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ORIGINAL RESEARCH

Quercetin Inhibits Adenomyosis by Attenuating Cell Proliferation, Migration and Invasion of Ectopic Endometrial Stromal Cells

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Purpose: To evaluate the effects of quercetin on proliferation, invasion and migration of endometrial stromal cells (ESCs) from adenomyosis patients.

Methods: Primary ectopic ESCs (EESCs) and eutopic ESCs (EuESCs) were obtained and purified from patients undergoing total hysterectomy for adenomyosis and identified by immunocytochemistry staining. The cytotoxicity and inhibition rate were determined by CCK-8 assay to obtain the IC_{50} value. Cell proliferative, migratory, and invasive abilities were detected by BrdU, wound scratch, transwell assays, respectively. Western blot analysis was employed to explore the effects of quercetin on the expression of MMP-2, MMP-9, Ezrin and Fascin proteins in cells.

Results: Both EESCs and EuESCs were characterized with strongly positive staining for vimentin and almost negative for cytokeratin. Quercetin inhibited the viability of EESCs and EuESCs in a dose- and time-dependent manner, with an IC_{50} = 33.00 μM for EuESCs and IC_{50} = 74.88 μM for EESCs at 72 h. Thus, the final concentrations and action time of quercetin in EuESCs (0, 20, 40, and 80 μM for 72 h) and EESCs (0, 40, 80, and 160 μM for 72 h) were selected. BrdU assay showed that quercetin dose-dependently suppressed the proliferation of EESCs and EuESCs, while the inhibition rate in EESCs was higher. Similarly, administration of quercetin in EESCs and EuESCs significantly decreased the motility and invasiveness in a dose-dependent fashion, with stronger inhibitory effects on EESCs. Finally, Western blot analysis demonstrated that invasion- and migration-related proteins (MMP-2, MMP-9, Erzin, and Fascin) were significantly downregulated with the quercetin concentration increasing. Moreover, the decreased level of these proteins in EESCs under quercetin exposure was greater than that in EuESCs.

Conclusion: Quercetin can inhibit the proliferation of EESCs in adenomyosis and reduce their mobility and invasiveness. These inhibitory effects may be related to the downregulation of MMP-2, MMP-9, Fascin, and Erzin proteins.

Keywords: quercetin, adenomyosis, proliferation, migration, invasion

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Introduction

Adenomyosis, characterized as the deep infiltration of endometrial glands and stroma into the myometrium, is a common benign uterine disorder in women worldwide.[1] Approximately 20% of adenomyosis cases occur in women younger than 40 years, with the remaining 80% being reported in women of late reproductive age (40–50 years).[2,3] Despite some patients may be asymptomatic, it often causes abnormal uterine bleeding, dysmenorrhea, menorrhagia, dyspareunia, and infertility, giving a negative impact on the

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quality of life.[4–7] However, the management of adenomyosis is a major challenge because few drugs have been developed exclusively for this disease.[8] Currently, oral contraceptive drugs, gonadotrophin-releasing hormone (GnRH) agonists, and total or subtotal hysterectomy are the common therapeutic methods for adenomyosis patients.[9,10] Unfortunately, although surgical treatment is regarded as the definitive therapy for adenomyosis, it can be traumatic for women who are symptomatic but have a strong desire to conceive.[11,12] In addition, evidence has shown several limitations of the medical approaches, including severe premenopausal symptoms, frequent recurrence after medicine withdrawal and a huge economic burden.[9,10,13] Thus, it is paramount to identifying novel efficacious medical therapies for adenomyosis.

Quercetin (3, 3′, 4′, 5, 7-pentahydroxyflavone), a naturally occurring polyphenolic flavonoid, is widely distributed in various dietary fruits and vegetables.[14,15] Notably, quercetin is well known to provide us with diverse pharmacological functions including anti-oxidative, anti-inflammatory, anti-proliferative, and anti-tumoral effects.[16] Based on these activities, numerous studies have suggested the potential benefits of quercetin in treating cancers and endometriosis with no obvious side effects.[17,18] Nevertheless, the ability of quercetin to inhibit adenomyosis remains poorly understood.

Therefore, using both primary human ectopic endometrial stromal cells (EESCs) and eutopic ESCs (EuESCs), we first investigated the influence of quercetin on the growth, invasion and migration of EESCs and EuESCs. We also investigated the effect of quercetin on the expression of invasion- and migration-related proteins in EESCs and EuESCs. This study can offer an in vitro evidence for applications of quercetin in anti-adenomyosis treatment.

Materials and Methods

Ethics Statement

A written informed consent was obtained from each patient before surgery for collecting uterine tissues in the present study. The use of uterine tissues for culture experiments was approved by the Medical Ethical Committee of the Second Affiliated Hospital of Wenzhou Medical University. This study was in accordance with the principles of the Declaration of Helsinki.

Reagents and Antibodies

Quercetin dihydrate (Sigma, USA) was dissolved in dimethyl sulfoxide (DMSO; Corning, USA) and further diluted with medium to reach 0, 20, 40, 50, 80, 100, 150, 160, and 200 μM before treatment. The following primary antibodies were used in the present study: rabbit anti-human Fascin antibody (1:1000; Abcam, USA), rabbit anti-human Erzin antibody (1:1000; Cell Signaling Technology, USA), mouse anti-human MMP-2 antibody (1:1000; Cell Signaling Technology, USA), rabbit anti-human MMP-9 antibody (1:1000; Cell Signaling Technology, USA), mouse anti-human β-actin antibody (1:3000; Abcam, USA), rabbit anti-human GAPDH antibody (1:3000; Abcam, USA), mouse anti-human vimentin antibody (1:400; Proteintech, USA), and mouse anti-human cytokeratin antibody (1:400; Proteintech, USA). The secondary antibodies used in this study were listed as follows: peroxidase-conjugated goat anti-rabbit antibody (1:5000; Biosharp, China) and anti-mouse antibody (1:5000; Biosharp, China).

Primary Cell Isolation and Culture

Human endometrium tissues were obtained from twenty patients who underwent total hysterectomy for adenomyosis in the Second Affiliated Hospital of Wenzhou Medical University from November 2018 to October 2019, with a median age of 43 years (range 33–55 years). The eutopic and ectopic endometrium tissues of each patient were dissected from the uterus and placed in an ice-cold 1:1 mixture of Dulbecco's modified Eagle's medium (DMEM; Gibco, USA) and 1% penicillin-streptomycin antibiotics (Solarbio, China) for transport. After washing with phosphate-buffered saline (PBS), the tissues were gently cut into small pieces (1 mm^3) and digested in 0.5% collagenase Ⅱ (diluted with DMEM) for 1 h at 37 °C in a shaking water bath. The dispersed cells were filtered through a 100-μm filter screen to remove the undigested tissue pieces. The EuESCs and EESCs were collected by centrifugation at 1000 rpm for 5 min and washed 2–3 times with PBS sterile.

The isolated EuESCs and EESCs were all cultured in DMEM supplemented with 10% fetal bovine serum (FBS; Gibco, USA) and 1% penicillin-streptomycin antibiotic solution. These cells in culture dishes were incubated at 37 °C in a humidified atmosphere of 5% CO_2-95% air. Cells from the third to the sixth passages were used for further experiments.

Cell Characterization

Identification of the isolated EuESCs and EESCs was performed by immunocytochemistry staining. In brief, cells were seeded on chamber slides and fixed with 4% paraformaldehyde for 30 min at 4 °C, followed by

blockage of endogenous peroxidase activity with 3.0% H_2O_2 for 10 min at room temperature. Thereafter, cells were immunostained with primary mouse anti-human vimentin (1:400; Abcam, USA) and mouse anti-human cytokeratin (1:400; Abcam, USA) antibodies for 1 h, respectively, followed by incubated with the secondary antibody for 30 min at room temperature. Immunoreactivity was visualized with the 3,3′-diaminobenzidine (DAB; Biosharp, China). Both vimentin and cytokeratin immunoreactivities were observed in the cytoplasm and cells that showed yellowish brown were recognized as positive.

Determination of Cytotoxicity

The cytotoxic effect of quercetin on EuESCs and EESCs was tested by Cell Counting Kit-8 (CCK-8; Dojindo, Japan) assay. Briefly, EuESCs and EESCs were dispensed into 96-well plates (3×10^3 cells/well) in 200 μL of complete DMEM and various concentrations of quercetin were added (0, 20, 50, 100 and 200 μM for EuESCs; 0, 50, 100, 150 and 200 μM for EESCs). After incubated at 37 °C for 24, 48 and 72 h, 10 μL of CCK-8 solution in 100 μL complete DMEM was added to each well and incubation continued for 4 h. The absorbance of each well was determined at 450 nm by Microplate Reader (Bio-Tek Instruments, USA). The cell viability was calculated using the following equation:

$$\text{Cellviability}(\%) = \frac{\text{Absorbance individual test group} - \text{Absorbance blank group}}{\text{Absorbance control group} - \text{Absorbance blank group}}$$

The IC_{50} values (concentration that inhibits cell growth by 50%) were determined using regression analysis. According to the IC_{50} values measured in this study, the final concentrations and exposure time of quercetin (0, 20, 40, and 80 μM for 72 h) and (0, 40, 80, and 160 μM for 72 h) were used on EuESCs and EESCs in subsequent experiments, respectively.

Cell Proliferation Assay

BrdU incorporation assay (Beyotime, China) was conducted to measure the proliferation of EuESCs and EESCs after quercetin treatment. Briefly, EuESCs and EESCs were seeded into 6-well plates with a density of 1×10^5 cells/well. Meanwhile, EESCs in each well were treated by quercetin for 72 h with varying concentrations (0, 40, 80, and 160 μM), while smaller gradients of quercetin (0, 20, 40, and 80 μM) were added to treat EuESCs for 72 h in the 6-well plates. BrdU (1 mg/mL) was added into culture medium of each well after quercetin treatment for 4 h. Finally, the number of BrdU positive cells in each well was counted under a microscope (Nikon, Japan), which represented the ability of cells to proliferate.

Cell Wound Scratch Assay

Prior to the experiment, EuESCs and EESCs were pretreated with different concentrations of quercetin (0, 20, 40, and 80 μM for EuESCs; 0, 40, 80, and 160 μM for EESCs) for 72 h. Subsequently, cells were plated at a density of 2.5×10^5 cells/well onto 6-well plates and cultured at 37 °C in 5% CO_2 until a monolayer was formed. After 24 h, artificial wound tracks were created in the confluent monolayers with a sterile micropipette tip. Cells were then gently washed with PBS to remove debris and further incubated with serum-free DMEM for 12 and 24 h. The migration distance was measured and analyzed using Image J software version 1.8.0 (National Institutes of Health, USA).

Transwell Invasion Assay

Cell invasion assay was performed using transwell chambers with polycarbonate filters (8.0 μm pore size; Costar, USA) in 24-well plates. In brief, the upper Transwell chambers were pre-coated with 100 μL Matrigel (BD Biosciences, USA) and serum-free DMEM medium mixtures at a ratio of 1:8. Before the experiment, EuESCs and EESCs were pretreated with different concentrations of quercetin (0, 20, 40, and 80 μM for EuESCs; 0, 40, 80, and 160 μM for EESCs) for 72 h. Then, 1×10^5 of EuESCs and EESCs were resuspended in 200 μL serum-free DMEM and plated on the upper side of the filter, while 600 μL complete DMEM containing 10% FBS was placed in the lower plate. Cells were then incubated at 37 °C in a 5% CO_2 for 24 h, after which non-invasive cells on the upper surface of the membrane were gently removed with a cotton swab. The invaded cells in the lower chamber were fixed with 4% paraformaldehyde for 20 min and stained with 0.25% crystal violet (Beyotime, China) for 15 min at room temperature. Next, the microscope (Nikon, Japan) was employed to capture the images of the cells invaded to the lower chamber. Five fields per filter were randomly selected under the microscope to count the invaded cell numbers.

Western Blot Analysis

The protocol of Western blot analysis was described previously.[19] In brief, EuESCs and EESCs seeded into 6-well plates at a density of 2.5×10^5 cells/well and treated by quercetin with varying concentrations described above (0, 20, 40, and 80 μM for EuESCs; 0, 40, 80, and 160 μM for EESCs). After 72 h incubation, total proteins in cells were extracted using radioimmunoprecipitation assay (RIPA) lysis buffer (60 mM Tris-HCl, pH 6.8, 5% glycerol, 2% SDS) on ice and quantified using Bicinchoninic Acid (BCA) Protein Assay kit (Thermo, USA) according to the manufacturer's instruction. Subsequently, proteins were electrophoresed in polyacrylamide gels, transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, USA), and incubated with primary antibodies at 4 °C overnight. After washing with Tris Buffered Saline Tween (TBST) for 3 times, the PVDF membranes were then incubated with horseradish peroxidase-conjugated secondary antibodies at room temperature for 1.5 h. The β-actin signal was used as a loading control. Finally, the bound antibodies were detected with Immobilon Western Chemiluminescent HRP Substrate (Millipore, USA). Images were obtained with Bio-Rad ChemiDoc^{TM} XRS system (Bio-Rad, USA). The intensity of the target proteins blots was quantified by Image J software version 1.8.0 (National Institutes of Health, USA).

Statistical Analysis

All statistical analyses were performed using SPSS 22.0 software (Chicago, USA). Before comparing, all data sets were first analyzed with Shapiro–Wilk normality test and considered normally distributed with $P > 0.05$. All normally distributed values were expressed as mean ± standard deviation (SD) and were evaluated by Student's *t*-test (two groups) or ANOVA (three or more groups) for the difference. If variances were homogeneous, then the least significance method was employed to compare between two groups. Dunnett's T3 method was used to compare between two groups if variances were nonhomogeneous. Non-normally distributed data were presented as median (range) and were analyzed comparably by Mann–Whitney testing. A two-tailed *P*-value less than 0.05 was defined as statistically significant.

Results

Phenotypic Characterization of EuESCs and EESCs Isolated from Human Uterine Tissues

Immunocytochemical staining for vimentin and cytokeratin was performed to identify whether the isolated cells were ESCs. As shown in Figure 1, both EuESCs and EESCs were strongly positive for vimentin (cytoplasm), a stromal cell marker, and nearly negative for cytokeratin (cytoplasm), which confirmed that these cells were actually ESCs.

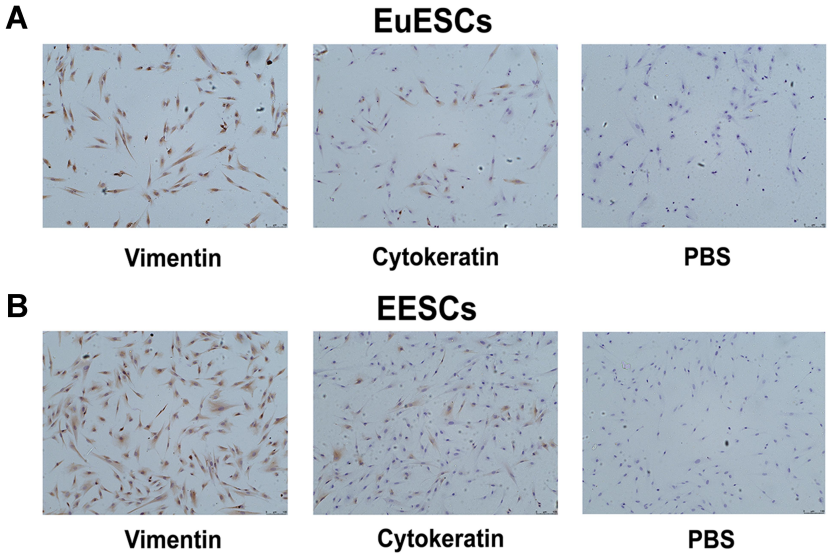

Figure 1 Immunocytochemical staining for vimentin and cytokeratin expression. (**A**) The cytoplasm of EuESCs was immunopositive for vimentin and immunonegative for cytokeratin. The nuclei were stained with hematoxylin (Magnification ×100). (**B**) The cytoplasm of EESCs was immunopositive for vimentin and immunonegative for cytokeratin. The nuclei were stained with hematoxylin (Magnification ×100).

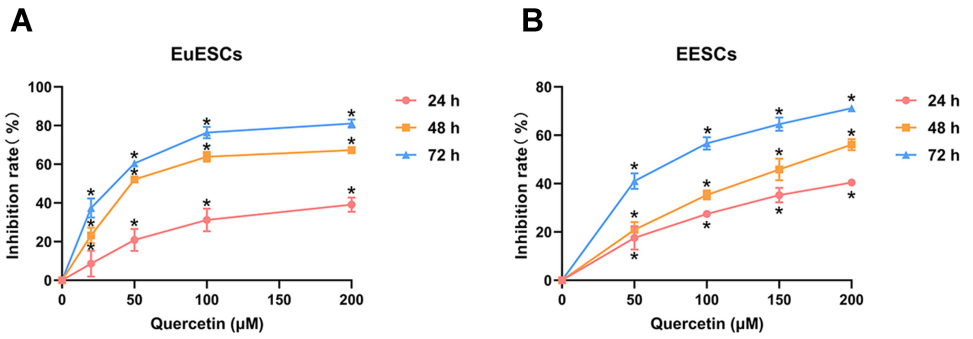

Figure 2 Quercetin shows the cytotoxic effect on the viability of EuESCs and EESCs in vitro. (**A**) EuESCs were exposed to quercetin with various concentrations (0, 20, 50, 100, and 200 μM) for 24, 48 and 72 h. (**B**) EESCs were exposed to quercetin with various concentrations (0, 50, 100, 150, and 200 μM) for 24, 48 and 72 h. Cell viability was measured by CCK-8 assay. Data were presented as mean ± SD; *P <0.05 vs 0 μM group. Each experiment was performed three times.

Cytotoxic Effects of Quercetin on EuESCs and EESCs

In order to confirm the cytotoxic activity of these extracts on EuESCs and EESCs, the concentrations of quercetin at different incubation times that inhibit cell growth by 50% were determined. The cytotoxic effect of quercetin on EuESCs and EESCs is shown in Figure 2. Compared with the control group, quercetin treatment at concentrations between 0 and 200 μM significantly increased the inhibition rate in both EuESCs and EESCs in a dose- and time-dependent manner. The IC_{50} values of quercetin for EuESCs were 315.1, 59.86 and 33.00 μM at 24 h, 48 h and 72 h, respectively, which were higher than those in EESCs at each time (332.8, 167.6 and 74.88 μM at 24 h, 48 h and 72 h, respectively). The cytotoxic results of quercetin were listed in Tables 1 and 2. Thus, the final concentrations and action time of quercetin in EuESCs (0, 20, 40, and 80 μM for 72 h) and EESCs (0, 40, 80, and 160 μM for 72 h) were selected in further experiments.

Quercetin Inhibits the Proliferation of EuESCs and EESCs

BrdU assay was performed to evaluate the effects of quercetin on the proliferation of EuESCs and EESCs. Both EuESCs and EESCs were exposed in quercetin with different concentrations for 72 h. As presented in Figure 3, quercetin significantly inhibited the proliferation of EuESCs and EESCs in a dose-dependent manner. Furthermore, our results showed that the number of BrdU positive cells of EESCs was significantly lower than EuESCs after 40 and 80 μM quercetin treatment, suggesting that quercetin displayed a higher inhibitory effect on the proliferation of EESCs compared with EuESCs.

Quercetin Suppresses the Migration and Invasion of EuESCs and EESCs

To determine whether quercetin affected the migration of EuESCs and EESCs, wound healing assay was performed, respectively. EuESCs and EESCs were pretreated with varying concentrations of quercetin for 72 h before the cell scratch experiment. Figure 4 demonstrates that quercetin decreased the wound healing of both EuESCs and EESCs in a dose-dependent manner. In addition, the result was that compared with EuESCs, the migration of EESCs were significantly reduced under 40 and 80 μM quercetin treatment.

Table 2 The Cytotoxic Results of Quercetin on EESCs

Quercetin Concentration (μM)	Inhibition Rate (%)		
	24 h	48 h	72 h
0	0 ± 0	0 ± 0	0 ± 0
50	17.58 ± 4.85	20.88 ± 3.23	41.06 ± 3.23
100	27.46 ± 0.78	35.29 ± 1.84	56.61 ± 2.53
150	35.26 ± 3.06	45.81 ± 4.52	64.57 ± 2.76
200	40.43 ± 0.58	56.08 ± 2.26	71.15 ± 0.41

Abbreviation: EESCs, ectopic endometrial stromal cells.

Table 1 The Cytotoxic Results of Quercetin on EuESCs

Quercetin Concentration (μM)	Inhibition Rate (%)		
	24 h	48 h	72 h
0	0 ± 0	0 ± 0	0 ± 0
20	8.59 ± 6.72	23.21 ± 4.00	37.36 ± 4.90
50	20.91 ± 5.07	52.14 ± 1.64	60.44 ± 1.89
100	31.18 ± 5.84	63.91 ± 2.55	76.35 ± 2.94
200	39.11 ± 3.68	67.27 ± 1.34	81.02 ± 2.13

Abbreviation: EuESCs, eutopic endometrial stromal cells.

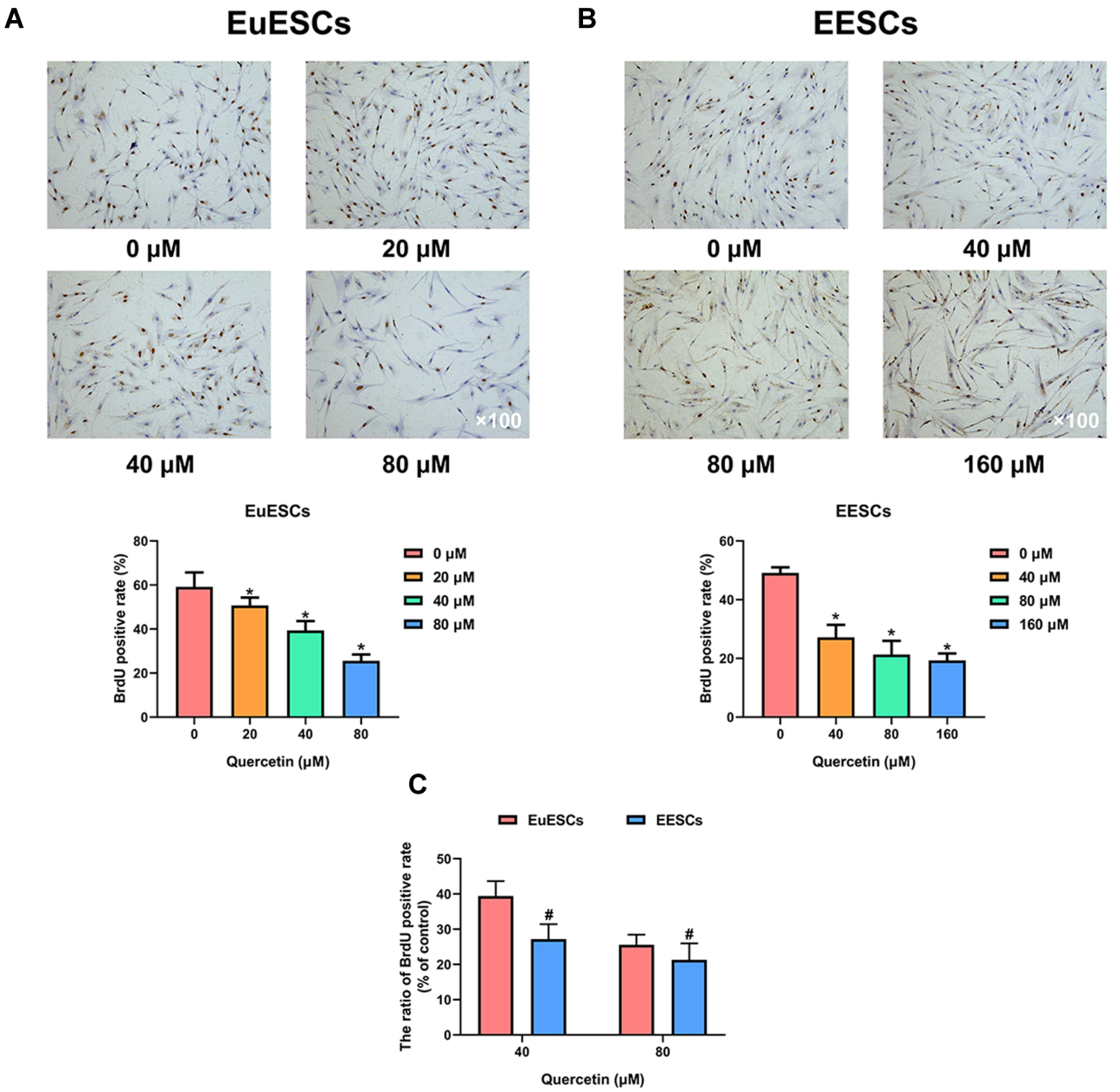

Figure 3 Quercetin inhibits the proliferation of EuESCs and EESCs in vitro. (**A**) Representative images for BrdU assay on EuESCs under a microscopy (Magnification ×100) after treatment with increasing concentrations of quercetin (0, 20, 40, and 80 μM) for 72 h. The bar graphs represented the BrdU positive rate of EuESCs. (**B**) Representative images for BrdU assay on EESCs under a microscopy (Magnification ×100) after treatment with increasing concentrations of quercetin (0, 40, 80, and 160 μM) for 72 h. The bar graphs represented the BrdU positive rate of EESCs. (**C**) The bar graph represented the ratio of BrdU positive rate between quercetin treatment group (40 or 80 μM) and control group (0 μM). Data were presented as mean ± SD; $^{*}P$ <0.05 vs 0 μM group; $^{\#}P$ <0.05 vs EuESCs group. Each experiment was performed three times.

Transwell chambers covered with Matrigel were employed to detect the effects of quercetin on ESCs invasion. Both EuESCs and EESCs were treated by quercetin in the same concentration gradient as that prior to the migration study for 72 h. The results in Figure 5 revealed that the number of invaded cells was significantly decreased following quercetin treatment in a dose-dependent manner in both EuESCs and EESCs groups. In comparison with EuESCs group, quercetin exerted a stronger inhibition on EESCs invasion at the concentration of 40 and 80 μM.

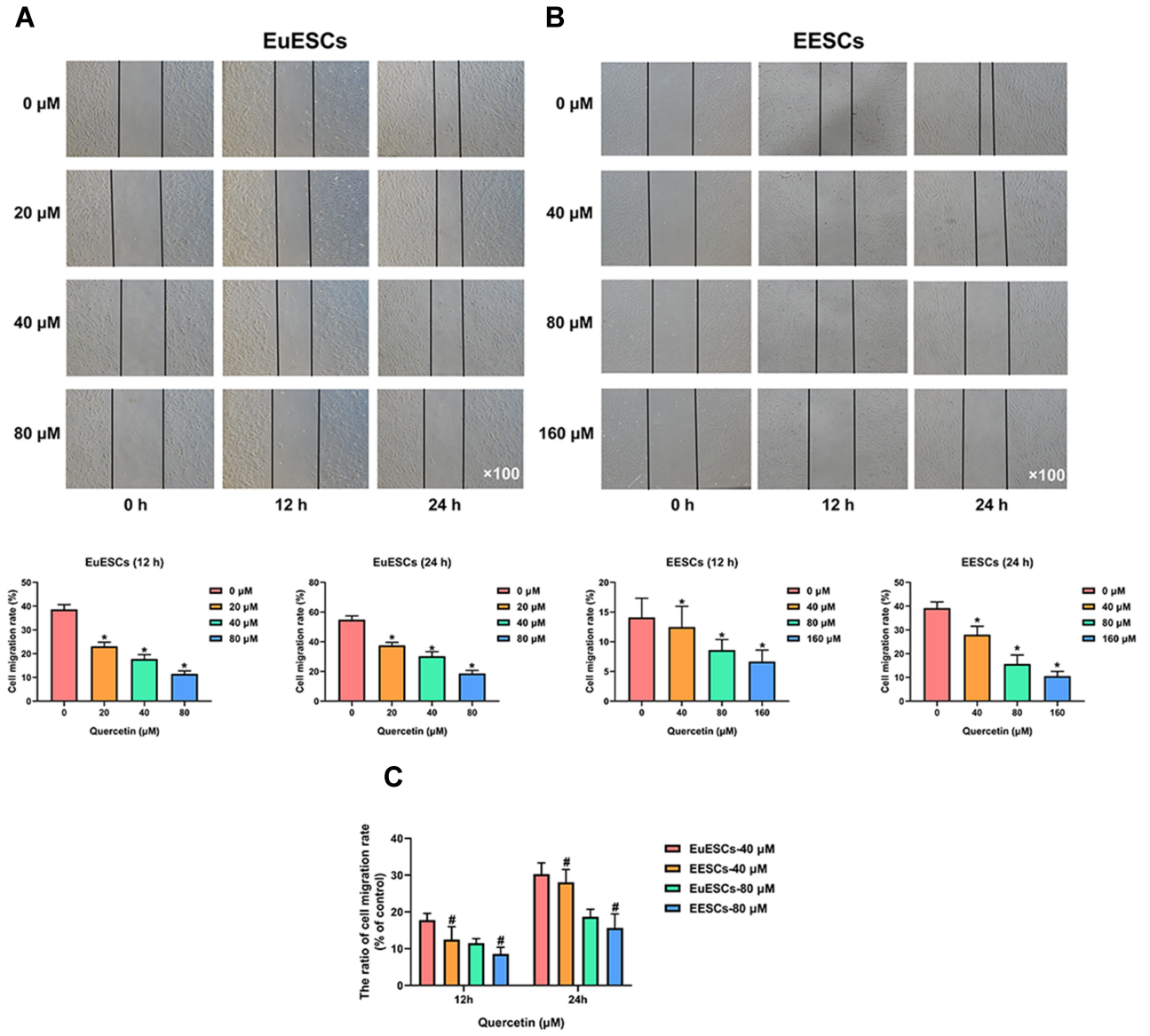

Figure 4 Quercetin inhibits the migration of EuESCs and EESCs in vitro. (**A**) EuESCs were exposed to increasing concentrations of quercetin (0, 20, 40, and 80 μM) for 72 h prior to the experiment. Representative images for cell scratch assay on EuESCs under a microscopy (Magnification ×100) at 0, 12 and 24 h. The bar graphs represented the cell migration rate of EuESCs. (**B**) EESCs were exposed to increasing concentrations of quercetin (0, 40, 80, and 160 μM) for 72 h prior to the experiment. Representative images for cell scratch assay on EESCs under a microscopy (Magnification ×100) at 0, 12 and 24 h. The bar graphs represented the cell migration rate of EESCs. (**C**) The bar graph represented the ratio of cell migration rate between quercetin treatment group (40 or 80 μM) and control group (0 μM). Data were presented as mean ± SD; $^{*}P$ <0.05 vs 0 μM group; $^{\#}P$ <0.05 vs EuESCs group. Each experiment was performed three times.

Quercetin Downregulates the Expression of Migration- and Invasion-Related Proteins in EuESCs and EESCs

To further explore the effect of quercetin on the migration and invasion of EuESCs and EESCs, the expression of several proteins which were crucial for the migration (Fascin and Ezrin) and invasion (MMP-2 and MMP-9) was detected after treatment with different concentrations of quercetin for 72 h. As shown in Figure 6, Western blot analysis showed that quercetin dose-dependently decreased the expression of Fascin, Ezrin, MMP-2, and MMP-9 proteins in both EuESCs and EESCs. In comparison with the EuESCs group, the decreased level of these proteins in EESCs group was significantly higher at 80 μM quercetin treatment. No significant difference of quercetin on reducing MMP-9 expression was observed between EuESCs and EESCs under 40 μM quercetin treatment. These results further indicated that quercetin played a stronger suppressive role in EESCs migration and invasion compared with EuESCs.

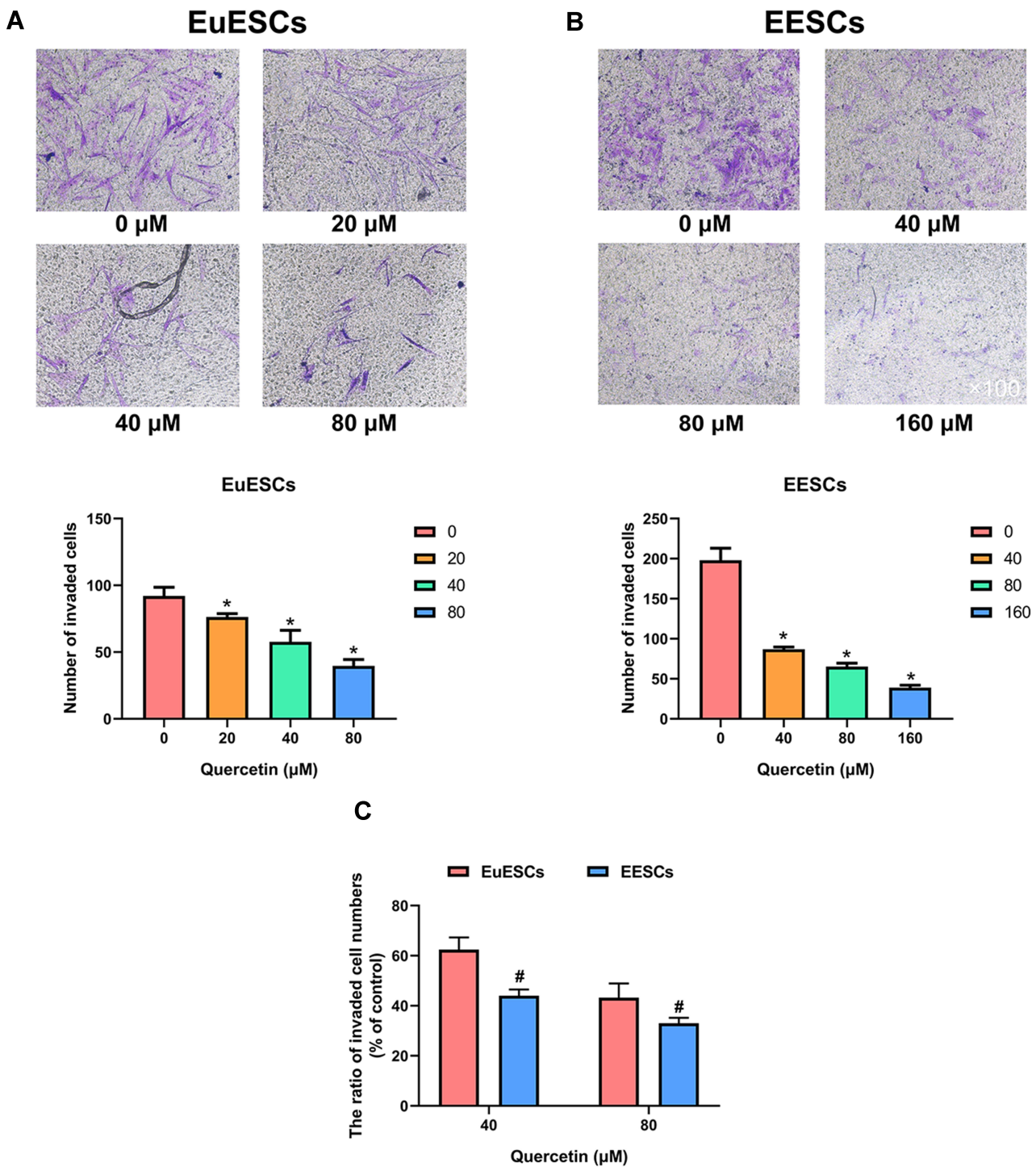

Figure 5 Quercetin inhibits the invasion of EuESCs and EESCs in vitro. (**A**) EuESCs were exposed to increasing concentrations of quercetin (0, 20, 40, and 80 μM) for 72 h prior to the experiment. Representative images for transwell invasion assay on EuESCs under a microscopy (Magnification ×100) at 24 h. The bar graphs represented the invaded cell numbers of EuESCs. (**B**) EESCs were exposed to increasing concentrations of quercetin (0, 40, 80, and 160 μM) for 72 h prior to the experiment. Representative images for transwell invasion assay on EESCs under a microscopy (Magnification ×100) at 24 h. The bar graphs represented the invaded cell numbers of EESCs. (**C**) The bar graph represented the ratio of invaded cell numbers between quercetin treatment group (40 or 80 μM) and control group (0 μM). Data were presented as mean ± SD; **P* <0.05 vs 0 μM group; #*P* <0.05 vs EuESCs group. Each experiment was performed three times.

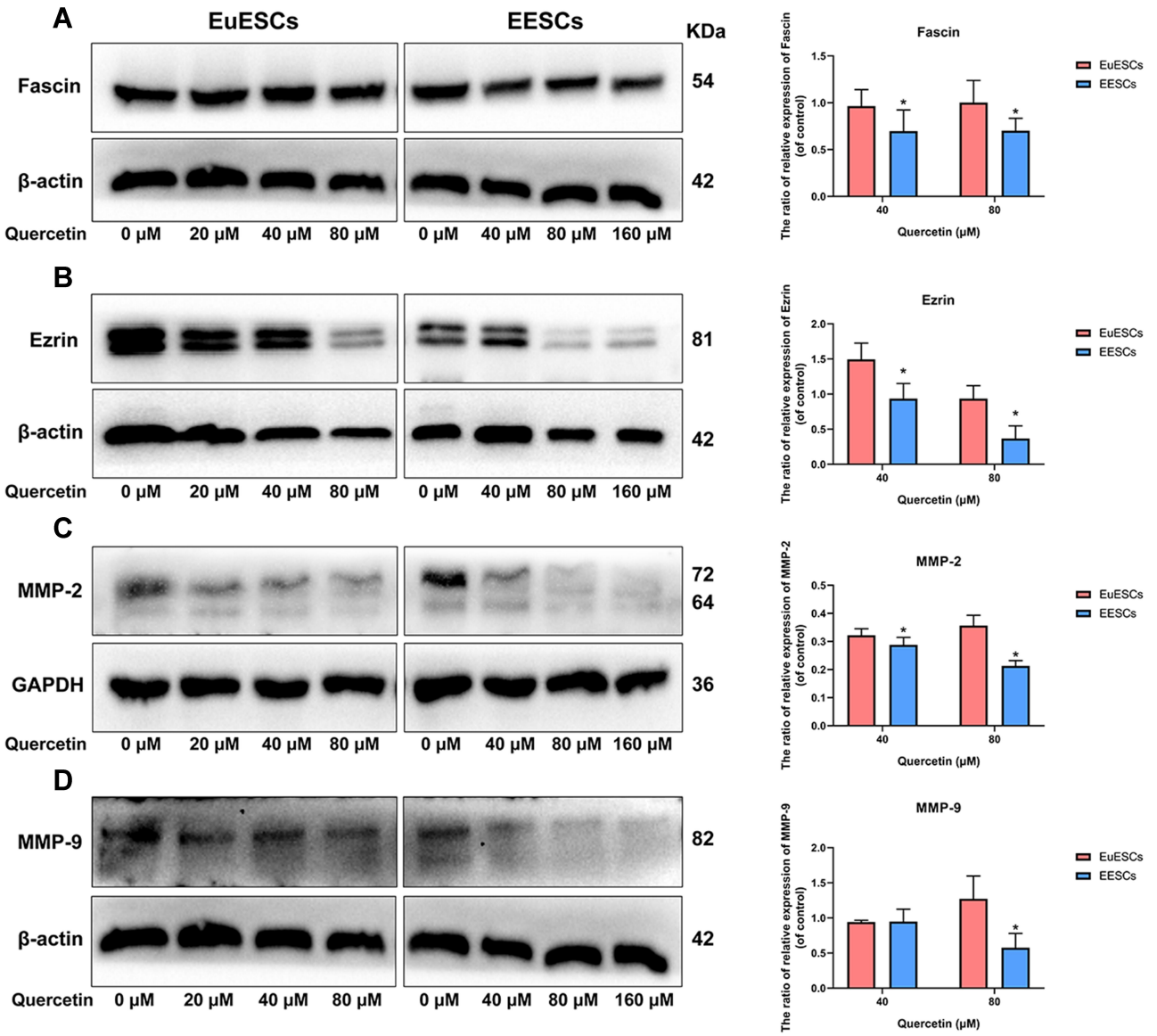

Figure 6 Quercetin downregulates the expression of migration- and invasion-related proteins in EuESCs and EESCs. (**A, B**) Western blot for the detection of cell migration markers (Fascin and Ezrin) in EuESCs and EESCs after treatment of different concentrations of quercetin (EuESCs: 0, 20, 40, and 80 μM; EESCs: 0, 40, 80, 160 μM) for 72 h. The bar graphs represented the ratios of Fascin and Ezrin relative expressions between quercetin treatment group (40 or 80 μM) and control group (0 μM). (**C, D**) Western blot for the detection of cell invasion markers (MMP-2 and MMP-9) in EuESCs and EESCs after treatment of different concentrations of quercetin (EuESCs: 0, 20, 40, and 80 μM; EESCs: 0, 40, 80, 160 μM) for 72 h. The bar graphs represented the ratios of MMP-2 and MMP-9 relative expressions between quercetin treatment group (40 or 80 μM) and control group (0 μM). Data were presented as mean ± SD; **P* <0.05 vs EuESCs group. Each experiment was performed three times. Data were presented as mean ± SD; **P* <0.05 vs EuESCs group. Each experiment was performed three times.

Discussion

Uterine adenomyosis manifests with the presence of endometrial glands and stroma within the myometrium. Although adenomyosis often occurs in women of childbearing age as a benign disorder, it is also a multifactorial and refractory gynecological disease with controversial pathogenesis and compromised reproductive outcomes.[20] Worthy of note, adenomyosis is common in endometrial adenocarcinoma hysterectomy specimens, which strongly suggests the potential for malignant transformation about this disease.[21] Indeed, accumulated evidences have reported the malignant features of adenomyosis in some respects based on several clinical evidences and pathological mechanisms.[22–24] Therefore, therapeutics used to treat malignant tumors may also be applicable for treating adenomyosis. Quercetin, as the most frequently investigated flavonoid extracted from various plants, has shown powerful anticancer properties through antioxidant, anti-inflammatory, anti-proliferative, anti-angiogenesis, and anti-metastasis activities.[17,18] Thus, the current study first

explored the effect of quercetin treatment on adenomyosis in vitro.

Cell proliferation in adenomyosis tissues is a commonly observed event, as evidenced by the hyperplasia and hypertrophy of the adjacent ectopic myometrium.[25] A cytotoxic test by CCK-8 assay was initially conducted for selecting the optimal concentration of quercetin in this study. Results found that the IC_{50} values of quercetin for EESCs were higher than those in EuESCs at all time sets. This indicated that a lower dose of quercetin cannot significantly inhibit EESCs growth but may be cytotoxic for EuESCs. However, we further showed that quercetin significantly inhibited the cell proliferation of EuESCs and EESCs in a dose-dependent fashion using BrdU assay. Moreover, 40 or 80 μM of quercetin could lead to a higher inhibitory effect on the growth of EESCs than that in EuESCs. Therefore, despite the higher IC_{50} values of quercetin for EESCs, these results suggest that quercetin is not only an efficient promising drug for treating adenomyosis but also a safe agent for normal endometrium.

Our findings are not in accordance with a previous study based on animal models.[26] Specifically, Nie et al[26] demonstrated that intragastric administration of quercetin caused no significant body weight loss in neonatal Imprinting Control Region (ICR) mice induced with adenomyosis compared with the untreated control group. However, this result found by Nie et al may be due to a pain-suppressed behavior and concomitant-reduced food intake.[26,27] Thus, whether quercetin could inhibit the EESCs proliferation in adenomyosis in vivo also needs to be investigated in the future.

Adenomyosis also exhibits a metastatic feature which is characterized by the deep infiltration of endometrial glands and stroma into the myometrium, suggesting that suppressing cell migration and invasion may be pivotal for adenomyosis therapy.[28,29] A growing number of evidences have revealed the suppressive role of quercetin on the metastatic abilities of multiple cancer cells, such as prostate,[30] oral,[31] lung,[32] liver,[33] and breast cancers.[34] Similar to these results in human cancer cells, our study also demonstrated that quercetin significantly decreased the migratory and invasive abilities in both EuESCs and EESCs in a dose-dependent manner, and this inhibitory effect was also higher in EESCs group compared to that in EuESC group. It is well known that several molecules such as Fascin, Erzin, MMP-2 and MMP-9 are critically involved in cell migration and invasion.[35–38] Herein, this study showed that quercetin significantly decreased the expression of Fascin, Erzin, MMP-2, and MMP-9 proteins in a dose-dependent manner. Moreover, the reduced levels of these proteins are more significant in EESCs compared with EuESCs. Notably, these results are consistent with a recent study[26] showing that oral treatment of quercetin significantly inhibited the infiltration of myometrium induced by tamoxifen in neonatal mice. Up to date, several Chinese herbs such as berberine and tanshinone IIA have been reported to inhibit the growth, invasion and migration through regulating NF-κB/p65,[39] LPS/TLR4,[40] and 14-3-3ζ signaling pathways.[41] However, whether quercetin can exhibit its anti-adenomyosis ability by targeting these pathways remains unknow, and needs to be further clarified by more studies.

Conclusion

Collectively, our study first showed that quercetin could inhibit the proliferation, migration, and invasion of ESCs in a dose-dependent manner, and these effects are significantly higher in EESCs compared with EuESCs. Worthy of note, we obtained uterine tissues from the same adenomyosis patients who received hysterectomy and collected the primary EESCs and EuESCs for experiments, which greatly improved the reliability of this study. Furthermore, to the best of our knowledge, the present study is the first to report the ability of quercetin to inhibit the proliferation, migration and invasion of EESCs in vitro, which provided an experimental evidence for alleviating adenomyosis by quercetin. However, the potential therapeutic effect of quercetin in adenomyosis must be further evaluated in vivo experiments. Moreover, more biochemical studies should be carried out to explore the potential signaling pathways involved in anti-adenomyosis function of quercetin.

Author Contributions

Xueqiong Zhu and Biyun Zhang conceived and designed the study. Wenbin Xu and Yizuo Song collected the tissues from patients and did the experiments. Kehan Li analyzed and interpreted the data. Wenbin Xu, Yizuo Song and Kehan Li drafted the manuscript. Xueqiong Zhu and Biyun Zhang revised the manuscript. All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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