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AKADÉMIAI KIADÓ

Exosomes from bone mesenchymal stem cells alleviate mifepristone-induced human endometrial stromal cell injury by inhibiting TLR3 via delivering miR-941

YU WANG, XIAOFEI SUN, QING YANG and LILI YIN*

Department of Obstetrics and Gynecology, Shengjing Hospital Affiliated to China Medical University, Shenyang, Liaoning, 110004, China

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ABSTRACT

Objective: We aim to investigate the protective effect and underlying mechanisms of BMSCs-exo on human endometrial stromal cells (HESCs) induced by mifepristone in this study. *Methods:* BMSCs-exo were extracted and then identified by transmission electron microscopy and western-blot assay. RT-PCR assay was used to determine the level of miR-941. MiR-941 mimics or inhibitor were transfected into BMSCs and the exosomes were extracted. Then, Cell activity, apoptosis rate, cell migration and invasion, as well as the expression of angiogenic proteins were determined in HESCs stimulated by mifepristone and BMSCs-exo. Next, Dual-luciferase reporting assay was used to verify the targeted binding of miR-941 to TLR3, and the TLR3 expression in HESCs was detected by RT-PCR and western-blot. Finally, TLR3 was overexpressed to evaluate the effects of miR-941 from BMSCs-exo on cell apoptosis, cell invasion and angiogenesis in HESCs induced by mifepristone. *Results:* miR-941 was highly expressed in BMSCs-exo. Exosome miR-941 in BMSCs-exo inhibited the cell apoptosis, and promoted cell activity, cell migration, invasion as well as angiogenesis were also improved in HESCs induced by mifepristone. TLR3 was a target of miR-941, which was up-regulated in mifepristonetreated HESCs. We further found that miR-941 derived from BMSCs-exo down-regulated the expression of TLR3 in HESCs treated by mifepristone. In addition, TLR3 over-expression blocked the inhibition of miR-941 on mifepristone-induced cell apoptosis, as well as cell

* Corresponding author. Department of Obstetrics and Gynecology, Shengjing Hospital Affiliated to China Medical University, No. 36, Sanhao Street, Heping District, Shenyang, Liaoning, China. Tel.: +86 024 9661544211. E-mail: ll_yin0621@163.com

migration and angiogenesis in HESCs. *Conclusions:* Thus, we concluded that BMSCs-exo has protective effect on mifepristone-induced cell damage by delivering miR-941 which targeted TLR3 and regulated cell activity, migration, and angiogenesis in HESCs.

KEYWORDS

bone mesenchymal stem cells, exosome, mifepristone, human endometrial stromal cell, miR-941, TLR3

INTRODUCTION

Endometrium is a highly renewable tissue which undergoes monthly growth, differentiation and shedding during a woman's reproductive life [1]. Human endometrial stromal cells (HESCs) are responsible for endometrial decidualization, vascular remodeling, immune cell recruitment and abundant molecular production, and play an important role in repairing endometrial damage and maintaining endometrial homeostasis [2, 3]. Apoptosis of HESCs and decreased activity of HESCs are the main characteristics of endometrial damage, which may affect their migration and angiogenesis, thus hindering endometrial regeneration and resulting in endometrial homeostasis imbalance [4, 5].

Exosomes are known as the important transport vehicles for communication between cells by delivering cargos such as proteins, lipids and microRNAs (miRNAs) [6]. As a source of exosomes, bone mesenchymal stem cells (BMSCs) are considered as an emerging therapeutic strategy for a variety of diseases, including endometrial injury [7, 8]. BMSCs-derived exosomes (BMSCs-exo) contribute to the repair of damaged endometrium by regulating the TGF-β1/Smad pathway [9]. It was found that BMSCs-exo promoted the proliferation and migration of endometrial cells *in vitro*, as well as promoted the recovery of damaged endometrium in rats [10]. In addition, miR-229 delivered by BMSCs-exo inhibits fibrosis during endometrial repair of intrauterine adhesions [10]. Liu et al. [11] found that exosome mediated delivery of miR-223-3p promoted the interaction of BMSCs with endothelial progenitor cells, which improved lipopolysaccharides-induced acute uterine injury. Moreover, exosomes from human umbilical cord mesenchymal stem cells mediated delivery of miR-7162-3p and regulated cell proliferation and apoptosis in endometrial stromal cells treated by mifepristone [12]. Thus, the miRNAs delivered by BMSCs-exo might possess important roles in the treatment of endometrial injury.

Toll-like receptors 3 (TLR3), a key member of the innate immunity family, is involved in cell proliferation, apoptosis, angiogenesis, tissue remodeling and repair by recognizing endogenous and exogenous ligands [13, 14]. TLR3 has been found to be highly expressed in patients with endometriosis and associated with inflammatory response [15, 16]. But the underlying mechanism is unclear. MiR-941, one of the members of miRNAs, has been considered as a promising biomarker for acute coronary syndrome and is also associated with the progression of diseases such as cancer and arthritis [17–19]. Studies have shown that miR-94 overexpression protected human endometrial cells from oxidative stress and programmed necrosis induced by OGD/R by regulating the Keap1/Nrf2 pathway [20]. Interestingly, miR-941 was found to bind TLR3 predicted by TargetScan website. In this study, we found that miR-941 derived from BMSCs-exo inhibited mifepristone-induced cell apoptosis in HESCs by inhibiting the expression of TLR3,

and increased cell activity, promoted cell migration and vascular formation, thus promoting the repair of human endometrial injury.

MATERIALS AND METHODS

Cell culture

The immortalized HESCs and BMSCs were purchased from the American Type culture collection (ATCC, USA). HESCs cells were cultured in Dulbecco's Modified Eagle Medium: F12 (DMEM/F12; Thermo Fisher, USA) with 10% charcoal-stripped fetal calf serum (FCS; Gibco, USA) and 1% penicillin-streptomycin. BMSCs were cultured by DMEM (Gibco, USA) containing 10% fetal bovine serum (FBS; Gibco, USA) and 1% penicillin-streptomycin. All the cells were cultured in an incubator containing 5% CO_2 and a constant temperature of 37 °C. All cells were cultured for 2–3 days and passed by when reaching to 90% confluence.

MTT assay

Cell viability was detected by MTT assay. The cells were seeded into 96-well plates at a density of 2×10^3 cells/well, and then 60 μM mifepristone with or with without BMSCS-exo (100 μg mL^{-1}, 200 μL/well) which was transfected with miR-941 mimics, mimic NC miR-941 inhibitors or inhibitor NC (Shanghai GenePharma, Shanghai, China) were added. After incubation for 24 h in an incubator containing 5% CO_2 at 37 °C, and the absorbance was measured at 490 nm with a microplate reader (BioTek Instruments, USA). All experiments were performed in 3 replicates to ensure repeatability of results.

Transwell assay

After treatment with mifepristone with or with without BMSCS-exo (100 μg mL^{-1}, 200 μL/well) transfected with miR-941 mimics, mimic NC miR-941 inhibitors or inhibitor NC for 48 h, 3×10^3 HESCs cells were inoculated into the upper compartment of the Transwell plates and cultured by serum-free DMEM. DMEM with 10% FBS was added into the lower compartment. The Transwell system was precoated with Matrigel (BD Biosciences, MA). After incubation for 48 h, the cells were fixed with 4% paraformaldehyde for 10 min and stained with 0.5% crystal violet. Then, the cells were observed and counted in 5 fields under an inverted microscope (magnification, ×200).

Wound healing assay

HESCs cells were seeded into 6-well plates with 1×10^6 cells/well and incubated at 37 °C for 24 h to form a single layer. Then, the cells were treated with 60 μM mifepristone with or with without BMSCS-exo (100 μg mL^{-1}, 200 μL/well) transfected with miR-941 mimics, mimic NC miR-941 inhibitors or inhibitor NC, and next the pipette tip was used to scratch the central area and the cells were incubated. The scratch time was set at 0 h, and cells were observed and photographed after 48 h. The images were analyzed by ImageJ software and the wound healing rate was calculated.

qRT-PCR

Total RNA from HESCs cells was extracted with TRIzol regent (Invitrogen; USA) following the manufacturer's direction. Optical density (OD) 260/280 ratio and RNA concentration were measured using Nanodrop (Thermo fisher, USA). All samples' OD260/280 ratios were between 1.8 and 2.1.

For miRNA analysis, the Mir-X™ miRNA qRT-PCR TB Green kit (Takara; Japan) was used for RNA reversing transcription. Relative level of miR-941 was normalized to U6 expression. Total RNA (500 ng) in cells was reversely transcribed into cDNA by PrimeScript RT kit (TaKaRa, Dalian, China) according to the manufacturer's instructions. RT-PCR was performed using TB Green Premix Ex Taq (TaKaRa; Japan) on StepOne Plus RT-PCR System (Applied Biosystem; USA). Relative gene levels of TLR3 were normalized to GAPDH expression. The sequences of primers were presented in Table 1.

Western-blot assay

PMSF (100 X) was added to WB and IP lysates (Beyotime Biotech, China) several minutes before the experiment. The HESCs cells were collected and washed twice with PBS, then the cells were dealt with WB and IP lysates (150 μL for per well in 6-well plates). After the cells were fully lysed, lysate was absorbed into a clean EP tube and centrifuged at 12,000 g at 4 °C for 5 min. Then, the supernatant was collected and the total protein concentration in the supernatant was determined by BCA protein quantification kit (Kerui; Wuhan; China). The supernatant was boiled in 5X buffer solution (Beyotime, Shanghai, China) for 8 min. Proteins (30 mg) form different groups were separated by SDS-PAGE and transform onto PVDF (0.45 μm) membranes. Next, the membranes were soaked in 5% non-fat milk. After blocking, the blots were incubated with antibodies against TSG101 (Abcam, 1:1000), CD63 (Abcam, 1:1000) and CD9 (Abcam, 1:1000), TLR3 (CST, 1:1000), Cleaved caspase 3 (CST, 1:1000), Bax (Absin, 1:1000), Bcl-2 (Absin, 1:1000), MMP2 (CST, 1:1000), MMP9 (CST, 1:1000), VEGFA (Absin, 1:1000), VEGFR1 (Absin, 1:1000), VEGFR2 (CST, 1:1000), RAP1B (SantaCruz, 1:1000) and GAPDH (Absin, 1:1000) overnight at 4 °C. Finally, the bands were incubated with HRP-conjugated secondary antibody (CST, 1:2500) at room temperature for 1 h and quantitated using BioImaging. The protein levels were quantified using GAPDH as a loading control.

Preparation of BMSCs exosomes

Exosomes from BMSCs were isolated and purified by super-centrifugation. The cells were cultured in the medium without exosomes for 48 h. Then, the medium was collected and filtered by a 0.22 nm filter. The filtrate was then centrifuged as follows: 2 000 g 20 min^{-1}, 16,500 g 45 min^{-1}, 100,000 g 2 h^{-1}, 100,000 g 2 h^{-1}, centrifugal temperature set to 4 °C. Next, exosomes were isolated using the MagCaptureTM Exosome isolation kit PS (FUJIFIL M Wako, Japan) according to the manufacturer's instructions.

TEM

The isolated exosomes were observed by transmission electron microscopy and photographed. The exosomes were immobilized with 30 μL 2% paraformaldehyde, and then transferred onto the discharge copper grid. The copper grid containing exosomes was immersed in 3% glutaraldehyde for fixation, and then stained with 4% uranyl acetate. Finally, the exosomes were observed and photographed by transmission electron microscopy (FEI, Hillsboro, OR).

Flow cytometry

Flow cytometry was used for detecting cell apoptosis in different groups. Apoptosis was analyzed by Annexin V-FITC/PI apoptosis kit. After cells were treated with 60 μM mifepristone with or with without BMSCS-exo transfected with miR-941 mimics, mimic NC miR-941 inhibitors or inhibitor NC for 48h, HESCs cells were collected and cell apoptosis was analyzed by Annexin V-FITC/PI apoptosis kit (Liankebio, China) according to the kit instructions. The cells were incubated with Annexin V-FITC and PI in darkness at room temperature for 10 min and detected by BD LSR flow cytometry (BD Biosciences).

Targets prediction

The target gene of miR-941 was predicted by using TargetScan database (http://www.targetscan.org/vert_72/).

Dual-luciferase reporting assay

The wild type (pmirGLO-TLR3-wt) and mutant 3′-UTRs (pmirGLO-TLR3-mut) of TLR3 were amplified by PCR and inserted into pGL3 plasmid. HESC cells were inoculated in 24-well plates at 50% confluence and co-transfected with wild-type or mutant luciferase reporter genes (100 ng) and miR-941 mimics (20 nM) or negative controls (NC) using Lipofectamine 3000 (Invitrogen, CA, USA). After transfection for 48 h, the luciferase reporting system was used to measure relative luciferase activity (Promega, Madison, USA).

Statistical analysis

SPSS 21.0 software was used for data analysis. The data are presented as Mean ± SD and visualized using GraphPad Prim 8.0. The differences between two groups were performed by two-tailed Student's *t*-test, and multiple groups were performed with one-way ANOVA followed by Tukey's post hoc test. $P < 0.05$ was considered as statistically significant difference.

RESULTS

BMSCs-exo overexpressed miR-941 and promoted the expression of miR-941 in HESCs

We isolated exosomes from BMSCs (BMSCs-exo) culture medium by phosphatide serine (PS) affinity method and observed them under a transmission electron microscopy. As shown in Fig. 1a, the shape of BMSCs-exo was round or oval, with complete vesicles. Subsequently,

Table 1. The information of primers

Gene name	Forward (5′-3′)	Reverse (3′-5′)
miR-941	GCACCCGGCTGTGT	CTCAACTGGTGTCGTGGA
TLR3	GTATTGCCTGGTTTGTTAATTGG	AAGAGTTCAAAGGGGGCACT
U6	TCGCTTCGGCAGCACATAT	ATTTGCGTGTCATCCTTGC
Gapdh	TCAAGATCATCAGCAATGCC	CGATACCAAAGTTGTCATGGA

western blot was used to detect the expression of exosome specific proteins TSG01, CD63 and CD9 (Fig. 1b), which were highly expressed in BMSCs-exo.

To verify the speculation that miR-941 was overexpressed in BMSCs-exo, we detected the expression level of miR-941 in BMSCs-exo. As shown in Fig. 1c, miR-941 was highly expressed in BMSCs-exo. Moreover, the expression level of miR-941 in HESCs was significantly increased after co-culture with BMSCs-exo (Fig. 1d).

BMSCs-exo promoted cell proliferation and inhibited mifepristone induced cell apoptosis in HESCs via miR-941

Mifepristone can induce endometrial stromal cell injury and is a commonly used modeling drug to study the mechanism of endometrial injury *in vitro* [12]. In this study, we found that the expression of miR-941 was significantly reduced in HESCs after treatment with mifepristone (Fig. 2a). To clarify the effect of miR-941 on HESCs, BMSCs were transfected with miR-941 mimic or inhibitor, and then the BMSCs-exo were extracted and the expression of miR-941 in BMSCs-exo were detected (Fig. 2b). We found that miR-941 mimic transfected BMSCs-exo could further increase the expression of miR-941 in HESCs cells (Fig. 2c). While the expression of miR-941 were reduced in HESCs treated with miR-941 inhibitor transfected BMSCs-exo compared with miR-941 NC (Fig. 2c), which indicated that the BMSCs-exo derived miR-941 could regulate the expression of miR-941 in HESCs.

Secondly, we found that cell viability of HESCs were markedly decreased after treatment with mifepristone, which was reversed by BMSCs-exo and miR-941 mimic transfection (Fig. 2d). Besides, cell activity was recovered more significantly in miR-941 mimic transfected BMSCs-exo group.

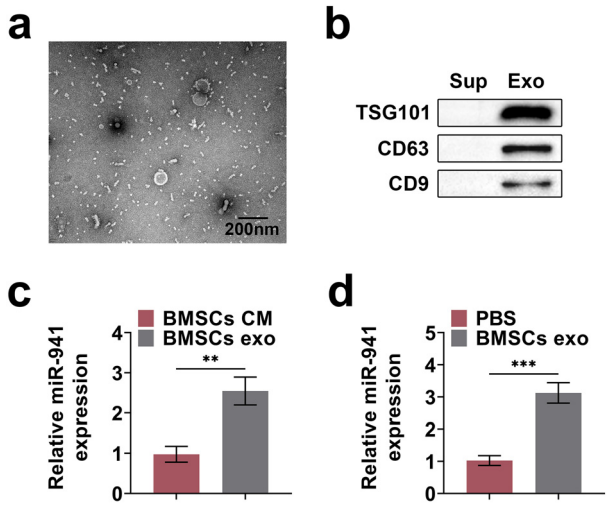

Fig. 1. miR-941 was highly expressed in BMSCs-exo. a, Morphology of BMSCs-exo under transmission electron microscopy. Bar = 200 nm. b, Western blot was used to analyze exosome-specific proteins such as TSG01, CD63 and CD9 in BMSCs-Exo. c, RT-PCR analysis of miR-941 in BMSCs-Exo. d, RT-PCR analysis of miR-941 in HESCs treated with PBS and BMSCs-Exo. $^{**}P < 0.01$ and $^{***}P < 0.001$. CM, conditioned medium; BMSCs-exo, exosomes from BMSCs

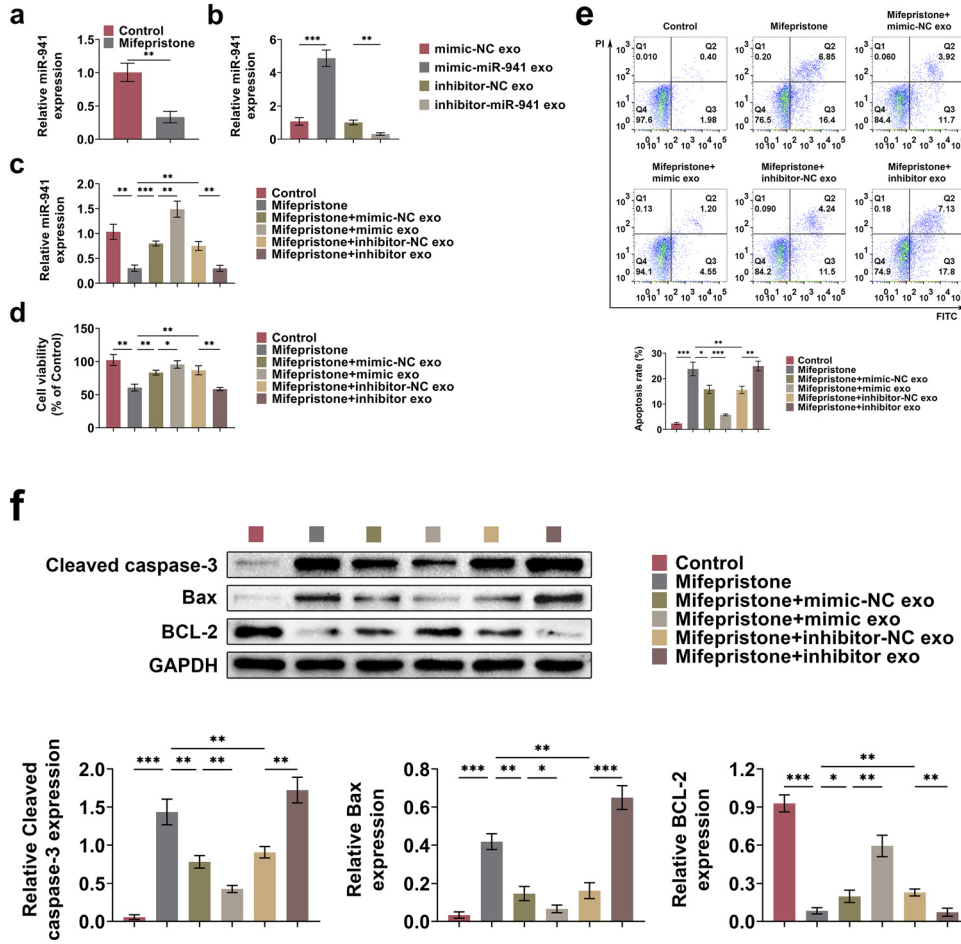

Fig. 2. BMSCs-exo derived miR-941 promoted cell proliferation and inhibited cell apoptosis in HESCs induced by mifepristone. a, The relative expression of miR-941 in HESCs treated with 60 μM mifepristone for 24 h. b, The level of miR-941 in BMSCs-exo extracted from BMSCs transfected with miR-941 mimics, mimic NC miR-941 inhibitors or inhibitor NC. c, RT-PCR analysis of miR-941 in HESCs treated with mifepristone and BMSCs-exo or BMSCs-exo transfected with miR-941 mimics, mimic NC miR-941 inhibitors or inhibitor NC. e, Cell apoptosis were detected by flow cytometry. f, Cell apoptosis rate was measured by flow cytometry. g, The protein expression of cleaved-caspase 3, Bax and Bcl-2 detected by western-blot assay. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$

Inversely, cell activity was also significantly decreased in HESCs after treatment with mifepristone and miR-941 inhibitor transfected BMSCs-exo (Fig. 2b). In addition, mifepristone induced cell apoptosis in HESCs, while BMSCs-exo and miR-941 mimic transfected BMSCs-exo significantly improved cell apoptosis induced by mifepristone (Fig. 2e and f). While BMSCs-exo treated with miR-941 inhibitor blocked the effect of BMSCs-exo on HESCs (Fig. 2e and f). The expression of apoptosis-related proteins (cleaved caspase-3 and Bax) was significantly increased in mifepristone

treated HESCs with or without miR-941 inhibitor transfected BMSCs-exo, which were significantly decreased in mifepristone treated BMSCs-exo or miR-941 mimic transfected BMSCs-exo group (Fig. 2g). The expression of anti-apoptotic proteins (Bcl-2) was absolutely on the contrary (Fig. 2g). Therefore, combined with the results of western-blot and flow cytometry in cell apoptosis, we concluded that BMSCs-exo derived miR941 alleviated cell apoptosis induced by mistafirone in HESCs.

BMSCs-exo promoted the migration and angiogenesis of HESCs induced by mifepristone through miR-941

We further examined the effect of BMSCs exosome-derived miR-941 on cell migration and invasion in HESCs. As shown in Fig. 3a–d, the migration and invasion in HESCs were significantly inhibited by mifepristone and mifepristone + miR-941 inhibitor treated BMSCs-exo (mifepristone + inhibitor exo) but were significantly reversed after treatment with BMSCs-exo and miR-941 mimic transfected BMSCs-exo (mifepristone + mimic exo). Also, we detected the expression of migration related proteins, which was consistent with the above results.

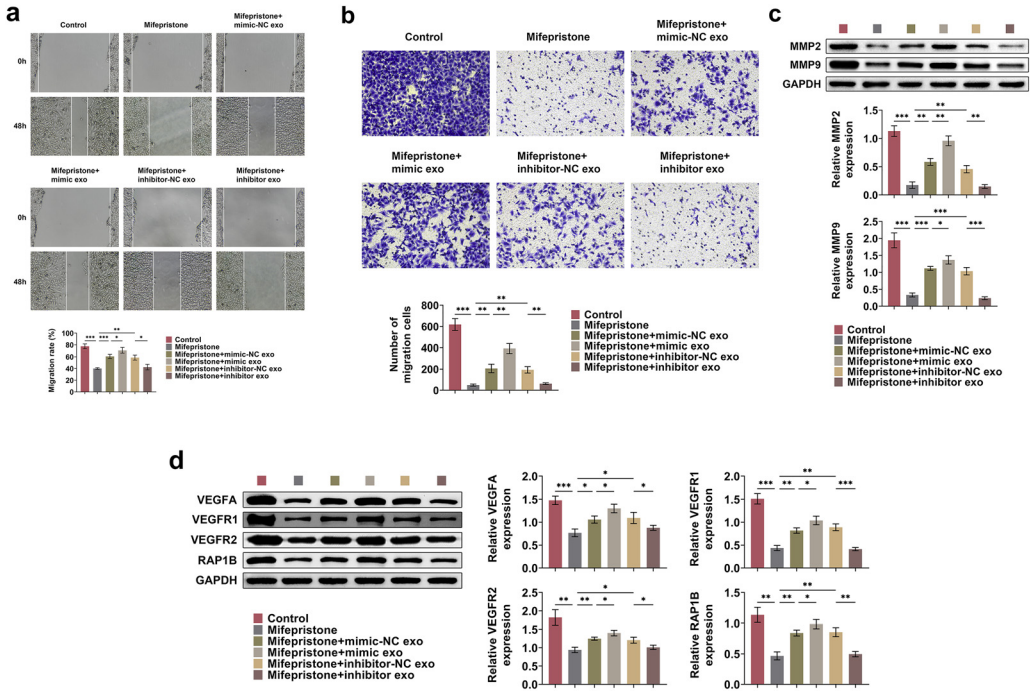

Fig. 3. BMSCs-derived exosomes deliver miR-941 to repress the invasion, migration and angiogenesis of HESCs. a, Cell migration was measured in HESCs by wound healing assay, reflected by quantification of the wound healing rate (b). c, Transwell assay was used to detect cell invasion in HESCs, with the number of invasion cells counted (d). e, Expression levels of EMT-related proteins in HESCs were determined by Western blot. f, Western-blot assay for detecting the expression levels of angiogenesis-related proteins in HESCs. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$

The migration related proteins, such as MMP2 and MMP9 were significantly decreased in mifepristone and mifepristone + inhibitor exo group but were significantly increased in mifepristone + BMSCs-exo and mifepristone + mimic exo group (Fig. 3e). In addition, the expression of angiogenic related proteins (VEGFA, VEGFR1, VEGFR2 and RAP1B) in HESCs treated with mifepristone + BMSCs-exo and mifepristone + mimic exo were also significantly increased compared with that in mifepristone group (Fig. 3f).

miR-941 in BMSCs-exo targeted TLR3 in HESCs

Previous studies have shown that TLR3 was upregulated in endometriosis patients [21]. We found that the expression of TLR3 gene and protein in HESCs cells induced by mifepristone was significantly up-regulated (Fig. 4a and b). Moreover, TLR3 was found to be one of the potential binding target proteins of miR-941 predicted by TargetScan website (Fig. 4c), and we further verified that miR-941 bound to TLR3 3′-UTR region by double luciferase reporting assay (Fig. 4d). Then, the HESCs were transfected with mimics NC, miR-941mimics, inhibitor NC and miR-941inhibitor to detect the effect of miR-941 on the expression of TLR3 (Fig. 4e). We found that the expression of TLR3 gene and protein was up-regulated by the treatment with miR-94 inhibitor (Fig. 4e and f). Contrarily, miR-941 mimics inhibited the expression of TLR3 genes and proteins (Fig. 4e and f), suggesting that miR-941 regulated the expression level of TLR3. Furthermore, our results further showed that BMSCS-exo could inhibit the expression level of TLR3 in HESCs treated by mifepristone, which was closely related to the expression of miR-941 (Fig. 4g and h).

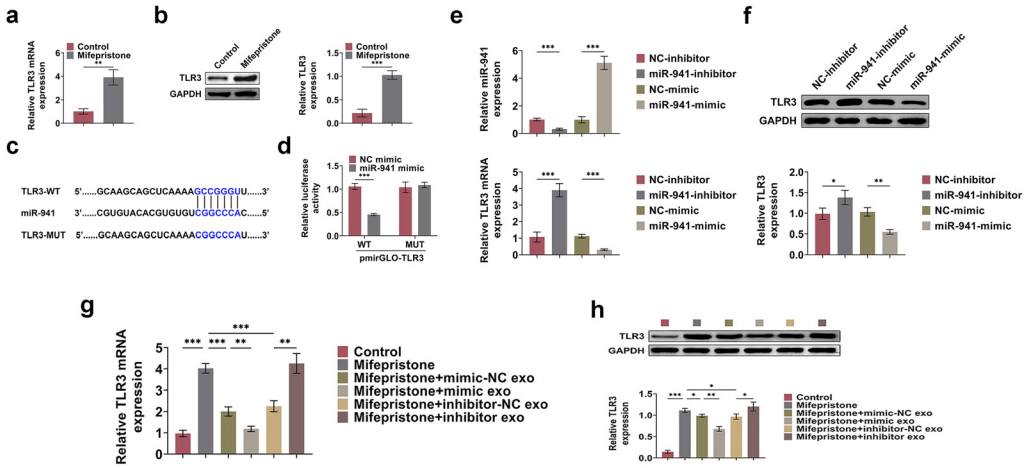

Fig. 4. MiR-941 from BMSCs-exo targeted TLR3. The mRNA (a) and protein (b) level of TLR3 in HESCs cells induced by mifepristone. c, The binding site of TLR3 and miR-941 predicted by TargetScan. d, Dual-luciferase reporting assay was used to verify the binding of miR-941 to TLR3. e, The relative expression of miR-941 and TLR3 in HESCs cells transfected with miR-941 mimics NC, miR-941 mimics, miR-941 inhibitor NC and miR-941 inhibitor. f, The protein level of TLR3 in HESCs cells transfected with miR-941 mimics NC, miR-941 mimics, miR-941 inhibitor NC and miR-941 inhibitor. The gene (g) and protein (h) level of TLR3 in HESCs treated with mifepristone, BMSCs-exo, BMSCs-mimic exo and BMSCs-inhibitor exo. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$

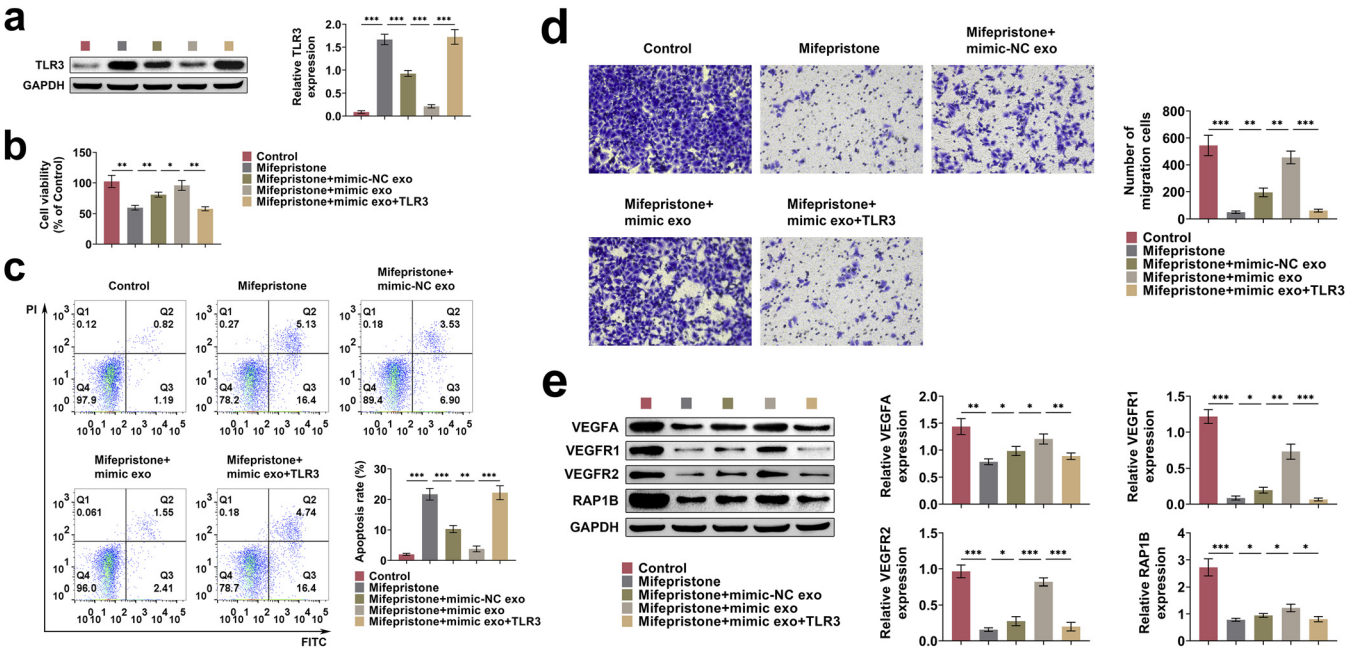

Fig. 5. Up-regulation of TLR3 blocked the effect of BMSCS-exo derived miR-941 on HESCs. HESCs were treated with mifepristone or TLR3 overexpressed plasmid, and co-cultured with BMSCS-mimic NC exo or BMSCS-mimic exo. a, The proteins level of TLR3 in HESCs. b, Cell activity was determined by MTT assay. c, Cell apoptosis was evaluated by flow cytometry. d, Cell migration ability was determined by Transwell assay. e, The expression levels of VEGFA, VEGFR1, VEGFR2 and RAP1B were measured by western-blot assay. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$

Overexpression of TLR3 reversed the effect of BMSCs-exo derived miR-941 on HESCs

Based on the above experimental results, we speculated that BMSCs-exo may regulate the expression of TLR3 by delivering miR-941 to improve mifepristone induced cell damage in HESCs cells. Therefore, we combined TLR3 overexpressed plasmid to detect the effect of BMSCS-exo on mifepristone induced cell apoptosis and migration in HESCs (Fig. 5a). The results showed that BMSCs-exo and BMSCs-mimics exo reversed the inhibitory effect on cell activity induced by mifepristone, while were blocked by TLR3 overexpression (Fig. 5b). Moreover, TLR3 overexpression blocked the inhibitory effect of BMSCs-mimics exo on mifepristone induced cell apoptosis and migration (Fig. 5c and d), and the expression level of angiogenesis related proteins was also increased (Fig. 5e).

DISCUSSION

At present, infertility is still an important problem plaguing many families. Despite significant advances in assisted reproductive technology, low embryo implantation rates remain a major obstacle to *in vitro* Fertilization success [22]. Successful implantation of a viable embryo from *in vitro* fertilization into the mother's uterus is a critical step in mammalian reproduction, which requires a receptive endometrium [23, 24]. Stromal cells are the main cellular component of the endometrium and are strictly regulated by hormones [25]. The differentiation of HESCs from fibroblast-like appearance to secretory decidua is a necessary transition from embryo implantation to maternal endometrium [26]. Inappropriate prolapse has been identified as the root cause of implantation failure and subsequent early embryo abortion [26]. Therefore, a deeper understanding of the molecular mechanism of endometrial decidua is helpful to improve endometrial receptivity and reduce adverse pregnancy outcomes.

BMSCs derived exosomes have a wide range of physiological and pathological effects, which ameliorated the progression of diseases such as osteoarthritis, cardiomyocyte hypoxic-reperfusion injury, and cognitive impairment [27, 28]. Studies have shown that BMSCs improved the endometrial thickness and promoted the regeneration of thin endometrium in rats, but the underlying mechanism remains unclear [29, 30]. Mifepristone is the antibody level hormone drug, with the termination of early pregnancy, anti-implantation, induction of menstruation and promotes cervical maturity and other effects. It was reported that exosomes from umbilical cord mesenchymal stem cell could reduce mifepristone induced HESCs injury [12, 31]. However, whether BMSCs-exo can ameliorate mifepristone induced HESCs injury has not been explored. In this study, we found that miR-941 was highly expressed in BMSCs-exo (Fig. 1c). In addition, BMSCs-exo induced the expression of miR-941 in HESCs (Fig. 1d). We found that Mifepristone reduced the level of miR-941 in HESCs with decreased cell activity, increased cell apoptotic rate, as well as decreased ability of migration and invasion (Fig. 2). Moreover, the expression of cell migration and angiogenesis related proteins was also significantly reduced in HESCs treated with mifepristone (Fig. 3). While these phenomena were reversed by BMSCs-exo, which was correlated with the level of miR-941 in BMSCs-exo (Figs 2–3). Our results showed that miR-941 mimics promoted the expression of miR-941 in BMSCs-exo, while miR-941 inhibitor had the opposite effect (Fig. 2b). Moreover, BMSCs-mimics exo was more effective in improving cell damage compared with BMSCs-exo (Figs 2–3). Thus, miR-941 derived from BMSCs-exo possessed therapeutic effect on mifepristone induced HESCs injury.

As we all know, miRNAs, one of the important cargos delivered by exosomes, exert an important influence on the physiological functions of cells by inhibiting the expression of target proteins [32, 33]. Using bioinformatics prediction and dual luciferase reporting assay, we found that miR-941 targeted TLR3, which was highly expressed in mifepristone induced HESCs (Fig. 4a–d). It was reported that activation of TLR3 signal stimulated the occurrence of chronic inflammation and promoted the progression of diseases such as cancers [34–36]. Besides, previous studies demonstrated that the expression level of TLR3 was increased in endometriosis patients with an intensive inflammatory state [21]. However, the role of TLR3 in HESCs remains to be elucidated. After further investigation, we found that miR-941 from BMSCs-exo significantly reversed the increase of TLR3 in HESCs, which may be related to its cellular protective effect against mifepristone (Fig. 4e–h). To verify this hypothesis, we transfected HESCs with TLR3 overexpression plasmid, and found that TLR3 overexpression in HESCs blocked the protective effect of BMSCs-exo and BMSCs-mimics exo on mifepristone -induced cell injury, which was manifested as decreased cell activity and migration, increased cell apoptosis, as well as significantly decreased expression of angiogenesis related proteins (Fig. 5).

In summary, BMSCs-exo attenuated mifepristone induced cell damage by reducing the expression of TLR3 via delivering miR-941 in HESCs.

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Ethics approval: Not applicable.

Author's contribution: All authors contributed to the study conception and design. Material preparation and the experiments were performed by Yu Wang. Data collection and analysis were performed by Xiaofei Sun and Qing Yang. The first draft of the manuscript was written by Lili Yin and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Not applicable.

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