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Bone Marrow Stromal Cells inhibited the growth and metastasis of human U87 cells through delivering exosomal miR-506

Liexiang Zhang[a], Yu Ding[a], Wei Zhou[a], Xiaohong Xu[b], Jing Zheng[a,*]

Abstract

Glioma is one of the malignancy brain tumors, which deeply threaten the health of patients. Although the traditional therapies for glioma have improved, the outcome is still far from satisfactory. Bone Marrow Stromal Cells (BMSC)-based therapy provided novel insight in the treatment for glioma. However, the detailed molecular mechanism is still not clear. The aim of present study is to discover the novel factor in BMSC-based therapy for glioma. The cell proliferation and apoptosis were identified by using CCK-8 and flow cytometry. The invasion of glioma cells was examined by using Transwell assay and wound-healing assay respectively. qRT-PCR was used to examine the expression of miR-506. Western blot was used to examine the protein levels of CD63, TSG101, NUR77 and CXCR4. Our data suggested that BMSC-derived exosome inhibited the proliferation and contributed to apoptosis of human U87 cells after culturing with miR-506 mimic. Overexpression of miR-506 in BMSC-derived exosome inhibited the invasion of human glioma U87 cells, while these effects were deeply suppressed in the presence GW4869. Our present study demonstrated that BMSC inhibited the growth and metastasis of human glioma U87 cells through delivering exosomal miR-506, and provided the evidences to develop the BMSC-based therapy for glioma.

Abbreviations: BMSC = bone marrow stromal cells, CCK-8 = cell counting kit 8, qRT-PCR = quantitative reverse transcriptase polymerase chain reaction, WB = Western blot.

Keywords: BMSC, exosome, Glioma, miR-506

1. Introduction

Gliomas belong to the intrinsic malignant brain tumors with diverse pathological and histological properties, which are caused by neuroglial progenitor cells.[1] In recently decades, the number of patients with glioma grows with a rapid speed.[2] Worse still, the prognosis of gliomas remains poor and far from our expectation.[3] Despite the outcome of tradition therapies for glioma, including surgery, chemotherapy, and radiotherapy, have achieved promising improvements in the prognosis of glioma patients, the 5-year relative survival of glioma is <5%.[4] Therefore, the novel treatment towards glioma is highly desired.

Exosomes are a group of cell-derived vesicle with the diameter of 30 to 200 nm, which represent a new means of intercellular communication by delivering various bioactive molecules, including proteins, lipids and nucleic acids, and participate in tumor initiation and progression.[5] Growing evidences have demonstrated the progression of glioma is closely associated with exosome.[6,7] Glioma cells can communicate with their surroundings to create a tumor-permissive microenvironment.[8] Moreover, exosome-based nanoimmunotherapy is identified as a promising strategy for glioma.[9] However, the molecule mechanism is still not clear.

Bone Marrow Stromal Cells (BMSCs) are a population of non-hematopoietic skeletal progenitor cells, which have been confirmed to play a key role for the hematopoietic microenvironment.[10] It has been reported that BMSCs have the ability to migrate into these tumors and even track infiltrating tumor cells, making them to be promising cellular vehicles for delivering therapeutic agents to glioma cells.[11,12] Moreover, BMSCs-derived exosomes harboring miR-506 sensitized LUAD cells to DDP/HT both in vitro and in vivo.[13] Meanwhile, miR-506 has associated with the progression of colorectal cancer and functioned as a potential biomarker for breast Cancer.[14,15] Recent evidence has suggested that exosome-transmitted miR-506 inhibits the malignancy of colorectal cancer cells[16] and miR-506 is also involved in the castration-resistant regulation of prostate cancer cells.[17] However, the detailed function of miR-506 in human glioma cancer is still not clear.

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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[a] Department of Neurosurgery, Suqian Hospital Affiliated to Xuzhou Medical University and Suqian Hospital of Nanjing Drum Tower Hospital Group, Suqian City, Jiangsu Province, China, [b] Department of Nephrology, Suqian Hospital Affiliated to Xuzhou Medical University and Suqian Hospital of Nanjing Drum Tower Hospital Group, Suqian City, Jiangsu Province, China.

** Correspondence: Jing Zheng, Department of Neurosurgery, Suqian Hospital Affiliated to Xuzhou Medical University and Suqian Hospital of Nanjing Drum Tower Hospital Group. Huanghenan Road No.158, Sucheng District, Suqian City, Jiangsu Province, 223800, China (e-mail: neurosurgery001@163.com).*

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In the current study, we examined the expression of miR-506 in BMSC-derived exosome and explore its function in human glioma cancer cells. Our findings not only gain a deep insight into the biological function of miR-506 but also provided preliminary evidences to indicate the value of miR-506 as a potential target in the therapy for human glioma.

2. Methods

2.1. Cell culture

Human BMSC and U87 were obtained from the American Type Culture Collection (ATCC, Manassas, USA).

2.2. Exosomes uptake by human U87 cells

Human BMSC-derived exosomes (50 μg/mL) were cultured with PKH67 at room temperature for 5 minutes. Then, the labeled exosomes were suspended and incubated with human U87 cells at 37°C for 12 hours. DAPI were used to stain the nuclei for 10 minutes. Then, observed by using IX53 fluorescence microscope (Olympus, Tokyo, Japan).

2.3. Cell counting kit (CCK)-8 assay and

Briefly, cells (3×10^3 cells/well) in 96-well plates were incubated with CCK-8 solution (10 μL/ cell) for 1 hour. The cell viability was examined by evaluating the value of OD450 nm. Three replications are performed for each reaction.

2.4. Cell apoptosis detection

Flow cytometry was used to determine the cell apoptosis as previously reported.[18] Cells in a 6-well plate (3×10^5 per well) were incubated with 5 μL Annexin-V-FITC for 15 minutes and 5 μL propidium iodide for 15 minutes, all from Beyotime Biotechnology (Shanghai, China), and finally, examined by a CytoFLEX flow cytometer (Beckman Coulter, USA). Three replications are performed for each reaction.

2.5. Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR)

In the present study, qRT-PCR was performed using SYBR green PCR master mix (Applied Biosystems, Foster, CA, USA) on an ABI 9700 real-time PCR system (Applied Biosystems). The primers used for PCR were as follows: miR-506-F: 5"-AGCCAGCGTATTCAGGAAGGT-3"; miR-506-R: 5'-GTCGTATCCAGTGCAGGGTCC-3'; U6-F: 5'-CTCGCTTCGGCAGC ACA-3'; U6-R: 5'-AACGCTTCA CGAATTTGCGT-3'.

2.6. Western blot analysis

After SDS-PAGE gel separation, proteins were transferred onto nitrocellulose membranes, which were then incubated with primary antibodies against Calnexin(ab227310; Abcam), CD63(ab271286; Abcam), TSG101(ab125011; Abcam), NUR77(ab283264, abcam), CXCR4 (ab124824, abcam), AKT1(# 75692, cell signaling), p-AKT1 (# 9018, cell signaling), and GAPDH (66009-1-Ig; Proteintech) followed by HRP-conjugated secondary antibodies (ZB-2305; ZSGB-BIO).

2.7. Wound-healing assay

The human U87 cells (4×10^5) were seeded in 6-well plates and cultured until they reached a density of 90%. A 200-μL micropipette tip was then used make a wound in the cells and the medium was replaced with serum-free medium. Electron microscopy was used to observe the shape of wound at 0 and 24 hours.

2.8. Transwell assay

Cell invasion were assayed in a 24-well plate with polycarbonate sterile chambers (8-μm filters; BD Biosciences, Franklin Lakes, NJ, USA) with or without Matrigel coating. The human U87 cells (2×10^4) were cultured with 100 μL of serum-free DMEM in the upper chamber and 600 μL of DMEM + 10% serum in the lower chamber. After 24 hours, the lower chamber was washed twice with PBS and crystal violet dye was added to the lower chamber incubated for 20 minutes. The lower chamber was washed with PBS 3 times, following which a cotton bud was used to remove cells and medium from the upper chambers. The invaded cells in the lower chambers were observed under an electron microscope.

2.9. Statistical analysis

All statistical analyses on quantitative data (mean ± SD) were done on GraphPad Prism 8.4.2 (GraphPad Software, San Diego, CA, USA). Overall survival (OS) was determined by Kaplan–Meier survival analysis and log-rank test. Comparison between different groups was performed with ANOVA test or Student *t* test, with P value < .05 as threshold of significance.

3. Results

3.1. BMSC-derived exosome inhibited the proliferation and contributed to apoptosis of human U87 cells after culturing with miR-506 mimic

The exosome of BMSC was isolated and purified as described previously.[19] As shown in Supplemental Digital Content, our data suggested that the exosomes are negative for Calnexin and positive for CD63 and TSG101 (Figure S1A, http://links.lww.com/MD/L28). Next, we chose human U87 cells as the recipient cells of the BMSC-derived exosomes. We labeled the exosomes with PKH67 (green) and added these exosomes into human U87 cells. Using confocal microscopy, we found that the human U87 cells absorbed the BMSC-derived exosomes (Figure S1B, http://links.lww.com/MD/L28).

Then, the BMSC and corresponding derived exosome (BMSC-exo) were cultured with GW4869 (a well-known inhibitor of exosome biogenesis/release). As shown in Figure 1A, the level of miR-506 showed no significant difference in BMSC with or without the treatment of GW4869. However, GW4869 deeply suppressed the expression of miR-506 in BMSC-derived exosome. To explore the function of miR-506, the miR-506 mimic was used to induce miR-506 overexpressed in BMSC and BMSC-exo (Fig. 1B). As illustrated in Figure 1C, the exosome derived from BMSC that pretreatment by miR-506 mimic (Exo-mimic) deeply suppressed the proliferation of human U87 cells compared with that in corresponding miNC treated exosome (Exo-miNC). Meanwhile, the apoptosis of human U87 cells was significantly upregulated after co-culturing with BMSC-derived exosome and Exo-mimic presented a stronger effect than that of Exo-miNC (Fig. 1D). Western blot showed that Exo-mimic also inhibited the protein contents of miR-506 potential targets in human U87 cells, including Nur77, CXCR4, and phosphorylated AKT(p-AKT) (Fig. 1E).

3.2. Exo-mimic inhibited the invasion of human U87 cells.

Next, we explore the function of Exo-mimic in the invasion of human U87 cells. As shown in Figure 2A and 2B,

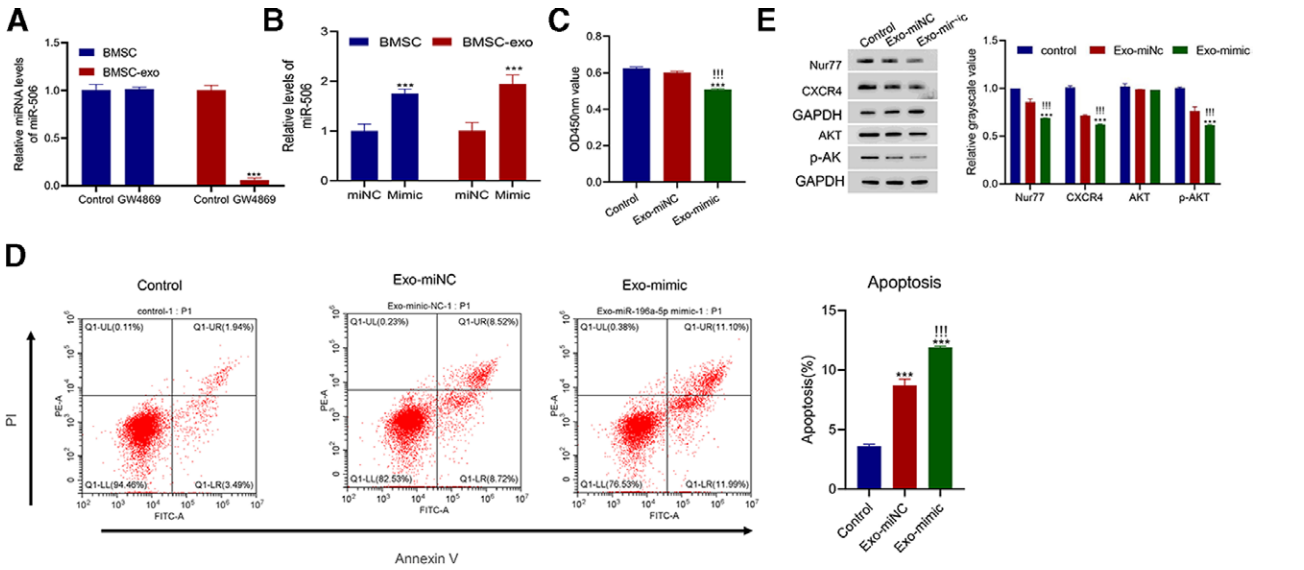

Figure 1. The miR-506 mimic BMSC derived exosome inhibited the proliferation and contributed to apoptosis of human U87 cells. (A) The relative level of miR-506 in BMSC and corresponding exosome. ***$P < .001$ vs Control. (B) miR-506 mimic was used to induce miR-506 overexpression in BMSC and corresponding exosome. ***$P < .001$ vs miNC. (C) The proliferation of human U87 cells after co-culturing with Exo-miNC or Exo-mimic. ***$P < .001$ vs control,!!! $P < .001$ vs Exo-miNC. (D) The apoptosis of human U87 cells after co-culturing with Exo-miNC or Exo-mimic. ***$P < .001$ vs control,!!! $P < .001$ vs Exo-miNC. (E) Western blot was used to determine the protein levels of Nur77, CXCR4, AKT and p-AKT, ***$P < .001$ vs. control,!!! $P < .001$ vs. Exo-miNC.

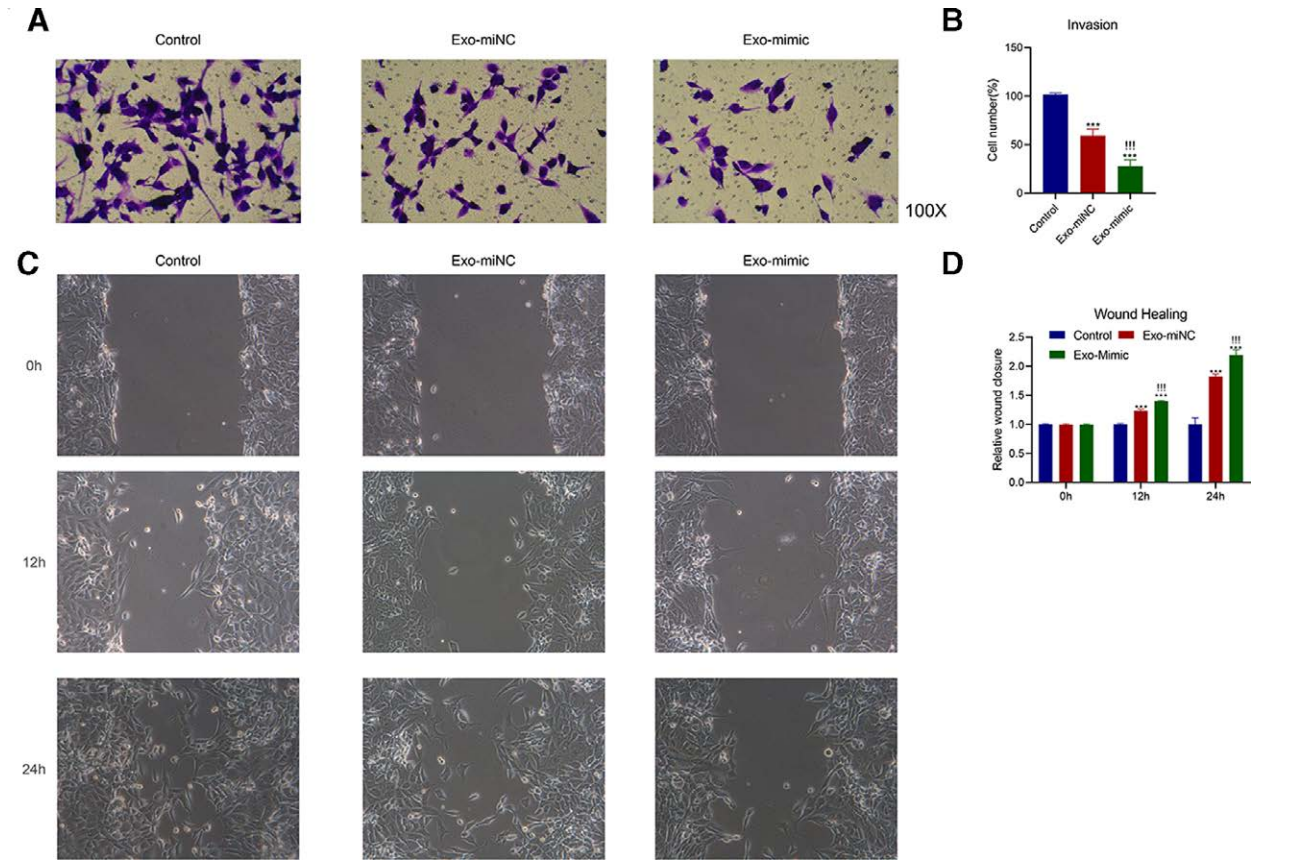

Figure 2. Exo-mimic inhibited the migratory ability of human U87 cells. (A, B) Transwell assay was used to examine the invasion of human U87 cells after co-culturing with Exo-miNC and Exo-mimic. ***$P < .001$ vs control,!!! $P < .001$ vs Exo-miNC. (C, D) Wound healing assay indicated that Exo-mimic suppressed the migratory ability of human U87 cells. ***$P < .001$ vs control,!!! $P < .001$ vs Exo-miNC.

the invasion cells of human U87 were significantly decreased after co-culturing with Exo-miNC or Exo-mimic. Moreover, results that obtained from wound healing assay indicated that the migratory ability of human U87 cells was deeply suppressed after co-culturing with Exo-mimic at 12 or 24h (Figs. 2C and 2D).

3.3. The BMSC promoted the apoptosis and inhibited the proliferation of human U87 cells through delivering exosomal miR-506

To further explore the role of BMSC-derived exosome, the BMSC, miR-506 mimic and GW4869 were used to culture human U87 cells. As shown in Figures 3A and 3B, the apoptosis of human U87 cells was increased after co-culturing with BMSC and further enhanced in the absence of miR-506 mimic, while this function was deeply abolished by an inhibitor of exosome biogenesis/release GW4869. The similar results were also obtained from in proliferation analyzing (Fig. 3C). Moreover, the protein contents of Nur77 and CXCR4 were deeply downregulated by miR-506 mimic in BMSC co-culturing human U87 cells, while this effect was disrupted by GW4869. The phosphorylation of AKT was also suppressed by miR-506 mimic, while remarkably recovered by GW4869 in BMSC co-culturing human U87 cells (Fig. 3D).

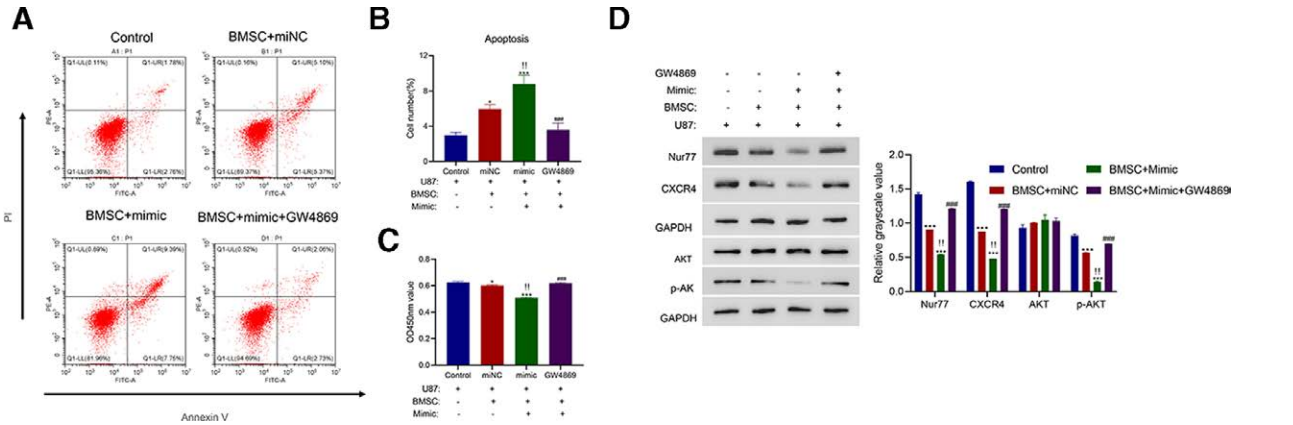

Figure 3. BMSC inhibited the growth of human U87 cells through delivering exosomal miR-506. (A, B) Flow cytometry was used to examine the apoptosis of cells as indicated above. *$P < .05$ vs control, ***$P < .001$ vs control, !!$P < .01$vs miNC, ### $P < .001$ vs GW4869. (C) CCK-8 assay was used to examine the proliferation in cells as indicated above. * $P < .05$ vs control, *** $P < .001$ vs control, !!$P < .01$vs miNC, ### $P < .001$ vs GW4869. (D) Western blot was used to determine the protein contents of Nur 77, CXCR4, AKT and p-AKT in different cells. *** $P < .001$ vs control, !!$P < .01$ vs miNC, ### $P < .001$ vs GW4869.

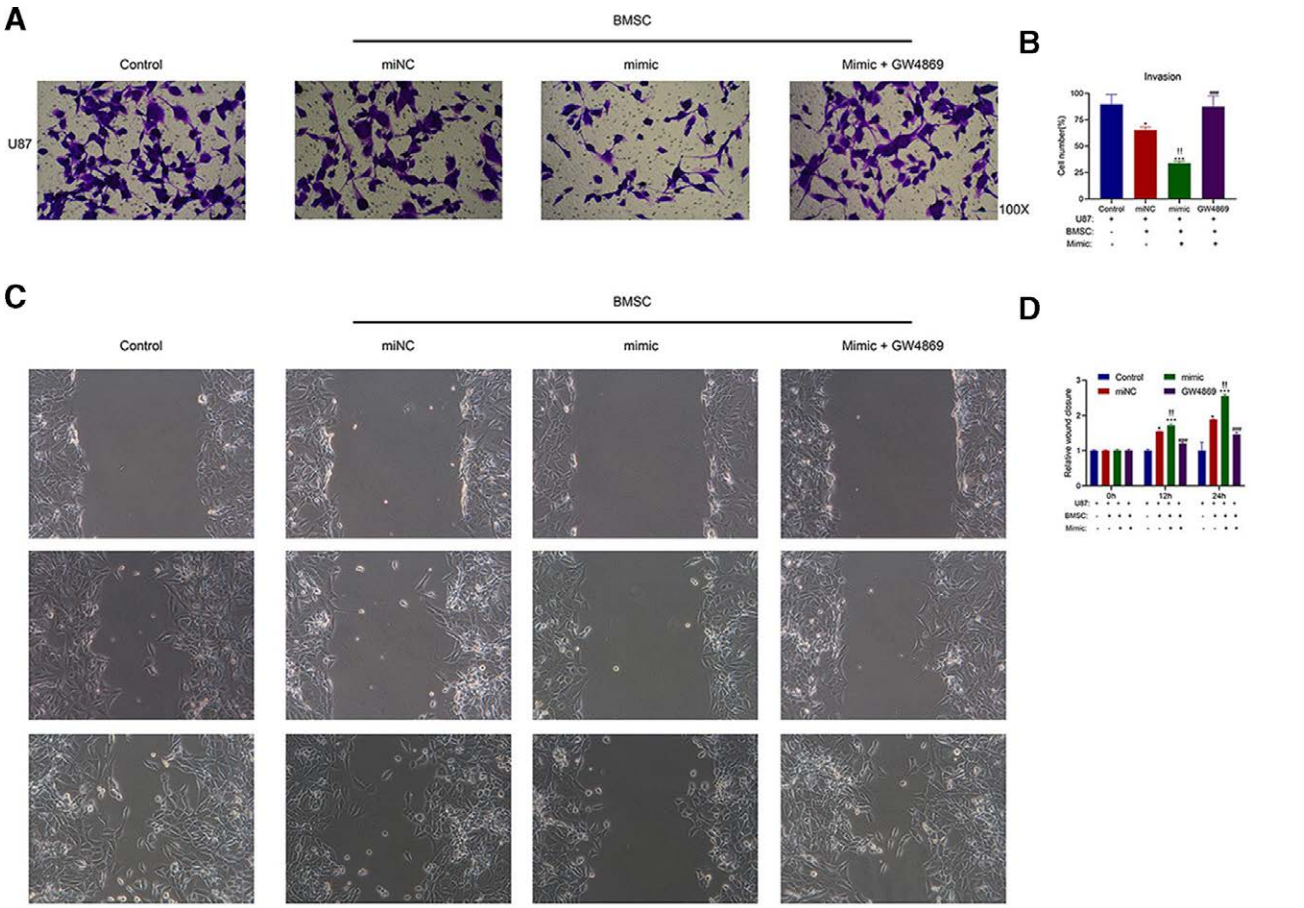

Figure 4. The BMSC disrupted the migratory ability of human U87 cells U87 cells through delivering exosomal miR-506. (A, B) Transwell assay was used to examine the invasion of human U87 cells that co-culturing with or without mimic or GW4869. *$P < .05$ vs control, ***$P < .001$ vs control, !!$P < .01$vs miNC, ###$P < .001$ vs GW4869. (C, D) Wound healing assay was used to examine the migratory ability.

3.4. The BMSC disrupted the migratory ability of human U87 cells U87 cells through delivering exosomal miR-506

As presented in Figures 4A and 4B, our data suggested that the miR-506 mimic significantly inhibited the invasion of human U87 cells that co-culturing with BMSC, while this effect was deeply abolished by GW4869. Then, results that from wound healing indicated that the migratory ability of human U87 cells was remarkably downregulated after co-culturing with BMSC in the presence of miR-506 mimic (Fig. 4C and 4D). Meaningfully, this effect also disrupted by GW4869. Together, present data suggested that BMSC inhibited the growth and metastasis of human U87 cells through delivering exosomal miR-506.

4. Discussion

In this study, our data suggested that BMSC-derived exosome contributed to inhibited the growth and invasion of human U87 cells. Here, the expression of miR-506 was induced upregulated in BMSC-derived exosome by using its corresponding mimic (Exo-mimic). Importantly, the Exo-mimic presented a stronger effect than that of corresponding Exo-miNC exosome, while this function was deeply disrupted in the presence of GW4869. Together, all these results demonstrated that overexpressed the expression of miR-506 in BMSC-derived exosome is a promising way in the therapy for human glioma cancer.

BMSCs have recognized as an important component of the glioma microenvironment, and the malignant transformation of BMSCs is closely related to glioma progression.[20] BMSC-derived exosomal has reduced cisplatin resistance of non-small cell lung cancer via delivering miR-193a.[21] Moreover, BMSC-derived exosomal miR-512-5p contributed to inhibit the progression of glioma.[22] In the current study, our results demonstrated that BMSC-derived exosomal miR-506 has the function to suppress the growth and invasion of glioma. Hence, our present findings demonstrated that the BMSC-based therapy for glioma via delivering microRNA.

Exosomes are a group of extracellular vehicles (EVs) with diameters ranging from 40 to 100 nm, which have the ability to deliver signaling molecules, including RNA, DNA, and proteins to surrounding cells.[23,24]

Previous report has identified that early invasion is identified as a major cause for poor therapeutic outcome of patient with glioma.[25] Here, our data illustrated that miR-506 presented as an anti-invasion factor in glioma, which can translocate from BMSC to glioma via exosome.

Moreover, our data indicated that the Exo-mimic inhibited the expression of several potential targets, including Nur77, CXCR4 and AKT. These results highlighted that Nur77, CXCR4 and AKT involved in the regulation of glioma metastasis. Further study will be meaningful to discover the detailed molecular mechanism. In the current time, the data from in vivo experiment and clinic part are still undergoing, which limited the significance of our findings. Hence, future researches are needed to provide these data to enhance our conclusion.

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Author contributions

Conceptualization: Liexiang Zhang, Yu Ding, Wei Zhou, Jing Zheng.
Data curation: Liexiang Zhang, Yu Ding, Wei Zhou, Jing Zheng.
Formal analysis: Wei Zhou.
Funding acquisition: Xiaohong Xu.
Investigation: Xiaohong Xu.
Writing – original draft: Jing Zheng.
Writing – review & editing: Jing Zheng.

References

[1] Xu S, Tang L, Li X, et al. Immunotherapy for glioma: Current management and future application. Cancer Lett. 2020;476:1–12.
[2] Nabors LB, Portnow J, Ahluwalia M, et al. Central Nervous System Cancers, Version 32020, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2020;18:1537–70.
[3] Kluckova K, Kozak J, Szaboova K, et al. TREM-1 and TREM-2 expression on blood monocytes could help predict survival in high-grade glioma patients. Mediators Inflamm. 2020;2020:1798147.
[4] Ostrom QT, Bauchet L, Davis FG, et al. The epidemiology of glioma in adults: a "state of the science" review. Neuro Oncol. 2014;16:896–913.
[5] He C, Zheng S, Luo Y, et al. Exosome Theranostics: Biology and Translational Medicine. Theranostics. 2018;8:237–55.
[6] Pan Z, Zhao R, Li B, et al. EWSR1-induced circNEIL3 promotes glioma progression and exosome-mediated macrophage immunosuppressive polarization via stabilizing IGF2BP3. Mol Cancer. 2022;21:16.
[7] Karami Fath M, Azami J, Masoudi A, et al. Exosome-based strategies for diagnosis and therapy of glioma cancer. Cancer Cell Int. 2022;22:262.
[8] Cheng J, Meng J, Zhu L, et al. Exosomal noncoding RNAs in Glioma: biological functions and potential clinical applications. Mol Cancer. 2020;19:66.
[9] Luo H, Zhang H, Mao J, et al. Exosome-based nanoimmunotherapy targeting TAMs, a promising strategy for glioma. Cell Death Dis. 2023;14:235.
[10] Li H, Ghazanfari R, Zacharaki D, et al. Isolation and characterization of primary bone marrow mesenchymal stromal cells. Ann N Y Acad Sci. 2016;1370:109–18.
[11] Feng J, Yao Z, Yang H, et al. Bone marrow-derived mesenchymal stem cells expressing BMP2 suppress glioma stem cell growth and stemness through Bcl-2/Bax signaling. J Cancer Res Ther. 2022;18:2033–40.
[12] Huang Q, Liu XZ, Kang CS, et al. The anti-glioma effect of suicide gene therapy using BMSC expressing HSV/TK combined with overexpression of Cx43 in glioma cells. Cancer Gene Ther. 2010;17:192–202.
[13] Zhang K, Sun X, Sun W, et al. Exosomal microRNA-506 inhibits biological activity of lung adenocarcinoma cells and increases sensitivity to cisplatin-based hyperthermia. Cell Signal. 2022;100:110469.
[14] Shang A, Gu C, Wang W, et al. Exosomal circPACRGL promotes progression of colorectal cancer via the miR-142-3p/miR-506-3p- TGF-β1 axis. Mol Cancer. 2020;19:117.
[15] Du Y, Miao Z, Qiu L, et al. Clinical potential of miR-451 and miR-506 as a prognostic biomarker in patients with breast cancer. J Healthc Eng. 2022;2022:9578788.
[16] Xuan B, Wang Y. Exosome-transmitted miR-506-3p inhibits colorectal cancer cell malignancy via regulating GSTP1. Appl Biochem Biotechnol. 2023;195:2015–27.
[17] Gao F, Xu Q, Tang Z, et al. Exosomes derived from myeloid-derived suppressor cells facilitate castration-resistant prostate cancer progression via S100A9/circMID1/miR-506-3p/MID1. J Transl Med. 2022;20:346.
[18] Zeng C, Yuan G, Hu Y, et al. Repressing phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit gamma by microRNA-142-3p restrains the progression of hepatocellular carcinoma. Bioengineered. 2022;13:1491–506.
[19] Coumans FAW, Brisson AR, Buzas EI, et al. Methodological guidelines to study extracellular vesicles. Circ Res. 2017;120:1632–48.
[20] Liu L, Li X, Shi Y, et al. The long noncoding RNA FTX promotes a malignant phenotype in bone marrow mesenchymal stem cells via the miR-186/c-Met axis. Biomed Pharmacother. 2020;131:110666.
[21] Wu H, Mu X, Liu L, et al. Bone marrow mesenchymal stem cells-derived exosomal microRNA-193a reduces cisplatin resistance of non-small cell lung cancer cells via targeting LRRC1. Cell Death Dis. 2020;11:801.
[22] Yan T, Wu M, Lv S, et al. Exosomes derived from microRNA-512-5p-transfected bone mesenchymal stem cells inhibit glioblastoma progression by targeting JAG1. Aging (Albany NY). 2021;13:9911–26.
[23] S ELA, Mäger I, Breakefield XO, et al. Extracellular vesicles: biology and emerging therapeutic opportunities. Nat Rev Drug Discov. 2013;12:347–57.
[24] Kalluri R. The biology and function of exosomes in cancer. J Clin Invest. 2016;126:1208–15.
[25] Wang J, Xu SL, Duan JJ, et al. Invasion of white matter tracts by glioma stem cells is regulated by a NOTCH1-SOX2 positive-feedback loop. Nat Neurosci. 2019;22:91–105.