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# Exosomal miR-22-3p derived from peritoneal macrophages enhances proliferation, migration, and invasion of ectopic endometrial stromal cells through regulation of the SIRT1/NF-κB signaling pathway

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**Abstract. – OBJECTIVE: Exosomes play crucial roles in cell-cell communication, but few studies exist on the role of exosomal miRNA in the interaction between peritoneal macrophages (pMϕ) and human ectopic endometrial stromal cells (eESCs) in endometriosis (EMS). This study aimed to identify which exosomal miRNAs are significantly differently produced from EMS pMϕ and to investigate the functional role of exosomal miRNAs in eESCs.**

**PATIENTS AND METHODS: Exosomes were collected from the culture media of pMϕ by differential centrifugation. Confocal microscopy was used to identify whether the exosomes secreted by pMϕ can be delivered into eESCs. miRNA microarray and quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) were used to identify which exosomal miRNAs were specifically elevated in pMϕ-derived exosomes from EMS and delivered into eESCs via exosomes. The effect of pMϕ-derived miR-22-3p on the biological function of eESCs was assessed by Cell Counting Kit-8 (CCK-8), wound-healing, and transwell chamber assays. Bioinformatics analysis and Luciferase reporter assay were used to detect the binding of exosomal miR-22-3p to the 3'untranslated region of SIRT1. Western blot was utilized to detect the activity of SIRT1/NF-κB pathway.**

**RESULTS: Exosomes secreted by pMϕ can successfully be transported to eESCs. pMϕ-derived exosomes from EMS promoted the proliferation, migration, and invasion of eESCs. MiR-22-3p was significantly increased in pMϕ-derived exosomes from EMS and delivered from pMϕ to eESCs via exosomes. Mechanistic analyses revealed that exosomal miR-22-3p from pMϕ promoted the proliferation, migration, and invasion of eESCs by targeting SIRT1 and activating NF-κB pathway.**

**CONCLUSIONS: Exosomal miR-22-3p promotes the proliferation, migration, and invasion of eESCs by regulating SIRT1/NF-κB pathway and may serve as a novel target for the inhibition of EMS progression.**

*Key Words:*
Exosomes, Peritoneal macrophages, Ectopic endometrial stromal cells, MiR-22-3p, SIRT1, NF-κB.

## Introduction

Endometriosis (EMS), which is characterized by presence of endometrial tissue outside the uterine cavity, is a common gynecological disorder that results in pelvic pain and infertility[1]. This disease affects probably 6-10% of women of reproductive age, and up to 50% of infertile women[2]. Many theories have attempted to explain the development of EMS, but the underlying pathogenic mechanisms remain unclear. Peng et al[3] have suggested that human eESCs are involved in the development of EMS. Indeed, eESCs have been isolated from ectopic endometrial tissue. Moreover, local immune changes in the pelvic and abdominal cavity may cause adhesion, implantation, and growth of eESCs, which may lead to the formation of EMS lesions[4].

Capobianco et al[5] have found that peritoneal macrophages (pMφ) in abdominal immune microenvironment may be involved in the pathogenesis of EMS. Macrophage colony-stimulating factor produced by pMφ in EMS contributes to EMS lesion formation and progression[6]. Activated pMφ secretes inflammatory substances that promote the proliferation, adhesion, and angiogenesis of ectopic endometrial cells, lead to abnormalities in the immune system, pelvic local adhesion, and

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fibrosis in EMS[7]. Therefore, it is speculated that the interaction between pMφ and eESCs plays an important role in the development of EMS.

Exosomes, which are involved in cell-cell communication, are small vesicles (30-150 nm in diameter) derived from the endo-lysosomal pathway. These vesicles modulate the function of recipient cells by transportation of lipids, proteins, mRNAs, and miRNAs[8]. Lee et al[9,10] reported that exosomes derived from macrophage mediated tumor suppression and suppressed endothelial cell migration. Hence, exosomes may be channels for information exchange between pMφ and eESCs in EMS. MicroRNAs (miRNAs) which are 19-25 nucleotides IN length, regulate gene expression by binding to 3′-untranslated regions (3′-UTRs) of target mRNAs. Although the miRNAs in exosomes derived from macrophages participate in the pathogenesis of many diseases[11], not much is known about the role of exosomal miRNA in the interaction between pMφ and eESCs in EMS.

In this study, pMφ-derived exosomes were co-cultured with eESCs to confirm whether exosomes affect proliferation, migration, and invasion of eESCs. Then, miRNA microarrays and qRT-PCR were used to identify whether exosomal miR-22-3p could be delivered from pMφ to eESCs. Bioinformatic analysis combined with Luciferase assays revealed that miR-22-3p directly targets the 3′-UTR of SIRT1 mRNA and regulates the NF-κB signaling pathway. This study demonstrates that pMφ-derived exosomes from EMS promote proliferation, migration, and invasion of eESCs through the miR-22-3p/SIRT1/NF-κB signaling pathway.

## Patients and Methods

### *Patients*

This study was approved by the Ethics Committee of Qilu Hospital of Shandong University. Peritoneal fluid (PF) samples were acquired from 20 women with EMS who were diagnosed through histopathological examination at Qilu Hospital of Shandong University. Moreover, PF samples were taken from 20 control women undergoing laparoscopy for benign gynecological diseases, such as uterine myoma and tubal sterilization. Endometriotic tissues were obtained from EMS patients and used for isolation of eESCs. The included patients did not receive any hormonal therapy 6 months before the study period. The exclusion criteria were pregnancy, inflammation, and malignant tumor. Informed consent was obtained from each female participant.

### *Isolation of pMϕ*

The PF samples were obtained using an aspiration needle before pelvic surgery was performed under direct vision, and pMφ was isolated in primary culture. The PF of each participant was collected under sterile conditions and was used for the isolation of macrophages as previously described[12].

### *Isolation and culture of eESCs*

The eESCs from ectopic endometrial tissues were isolated and cultured as previously described[13]. Fresh ectopic endometrial samples were minced into small pieces, washed twice with Dulbecco's Modified Eagle's Medium/F12 (DMEM/F12; Gibco, Rockville, MD, USA) and digested with collagenase II (Life Technologies, Gaithersburg, MD, USA, USA) for 1 h at 37°C. Undigested tissue debris and epithelial cells were removed with sterile gauze pads. The filtered cells were resuspended in a DMEM/F12 medium containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA), 100 IU/mL of penicillin (Gibco, Rockville, MD, USA), and 100 μg/mL of streptomycin (Gibco, Rockville, MD, USA). Then, the cells were seeded into a 6-well plate and cultured at 37°C in humidified atmosphere of containing 5% $CO_2$.

### *Isolation and Characterization of Exosomes*

Exosomes were isolated from macrophage cell culture medium using differential centrifugation as previously described[14]. The exosomes were fixed in 2% PFA and loaded onto carbon-coated copper grids. The copper grids were floated on the drop for 2 min and stained with 2% sodium phosphotungstate (Solarbio, Beijing, China). The samples were allowed to dry for several minutes and examined under transmission electron microscopy (TEM; JEOL, Tokyo, Japan). Nanoparticle-tracking analysis (NTA) was used to measure the diameters and concentrations of the exosomes using the Nanosight NS300 System (Malvern Instruments, Malvern, Worcestershire, UK).

### *Western Blot Analysis*

Western blot assay was performed to quantify specific protein expression levels in pMφ-derived exosomes and SIRT1/NF-κB pathway. Equal quantities of protein were subjected to 10% sodium dodecyl sulphate-polyacrylamide gel elec-

trophoresis (SDS-PAGE) and then transferred to polyvinylidene difluoride membranes. Each membrane was blocked with 5% bovine serum albumin (BSA; Gibco, Rockville, MD, USA) and incubated overnight at 4°C with the appropriate primary antibodies. After washing, the membranes were incubated with peroxidase-conjugated goat anti-mouse secondary antibody (Invitrogen, Carlsbad, CA, USA). Image analysis was performed with an Image Quant LAS 4000 imager (GE Healthcare, Little Chalfont, Buckinghamshire, UK). The primary antibodies used were anti-CD9, anti-CD63, anti-SIRT1, anti-p65, anti-p-p65, anti-IκBα, anti-p-IκBα, and anti-β-actin (all from Abcam, Cambridge, MA, USA).

### Exosome Labeling and Internalization

The exosomes were labeled using PKH26 Red Fluorescent Cell Linker Kits (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions. Then, the eESCs were incubated with PKH26-labeled exosomes (10 µg) for 2 h, fixed in 4% paraformaldehyde for 10 min at room temperature, and washed thrice with phosphate-buffered saline (PBS; Solarbio, Beijing, China). Finally, the cells were incubated with 4',6-diamidino-2-phenylindole (DAPI) (1:500 dilution, Invitrogen, Carlsbad, CA, USA) for 5 min at room temperature, and subjected to confocal microscopy using a Zeiss LSM 780 confocal microscope (Zeiss, Jena, Germany) at 100x magnification.

### Cell Proliferation Assay

Cell proliferation was measured with Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Kumamoto, Japan) according to the manufacturer's instructions. The eESCs were seeded in 96-well plates at an initial density of 5,000 cells/well and cultured for 24 h. The medium was removed, and 100 mL of fresh medium with 2 mg of exosomes were added. At the $24^{th}$, $48^{th}$, $72^{nd}$, $96^{th}$, and $120^{th}$ hours after incubation, 10 mL of CCK-8 solution was added and the plates were incubated in the dark for 3 h. The absorbance of each sample was measured spectrophotometrically at 450 nm. The proliferation of the cells was calculated using corrected optical density (OD) values obtained by subtracting the blank well absorbance from those of the test wells.

### Cell Migration and Invasion Assays

Wound-healing and transwell chamber (Corning, NY, USA) assays were used to determine the migratory and invasion capacities of eESCs pretreated with pMφ-derived exosomes for 24 h before the assays. For the wound-healing test, the cells were seeded into six-well plates. After 24 h of incubation, each well was scraped with a 10-mL pipette tip to create two linear regions devoid of cells, and a medium without exosomes was added. At the time points of 0, 12, 24, and 48 h, photographs were taken to determine the cell number.

In the transwell chamber test, eESCs were inoculated into 24-well Falcon Migration inserts ($1 \times 10^3$ cells per well) and Matrigel Invasion inserts ($1 \times 10^3$ cells per well) with 200 mL of an exosome-free growth medium. The inserts were placed in Falcon companion plates and incubated for 16 h (for migration) and 24 h (for invasion). The migrated or invaded cells were counted under a light microscope.

### Extraction and Microarray Analysis of Exosomal RNA

Total RNA was extracted from the exosomes using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The quality and distribution of miRNAs were analyzed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The miRNA microarrays were performed using an Agilent Human miR 21.0 array (Agilent Technologies, Santa Clara, CA, USA), and the results were analyzed with GenePix Pro software v4.1 (Molecular Devices Corporation, San Jose, CA, USA). Differentially expressed miRNAs were identified using paired *t*-test with the cut-off criteria of $p < 0.05$ and $|\log_2$ fold change$| > 1$.

### qRT-PCR

The expressions of miR-22-3p in cells and exosomes were determined using a TaqMan miRNA reverse transcription kit and a TaqMan miRNA assay kit (Applied Biosystems, Foster City, CA, USA). Stem loop primers: GTCGTATCCAGTGCGT-GTCGTGGAGTCGGCAATTGCACTGGATAC-GACACAGTTCT. Forward primer (5'-3'): ACTC-CAGCAAGCT-GCCAGTTGA. Reverse primer (5'-3'): CAGT-GCGTGTCGTGGAGT. GAPDH and U6 were served as the internal controls. U6, forward: 5'-CTCGCTTCGGCAGCACA-3'; reverse: 5'-AACGCTTCACGAATTTGCGT-3'. GAPDH, forward: 5'-AGGTCGGTGTGAACGGATTTG-3', reverse: 5'-TGTAGACCATGTAGTTGAGGT-CA-3'. All reactions were performed in triplicate, and the results were analyzed using the $2^{-\Delta\Delta Ct}$ method.

#### miRNA Transfection

For miRNA studies, eESCs were transfected with an miR-22-3p mimic or inhibitor (Ribobio, Guangzhou, China) using Lipofectamine 2000 Transfection Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. At 24 h post-transfection, the cells were collected for use in subsequent studies.

#### Luciferase Reporter Assay

The target genes of miR-22-3p were predicted using TargetScan (http://www.targetscan.org/vert_72/). The putative miR-22-3p complementary site in the 3′-UTR of SIRT1 or its mutant sequence was cloned into the pGL3 vector (Promega, Madison, WI, USA). The plasmids were co-transfected into eESCs with miR-22-3p mimic, inhibitor, or negative control using Lipofectamine 2000 Transfection Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's specification. At 48 h after transfection, the luciferase activity was measured using the Dual-Luciferase reporter assay system (Promega, Madison, WI, USA).

#### Statistics Analysis

Statistical analysis was performed using Graph Prism 6.0 (GraphPad Software, San Diego, CA, USA) and Statistical Product and Service Solutions (SPSS) 19.0 (IBM Corp., Armonk, NY, USA). Data are presented as mean ± SD (standard deviation) of at least three independent experiments. The two-tailed Student's *t*-test was used for comparisons of two independent groups. In this study, statistical significance was assumed at $p<0.05$ (*$p<0.05$, **$p<0.01$, and ***$p<0.001$).

## Results

#### Characterization and Internalization of pMφ-Derived Exosomes

In this study, pMφ was isolated and cultured to determine whether pMφ secreted exosomes. The results from TEM revealed that exosomes from pMφ culture medium produced typical cup-shaped morphologies and were in the size range of less than 200 nm (Figure 1A). The exosomes had an average diameter of 105 ± 3.9 nm and a concentration of $4.1 \times 10^8$ particles/mL, as revealed from NTA (Figure 1B). Western blot analysis showed that the exosome-specific markers CD9 and CD63 were highly enriched in the isolated exosomes but not in the exosome-free supernatant (Figure 1C). Exosomes were labeled with PKH67 to determine whether pMφ-derived exosomes could be taken up by eESCs. After incubation with eESCs, confocal imaging showed the presence of PKH67 spots in recipient cells, indicating that labeled exosomes released by pMφ could be delivered to eESCs (Figure 1D).

#### pMφ-Derived Exosomes from EMS Promoted the Proliferation, Migration, and Invasion of eESCs

Results from CCK-8 assays showed that pMφ-derived exosomes from EMS significantly increased the proliferation of eESCs, when compared with exosomes from patients with benign gynecological diseases (Figure 2A). Moreover, wound-healing assay demonstrated that pMφ-derived exosomes affected cell motility by significantly increasing cell migration into the scraped areas (Figure 2B). Transwell chamber assay showed that eESCs incubated with pMφ-derived exosomes from EMS exhibited increased cell migration and invasion (Figure 2C). These findings indicate that exosomes secreted by pMφ from EMS enhanced the biological function of eESCs.

#### Exosomal MiR-22-3p was Delivered to eESCs from pMφ

Exosomal miRNA microarrays were carried out to identify which exosomal miRNAs were expressed differently in pMφ isolated from EMS. The results showed 15 upregulated and 5 downregulated exosomal miRNAs (Table I). Further studies were focused on miR-22-3p because qRT-PCR assays showed that its expression was significantly increased in pMφ-derived exosomes from EMS patients (Figure 3A). Treatment of eESCs with pMφ-derived exosomes from EMS induced increase in the cellular level of mature miR-22-3p, when compared with the corresponding level in cells treated with control exosomes (Figure 3B). In contrast, the expression level of pri-miR-22-3p did not differ from that in eESCs treated with pMφ-derived exosomes from EMS or control exosomes (Figure 3C), indicating that the upregulated expression of miR-22-3p in eESCs was caused by exosome-mediated miR-22-3p transfer and not by pri-miR-22-3p transcription.

#### MiR-22-3p Promoted the Biological Function of eESCs

The eESCs were transfected with miR-22-3p mimic or inhibitor so as to overexpress or suppress miR-22-3p expression in order to determine the biological role of miR-22-3p in eESCs. Upregulation of miR-22-3p in eESCs increased cell pro-

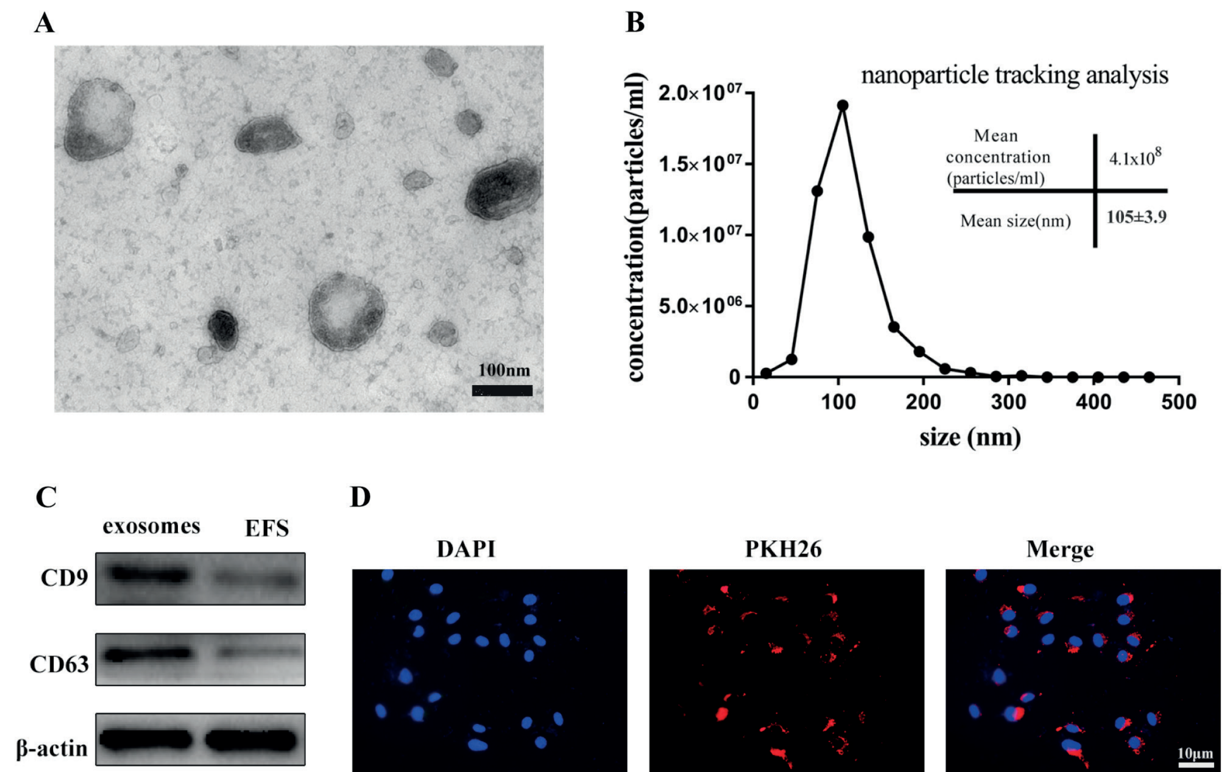


**Figure 1.** Characterization and internalization of pMφ-derived exosomes. **A,** Morphological characterization of exosomes by TEM. Scale bar = 100 nm (magnification: 100,000×). **B,** Size and concentration of exosomes analyzed by NTA. **C,** The protein markers of exosomes, CD9 and CD63, were detected by Western blot in exosomes and exosome-free supernatant (EFS). **D,** Confocal microscopy showed the internalization of PKH67-labeled exosomes (red) by eESCs. Cell nuclei were stained with DAPI (blue) (magnification: 400×).

**Table I.** Twenty exosomal miRNAs were statistically different dysregulated.

| Upregulated | Fold change | *p*-value | Downregulated | Fold change | *p*-value |
|---|---|---|---|---|---|
| miR-22-3p | 21.015 | 0.0093 | miR-133 | 0.1043 | 0.0117 |
| miR-28-5p | 9.0345 | 0.0143 | miR-296 | 0.3496 | 0.0335 |
| miR-302a | 7.4352 | 0.0297 | miR-1912 | 0.3751 | 0.0374 |
| miR-320b | 5.0106 | 0.0319 | miR-2113 | 0.4162 | 0.0388 |
| miR-3118 | 4.2730 | 0.0345 | miR-3188 | 0.4317 | 0.0401 |
| miR-3168 | 3.7643 | 0.0378 | | | |
| miR-425-5p | 3.4159 | 0.0385 | | | |
| miR-4256 | 4.5642 | 0.0127 | | | |
| miR-4447 | 6.7128 | 0.0116 | | | |
| miR-507 | 3.1445 | 0.0213 | | | |
| miR-596 | 5.3497 | 0.0169 | | | |
| miR-5582 | 2.5076 | 0.0381 | | | |
| miR-610 | 2.4391 | 0.0342 | | | |
| miR-663a | 2.3540 | 0.0410 | | | |
| miR-6720 | 2.1781 | 0.0418 | | | |
| Yes | 20 | | | | |
| No | 11 | | | | |

liferation (Figure 3D), and significantly increased cell migration into the scraped area (Figure 3E), whereas miR-22a-3p inhibitor decreased cell motility in eESCs (Figure 3F).

### *MiR-22-3p Targeted SIRT1 and Activated the NF-κB Pathway*

Mechanistic studies showed that miR-22-3p directly targeted the 3′-UTR of SIRT1 mRNA

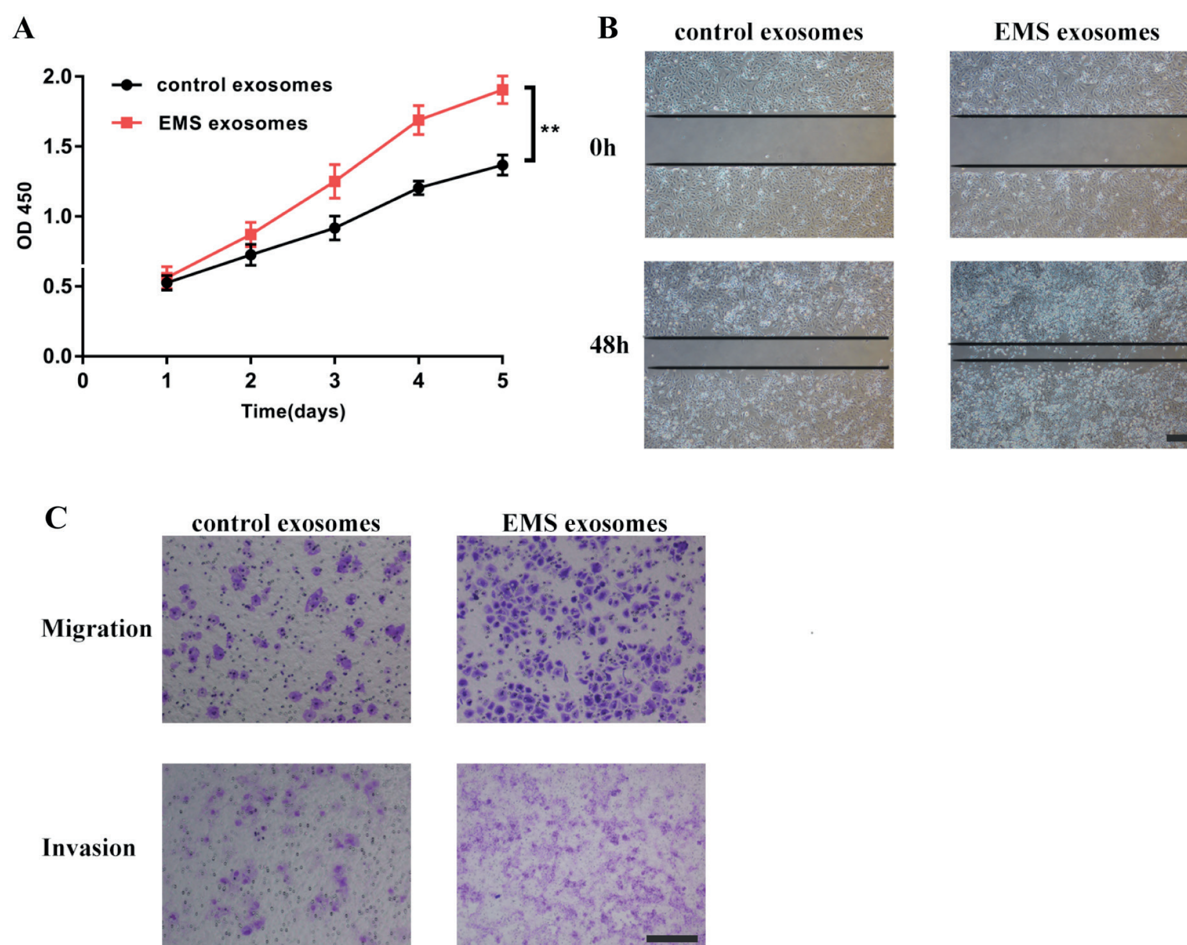


**Figure 2.** pMφ-derived exosomes promote proliferation, migration, and invasion of eESCs. **A,** Effects of pMφ-derived exosomes from EMS (EMS exosomes) and patients with benign gynecological diseases (control exosomes) on the growth of eESCs were compared by CCK-8 assay. **B**, Cell motility was assessed using a wound-healing assay (magnification: 40×). **C**, Migration and invasiveness were assessed by transwell chamber assay (magnification: 100×). Scale bar = 200 µm.

(Figure 4A). Relative Luciferase activity was noticeably decreased when miR-22-3p mimics were co-transfected, whereas miR-22-3p mimics had no effect on the Luciferase activity of mutant SIRT1 (Figure 4B). Then, mimic or inhibitor of miR-22-3p was transfected into eESCs to determine the relationship of miR-22-3p and SIRT1. The results demonstrated that the mRNA and protein expressions of SIRT1 were downregulated by miR-22-3p mimic but were upregulated by the inhibitor (Figure 4C). Based on the antagonistic crosstalk between NF-κB and SIRT1 in many biological progresses[15], the activity of NF-κB pathway was studied. It was revealed that the miR-22-3p mimic induced the phosphorylation of P65 and IκBα, which are the components of NF-κB pathway (Figure 4D). These findings suggest that miR-22-3p targeted SIRT1 and activated the NF-κB signaling pathway. The expression of SIRT1 was downregulated at the mRNA and protein levels in eESCs treated with pMφ-derived exosomes from EMS (Figure 4E). This result further indicates that pMφ-derived exosomes from EMS regulated the biological function of eESCs through the miR-22-3p/SIRT1/NF-κB axis.

## Discussion

Simeoli et al[16] have shown that exosomes play important roles in many diseases through the intercellular transfer of miRNA, protein, and lncRNA. However, there were no studies on exosome-mediated communication between pMφ and eESCs. Thus, the role of exosomes derived from pMφ in the pathogenesis of EMS

was investigated in the present study. The results showed that exosomal miR-22-3p derived from EMS pMφ modulated the pathogenesis of EMS through the miR-22-3p/SIRT1/ NF-κB signaling pathway. To the best of our knowledge, this is the first report to explore the effect of pMφ-derived exosomes on eESCs, which is the main novelty of this study.

Macrophages are crucial in the establishment of EMS[5]. Many studies have demonstrated the communication between macrophages and endometrial stromal cells. The communication be-

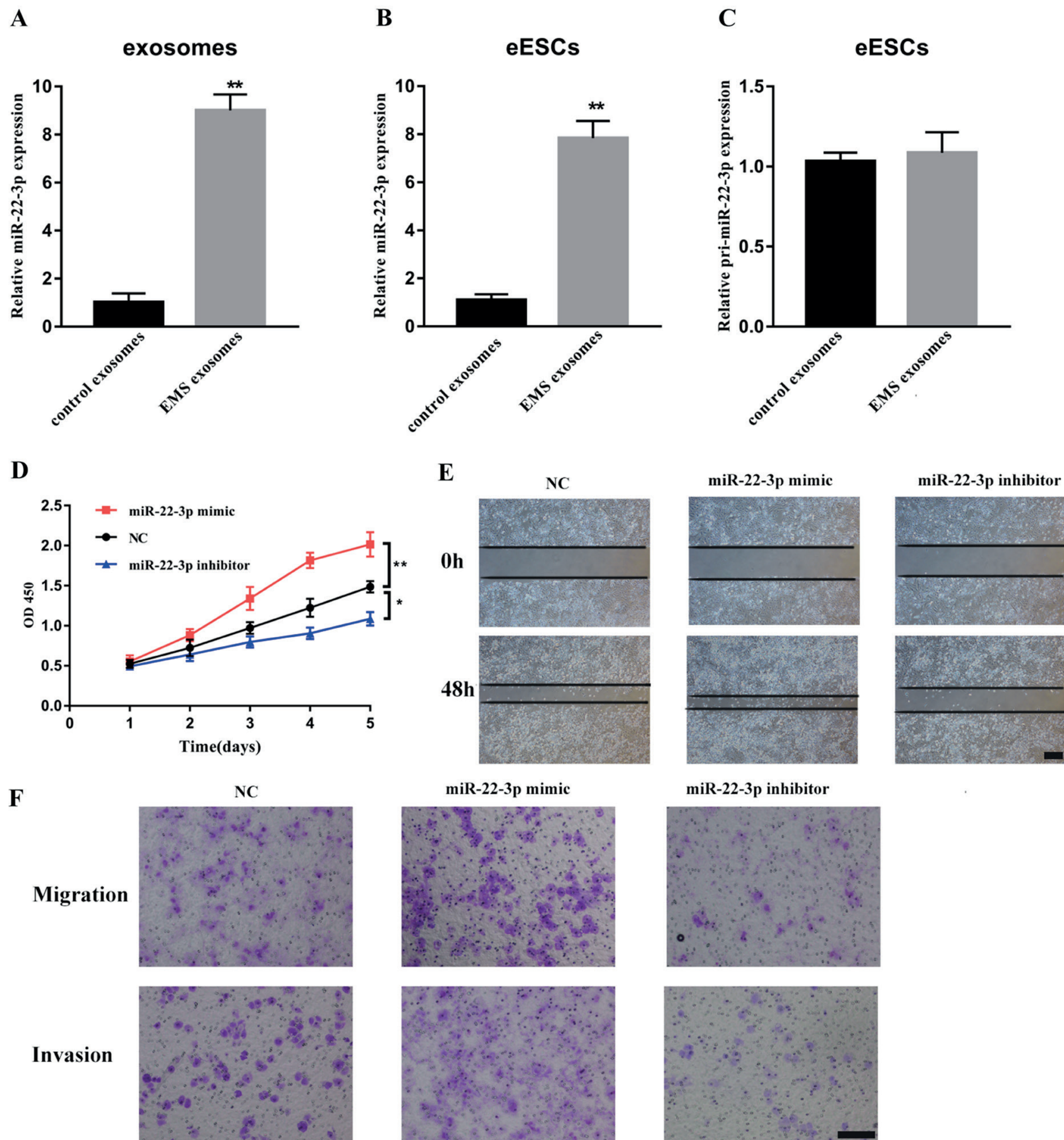


**Figure 3.** miR-22-3p is delivered from pMφ into eESCs *via* exosomes and promotes the biological function of eESCs *in vitro*. **A,** Relative expression levels of miR-22-3p in pMφ-derived exosomes are shown. **B,** Expression of miR-22-3p in eESCs cocultured with exosomes derived from pMφ. **C,** Expression of pri-miR-22-3p in eESCs cocultured with pMφ-derived exosomes. **D,** CCK-8 assay was used to detect the effects of miR-22-3p mimics, miR-22-3p inhibitors, and negative control (NC) on the proliferation of eESCs. **E,** Cell motility was assessed using a wound-healing assay (magnification: 40×*)*. **F,** Migration and invasiveness were assessed by transwell chamber assay (magnification: 100×) Scale bar = 200 μm.

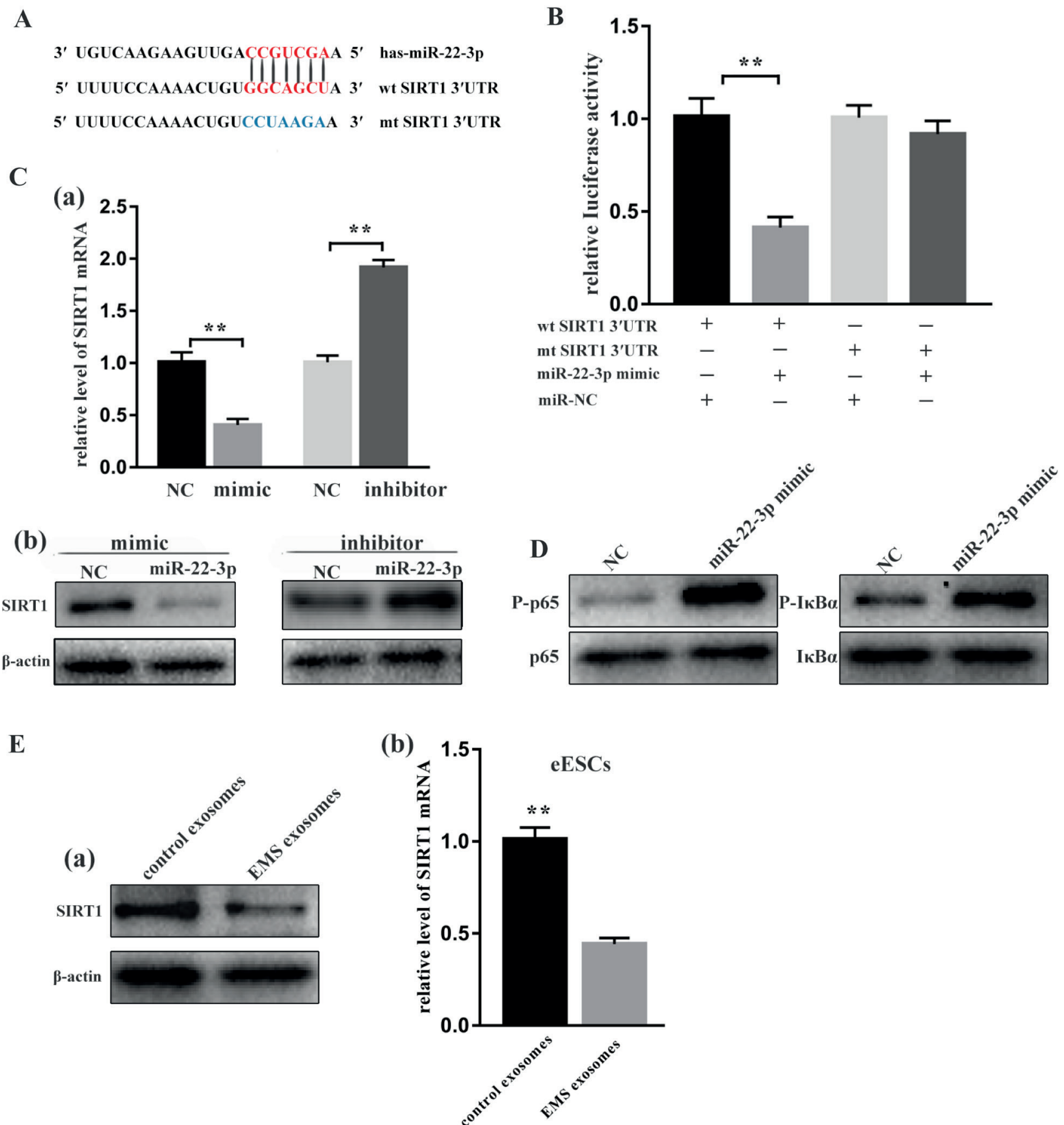


**Figure 4.** miR-22-3p targets SIRT1 and activates the NF-κB signaling pathway. **A,** Predicted binding sites of miR-22-3p within the 3′-UTR of SIRT1 mRNA. **B,** Luciferase reporter assays in eESCs with cotransfection of wt or mt SIRT1 3′-UTR. **C,** mRNA level (**a**) and protein expression (**b**) of SIRT1 in eESCs cells after the treatment with miR-22-3p mimic, miR-22-3p inhibitor, and NC. **D,** Effects of miR-22-3p mimics on the protein expressions of p65 and IκB*α*. **E,** Expressions of SIRT1 at the protein (**a**) and mRNA (**b**) levels in eESCs treated with pMφ-derived exosomes.

tween endometrial stromal cells and macrophages reduces the cytotoxicity of NK cells in EMS by secreting IL-10 and TGF-β[17]. Shao et al[18] reported that macrophages promote the growth and invasion of endometrial stromal cells by reducing IL-24. However, in the present study, it was found that exosomes derived from pMφ promoted the proliferation and migration abilities of eESCs, thereby providing a new insight into the intercellular crosstalk between pMφ and eESCs. These findings suggest that exosomes may serve as a new bridge between pMφ and eESCs. Exosomal

miRNAs secreted by macrophage have served as key regulators in many cellular processes. Exosomal miRNAs derived from adipose tissue macrophage regulate insulin sensitivity *in vivo* and *in vitro*[19]. In the present study, it was revealed that exosomal miR-22-3p derived from pMφ was significantly increased in EMS patients and transported from pMφ to eESCs.

Many reports established that miRNAs are important in the pathogenesis of EMS. Hsu et al[20] reported that miR-199a-5p enhances the pathogenesis of EMS by modulating VEGFA in endometrial mesenchymal stem cells. Although studies on the role of miR-22-3p in the pathogenesis of EMS are limited, various authors have demonstrated that miR-22 promotes cell survival and cell self-renewal. Feng et al[21] referred that exosomal miR-22 enhanced the protective effect of stem cells by targeting Mecp2 in cardiac diseases. The present report found that miR-22-3p promotes the proliferation and motility of eESCs, indicating that exosomal miR-22-3p acts as a critical inducer of EMS.

In this study, *SWIRT1* was selected as a target gene for investigating the mechanisms underlying the role of miR-22-3p in eESCs because of its close involvement in inflammation. It is known that SIRT1 is an NAD+-dependent class III histone deacetylase that regulates intracellular activities through control of transcription[22]. Antagonistic influence exists between SIRT1 and NF-κB. It has been reported[15] that SIRT1 supports oxidative respiration and anti-inflammatory responses, whereas NF-κB promotes inflammation. Bai et al[23] observed that miR-138 aggravates the inflammatory responses of macrophages by regulating SIRT1/NF-κB/AKT pathways. Liu et al[24] have shown that MiR-520c and miR-373 promote cell migration and growth in 3D type I collagen gels by targeting SIRT1 and activating the NF-κB pathway. In the present study, pMφ exosomes containing miR-22-3p reduced the expression of SIRT1 and upregulated the expression of NF-κB, indicating that miR-22-3p might be involved in EMS *via* the SIRT1/NF-κB pathway. Similarly, Wei and Shao[25] reported that EMS may be alleviated by downregulating the activity of NF-κB. Hence, miR-22-3p and SIRT1/NF-κB pathway may be used as novel targets for treating EMS.

This work has some limitations. Endometriosis (EMS) is modulated by multiple factors, and exosomes can deliver various molecules, such as lipids, lncRNA, miRNA, and proteins. Thus, further investigations are needed to determine whether other exosomal components play roles in the development of EMS. The role of SIRT1/NF-κB pathway in eESCs also requires further researches. Having found that exosomes from pMφ regulate the proliferation and angiogenesis of eESCs, it is necessary to study the effect of exosomes derived from eESCs on the biological characteristics of pMφ.

In this study, a novel mechanism underlying the communication between pMφ and eESCs was demonstrated, and a new role of pMφ-derived exosomes in EMS was revealed. These findings provide vital information and open new directions in EMS research. First, the cell-cell communication *via* exosomes may be a promising therapeutic strategy that may provide an alternative approach to the current treatments of EMS. Secondly, the exosomal components could serve as targets, including miR-22-3p and other molecules. Finally, macrophage-derived exosomes could be used to transport therapeutic agents.

## Conclusions

In summary, these data indicate that exosomal miR-22-3p derived from pMφ promote proliferation, migration, and invasion of eESCs by regulating the SIRT1/NF-κB pathway. Thus, the exosomes, miR-22-3p and the SIRT1/NF-κB pathway may be used as novel targets to treat EMS.


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#### Conflict of Interests
The Authors declare that they have no conflict of interests.


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