-MB-231 cells at 0 h and 24 h in scratch assay (magnification: 100×). $^*P$<0.05 *vs.* the NC group.

the increased expression of miR-101-3p inhibited the proliferation, migration, and invasion of colorectal cancer cells[20]. However, the effects of miR-101-3p and its target gene on tumor invasion and migration are not fully investigated yet. For example, tumor-associated fibroblasts promoted the secretion of vascular endothelial growth factor A mediated by miR-101-3p, and the AKT/eNOS pathway mediated the migration and invasion of non-small cell lung cancer cells[21]. In gallbladder cancer, miR-101-3p regulates the MAPK/Erk and Smad pathways by targeting zinc finger protein X-linked (ZFX), thus inhibiting the proliferation and apoptosis of tumor cells[22]. However, the role of miR-101-3p in breast cancer and the mechanism by which it influences the invasion and migration of cancer cells *via* DLG5 remain unclear.

DLG5 overexpression was detected in normal tissues as well as low-grade cancer tissues and cells, while in high-grade cancer tissues and cells, DLG5 expression was decreased or absent. As a primary target of progesterone, DLG5 overexpression was also detected in luminal breast cancer[23] and was associated with development, progression, and prognosis of breast cancer. Thus, DLG5 participation in multiple biological functions has been reported, including cell migration and invasion, epithelial polarity, and EMT, which might explain in part the inhibitory effects of M2 macrophage-secreted exosomes on breast cancer cell migration.

Our RNA sequencing analysis showed that miR-101-3p expression was upregulated in breast cancer MDA-MB-231 cells treated with exosomes. M2 macrophage-secreted exosomes exerted inhibitory effects on MDA-MB-231 cell migration. The binding site between miR-101-3p and *DLG5* was identified using Targetscan software. Our dual-luciferase reporter assay showed that DLG5 expression could be regulated by miR-101 *via* binding to DLG5 mRNA 3'-UTR. Moreover, upregulated miR-101-3p can inhibit DLG5 mRNA and protein expression.

In conclusion, this study suggests that exosomes derived from TAMs play an important role in the migration of breast cancer cells. Our findings further indicate that exogenous miR-101-3p from TAMs may inhibit the migration of breast cancer cells by regulating the expression of DLG5, which provides the necessary experimental basis for further research on exogenous exosomes of TAMs.

Conflict of interest statement

The authors declare that they have no competing interests.

Funding

This project was supported by the Key Research and Development Program of Hainan Province (ZDYF2020139, ZDYF2018158) and the Science and Technology Funding Project of Hainan Province (821MS129).

Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

YL conceptualized the study. CW, JX, YZ, SY, and QH performed experiments. YL and RY designed the experiments. YL, CW, and JX supervised experiments. All authors analyzed data and contributed to discussion. CW and YL wrote the paper. JX was the guarantor of this work and had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final manuscript.

References

[1] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2021; **71**(3): 209-249.

[2] Hanker AB, Sudhan DR, Arteaga CL. Overcoming endocrine resistance in breast cancer. *Cancer Cell* 2020; **37**(4): 496-513.

[3] Xu S, He X, Zhang L, Wang G, Luo T, Xu X, et al. Negative regulatory effect of microRNA-100 on the proliferation of breast cells and breast cancer cells. *Acta Universitatis Medicinalis Anhui* 2020; **55**(2): 177-182.

[4] Sangpairoj K, Settacomkul R, Siangcham T, Meemon K, Niamnont N, Sornkaew N, et al. Hexadecanoic acid-enriched extract of *Halymenia durvillei* induces apoptotic and autophagic death of human triple-negative breast cancer cells by upregulating ER stress. *Asian Pac J Trop Biomed* 2022; **12**(3): 132-140.

[5] Buranrat B, Junking M. Piperine suppresses growth and migration of human breast cancer cells through attenuation of Rac1 expression. *Asian Pac J Trop Biomed* 2022; **12**(1): 39-46.

[6] Cansaran-Duman D, Tanman Ü, Yangın S, Atakol O. The comparison of miRNAs that respond to anti-breast cancer drugs and usnic acid for the treatment of breast cancer. *Cytotechnology* 2020; **72**(6): 855-872.

[7] Han L, Chen WJ, Xia YM, Song YR, Zhao Z, Cheng H, et al. MiR-101 inhibits the proliferation and metastasis of lung cancer by targeting zinc finger E-box binding homeobox 1. *Am J Transl Res* 2018; **10**(4): 1172-1183.

[8] Zhao H, Cao H, Ning F. Mechanism of microRNA-101 targeting pre-B-cell leukemia transcription factor 3 affecting the proliferation and apoptosis of acute T lymphocyte leukzemia cells. *Chin J Cancer Prev Treat* 2020; **27**(20): 1605-1615.

[9] Franklin RA, Li MO. Ontogeny of tumor-associated macrophages and its implication in cancer regulation. *Trends Cancer* 2016; **2**(1): 20-34.

[10] Cheng H, Wang Z, Fu L, Xu T. Macrophage polarization in the development and progression of ovarian cancers: An overview. *Front Oncol* 2019; **9**: 421.

[11] Pegtel DM, Gould SJ. Exosomes. *Annu Rev Biochem* 2019; **88**: 487-514.

[12] Zhang J, Li S, Li L, Li M, Guo C, Yao J, et al. Exosome and exosomal microRNA: Trafficking, sorting, and function. *Genomics Proteomics Bioinformatics* 2015; **13**(1): 17-24.

[13] Yang F, Ning Z, Ma L, Liu W, Shao C, Shu Y, et al. Exosomal miRNAs and miRNA dysregulation in cancer-associated fibroblasts. *Mol Cancer* 2017; **16**(1): 148.

[14] Liu J, Li J, Li P, Wang Y, Liang Z, Jiang Y, et al. Loss of DLG5 promotes breast cancer malignancy by inhibiting the Hippo signaling pathway. *Sci Rep* 2017; **7**: 42125. doi: 10.1038/srep42125.

[15] Guen VJ, Chavarria TE, Kröger C, Ye X, Weinberg RA, Lees JA. EMT programs promote basal mammary stem cell and tumor-initiating cell stemness by inducing primary ciliogenesis and Hedgehog signaling. *Proc Natl Acad Sci U S A* 2017; **114**(49): e10532-e10539.

[16] Wen S, Tan W, Liu W. Research progress of microRNA-34a in malignant tumors. *Chin J Clin Res (Chin)* 2020; **33**(10): 1432-1434, 1438.

[17] Park J, Cho M, Cho J, Kim EE, Song EJ. MicroRNA-101-3p suppresses cancer cell growth by inhibiting the USP47-induced deubiquitination of RPL11. *Cancers (Basel)* 2022; **14**(4). doi: 10.3390/cancers14040964.

[18] Wang H, Xiao R, Yang B. MiR-101-3p suppresses progression of cervical squamous cell carcinoma by targeting and down-regulating KPNA2. *Technol Cancer Res Treat* 2021; **20**. doi: 10.1177/15330338211055948.

[19] Gu Z, You Z, Yang Y, Ding R, Wang M, Pu J, et al. Inhibition of MicroRNA miR-101-3p on prostate cancer progression by regulating Cullin 4B (CUL4B) and PI3K/AKT/mTOR signaling pathways. *Bioengineered* 2021; **12**(1): 4719-4735.

[20] Wang S, Sun H, Zhang S, Chen L, Chen X, Li W. lncRNA TDRG1 promotes proliferation, invasion and migration of colorectal cancer cells by regulating miR-101-3p. *Chin J Pathophysiol* 2021; **37**(5): 879-884.

[21] Guo X, Chen M, Cao L, Hu Y, Li X, Zhang Q, et al. Cancer-associated fibroblasts promote migration and invasion of non-small cell lung cancer cells *via* miR-101-3p mediated VEGFA secretion and AKT/eNOS pathway. *Front Cell Dev Biol* 2021; **9**. doi: 10.3389/fcell.2021.764151.

[22] Bao RF, Shu YJ, Hu YP, Wang XA, Zhang F, Liang HB, et al. miR-101 targeting ZFX suppresses tumor proliferation and metastasis by regulating the MAPK/Erk and Smad pathways in gallbladder carcinoma. *Oncotarget* 2016; **7**(16): 22339-22354.

[23] Smolen GA, Zhang J, Zubrowski MJ, Edelman EJ, Luo B, Yu M, et al. A genome-wide RNAi screen identifies multiple RSK-dependent regulators of cell migration. *Genes Dev* 2010; **24**(23): 2654-2665.

Publisher's note