hsa-miR-100-5p, an overexpressed miRNA in human ovarian endometriotic stromal cells, promotes invasion through attenuation of SMARCD1 expression

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Abstract

Background: A number of microRNAs are aberrantly expressed in endometriosis and are involved in its pathogenesis. Our previous study demonstrated that has-miR-100-5p expression is enhanced in human endometriotic cyst stromal cells (ECSCs). The present study aimed to elucidate the roles of has-miR-100-5p in the pathogenesis of endometriosis.

Methods: Normal endometrial stromal cells (NESCs) were isolated from normal eutopic endometrium without endometriosis. Using hsa-miR-100-5p-transfected NESCs, we evaluated the effect of hsa-miR-100-5p on the invasiveness of these cells by Transwell invasion assay and in-vitro wound repair assay. We also investigated the downstream signal pathways of hsa-miR-100-5p by microarray analysis and Ingenuity pathways analysis.

Results: hsa-miR-100-5p transfection enhanced the invasion and motility of NESCs. After hsa-miR-100-5p transfection, mRNA expression of SWItch/sucrose non-fermentable-related matrix-associated actin-dependent regulator of chromatin subfamily D member 1 (SMARCD1) was significantly attenuated. Whereas, the expression of matrix metallopeptidase 1 (MMP1) mRNA and active MMP1 protein levels was upregulated.

Conclusion: We found that SMARCD1/MMP-1 is a downstream pathway of hsa-miR-100-5p. hsa-miR-100-5p transfection enhanced the motility of NESCs by inhibiting SMARCD1 expression and MMP1 activation. These findings suggest that enhanced hsa-miR-100-5p expression in endometriosis is involved in promoting the acquisition of endometriosis-specific characteristics during endometriosis development. Our present findings on the roles of hsa-miR-100-5p may thus contribute to understand the epigenetic mechanisms involved in the pathogenesis of endometriosis.

Keywords: Endometriosis, Invasion, Hsa-miR-100-5p, SMARCD1, Matrix metallopeptidase 1

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Background

Endometriosis belongs to estrogen-dependent benign tumors and occurs in 6–10% of the women of reproductive age [1]. The microscopic features of endometriotic tissues resemble those of proliferative-phase endometrial tissues [1]; however, molecular studies have revealed a number of differences at the epigenetic, genetic, transcriptional, and posttranscriptional levels [2–5].

To understand the mechanism(s) responsible for the pathogenesis of endometriosis, we have previously investigated microRNA (miRNA) expression levels in endometriosis [4–7]. Our previous microarray study detected a repertoire of aberrantly expressed miRNAs in endometriosis [4]. Of these aberrantly expressed miRNAs, we demonstrated that upregulation of hsa-miR-210 [5] and downregulation of hsa-miR-196b [4] and hsa-miR-503 [6] contribute to the pathogenesis of endometriosis. Hsa-miR-210 induced the cell proliferation and vascular endothelial cell growth factor (VEGF) production of human normal endometrial stromal cells (NESC) and inhibited apoptosis of these cells [5]. Whereas, hsa-miR-196b induced the apoptosis of human endometriotic cyst stromal cells (ECSC) and inhibited the proliferation of these cells [4]; hsa-miR-503 also induced the cell-cycle arrest at G0/G1 phase and apoptosis and inhibited the cell proliferation, VEGF production, and contractility of ECSCs [6].

SWItch/sucrose non-fermentable (SWI/SNF)-related matrix-associated actin-dependent regulator of chromatin subfamily D member 1 (SMARCD1) belongs to the SWI/SNF chromatin remodeling complex family of proteins which regulate the target gene transcription by altering the local chromatin structure around those genes [8, 9]. SMARCD1 is often involved in somatic rearrangement in tumorigenesis [10]. The chromatin remodeling activity of SMARCD1 is essential for tumor suppression [11, 12]. We speculated that SMARCD1 supression may induce tumorigenesis in endometriosis.

Matrix metallopeptidase 1 (MMP1) is a key enzyme that promotes the breakdown of extracellular matrix during physiological and pathological processes such as embryonic development, reproduction, and tissue remodeling, as well as tumor invasion and metastasis. MMP-1 is the most ubiquitously expressed interstitial collagenase that cleaves the interstitial collagen, types I, II, and III [13]. MMP1 is overexpressed in endometriotic tissues, suggesting its involvement in the pathogenesis of endometriosis [14].

In the present study, we evaluated the role of hsa-miR-100-5p, a miRNA that is upregulated in ECSCs, regarding the pathogenesis of endometriosis [4]. Using hsa-miR-100-5p-transfected NESC, we assessed the effect of hsa-miR-100-5p on the invasiveness of these cells and the expression of SMARCD1 and MMP1, which are downstream targets of hsa-miR-100-5p, in these cells.

Methods

Human NESC and ECSC isolation procedures and cell culture conditions

Normal endometrial tissues were collected at the time of hysterectomies from patients with subserous or intramuscular leiomyoma who had regular menstrual cycle and had no evidence of endometriosis (n = 13, age 31–53 yrs.), as described previously [15]. Whereas, ovarian endometrioma tissues were obtained at the time of surgical treatment from patients with regular menstrual cycles (n = 6, age 22–42 yrs.), as described before [6, 7, 15]. None of the patients had received the hormonal treatments for at least 2 years prior to the surgery. Pathological examination and/or menstrual records confirmed that all the specimens were in the mid-to-late proliferative phases. This study was approved by the Institutional Review Board (IRB) of the Faculty of Medicine, Oita University (registration number: P-16-01), and written informed consent was obtained from all the patients.

ECSCs and NESC were isolated from ovarian endometrioma and normal endometrial tissues, respectively, by enzymatic digestion, and cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 100 IU/ml of penicillin (Gibco-BRL, Gaithersburg, MD, USA), 50 mg/ml of streptomycin (Gibco-BRL), and 10% charcoal-stripped heat-inactivated fetal bovine serum (FBS) (Gibco-BRL) at 37 °C in 5% CO2 in air, as described previously [6, 7, 15]. This culture condition is free of ovarian steroid hormones. Each experiment was performed in triplicate and was repeated at least three times with cells isolated from separate patients.

Quantitative reverse transcription-polymerase chain reaction (RT-PCR) for hsa-miR-100-5p

In our previous study, using a miRNA microarray technique, we demonstrated that hsa-miR-100-5p was upregulated in ECSCs [4]. For the validation of the microarray data, we performed quantitative RT-PCR with NESC (n = 6) and ECSCs (n = 6) as described previously [4–6]. hsa-miR-100-5p-specific (Assay ID: 000437, Applied Biosystems, Carlsbad, CA, USA) or endogenous control (RNU44)-specific (Assay ID: 001094, Applied Biosystems) reverse primers were used. The expression levels of hsa-miR-100-5p were normalized to those of RNU44, calculated by the ΔΔCT method, and were presented as the relative expression in ECSCs compared to that in NESCs.

Transfection of miRNA precursors and small interfering RNAs (siRNAs)

Precursor hsa-miR-100-5p (pre-miR miRNA precursor-hsa-miR-100-5p, Ambion, Austin, TX, USA), negative control precursor miRNA (pre-miR miRNA precursor-negative control #1, Ambion), SMARCD1 siener pre-designed siRNA (AM16708, Ambion) or Silencer® select
negative control #1 siRNA (Ambion) were transfected into NESCs using Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA, USA) and the reverse transfection method, as described before [4–6].

**Gene expression microarray**

Forty-eight hours after transfection, total RNA was extracted from cultured NESCs transfected with precursor hsa-miR-100-5p \((n = 4)\) and NESCs transfected with negative control precursor miRNA \((n = 4)\) using an RNeasy Mini kit (Qiagen, Valencia, CA, USA) and subjected to gene expression microarray analyses with a commercially available human miRNA microarray (G4851A, SurePrint G3 Human Gene Expression Microarray 8x60K v2, Agilent Technologies, Santa Clara, CA, USA), as described previously [5]. To identify the upregulated and downregulated genes, the Z-scores and ratios (non-log scaled fold-change) from the normalized signal intensities of each probe were calculated to compare between NESCs transfected with precursor hsa-miR-100-5p and NESCs transfected with negative control precursor miRNA [5]. We established the following criteria for the regulated genes: at least 3 out of 4 samples has Z-score \(\geq 2.0\) and ratio \(\geq 2.0\)-fold for upregulated genes, and Z-score \(\leq 2.0\) and ratio \(\leq 0.5\) for downregulated genes. All the gene expression microarray data are available at the Gene Expression Omnibus through the NCBI under Accession No. GSE139954 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE139954).

**miRNA target prediction and pathways analysis**

To elucidate the downstream target genes and signal pathways of hsa-miR-100-5p, datasets representing the genes with an altered expression profile derived from the microarray analyses were analyzed by the Ingenuity pathways analysis (IPA) software (Ingenuity Systems, Redwood City, CA, USA) with the IPA knowledgebase (IPA Summer Release 2015). Thereafter, predicted targets of hsa-miR-100-5p were confirmed by online public databases including miRDB (http://mirdb.org/miRDB/), TargetScanHuman (http://www.targetscan.org/, Release 7.0), PicTar (http://pic.tar.mdc-berlin.de/), and microRNA.org (http://www.microrna.org/microrna/getGeneForm.do).

**Transwell invasion assay**

The invasive properties of hsa-miR-100-5p-transfected NESCs were evaluated by Transwell invasion assay, as described previously [16, 17]. NESCs after miRNA transfection \((2 \times 10^5\) cells) were cultured in DMEM supplemented with 10% charcoal-stripped heat-inactivated FBS on the growth factor-reduced Matrigel-coated Transwell inserts with 8-μm pores (Corning Inc., New York, NY, USA). After 48 h, the membranes were fixed with 100% methanol, and the number of cells appearing on the undersurface of the polycarbonate membranes after Giemsa staining was scored visually at \(\times 200\) magnification using a light microscope.

The data from triplicate samples were calculated and presented as the percent values obtained for the NESCs transfected with precursor hsa-miR-100-5p relative to those transfected with the negative control precursor miRNA.

**In vitro wound repair assay**

Cell motility was also determined by an in vitro wound repair assay, as described previously [16, 17]. NESCs grown to confluence in 6-well plates (Corning Inc.) were challenged overnight with serum-free medium and then transfected with the miRNA precursor. The monolayer was wounded using a cell scraper and the plates were incubated in DMEM plus 0.1% BSA for 48 h. The cells were then fixed with 3% paraformaldehyde and stained with Giemsa solution. Areas with lesions were photographed, and wound repair was assessed by calculating the repaired area in square micrometers between the lesion edges at 0 h and 48 h using the public domain software Image J 1.44 developed at the U.S. National Institutes of Health (Bethesda, MD, USA).

The data from triplicate samples were calculated and presented as the percent values obtained for the NESCs transfected with precursor hsa-miR-100-5p relative to those transfected with the negative control precursor miRNA.

**RT-PCR for mRNA expression**

The effects of hsa-miR-100-5p on the expression levels of possible downstream target genes were evaluated in ECSCs by quantitative RT-PCR, as described [4–6]. SMARCD1 and MMP1 were selected as candidate genes because SMARCD1 was confirmed to be the predicted target of hsa-miR-100-5p in the online public database, TargetScanHuman (http://www.targetscan.org/, Release 7.2). MMP1 is known to be the downstream target of SMARCD1 [18] and promotes cell motility (Fig. 1).

In brief, 48 h after miRNA transfection, total RNA from miRNA-transfected NESCs was extracted as described above and subjected to quantitative RT-PCR with the following specific primers (all from Applied Biosystems): SMARCD1 (Assay ID: Hs00161980_m1), MMP1 (Assay ID: Hs00899658_m1), or glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Assay ID: Hs02758991_g1). The expression levels of candidate mRNAs relative to those of GAPDH mRNA were calculated using a calibration curve. The data were calculated from triplicate samples and are presented as percent values obtained for NESCs after hsa-miR-100-5p
transfection relative to those transfected with the negative control precursor miRNA.

**ELISA for active MMP1**
Culture media of miRNA-transfected NESCs were collected 48 h after miRNA transfection and subjected to Human Active MMP-1 Fluorescent Assay (F1M00, R&D Systems, Minneapolis, MN, USA), according to the manufacturer’s instructions. The data from triplicate samples were calculated and presented as the percent values obtained for NESCs transfected with precursor hsa-miR-100-5p relative to those transfected with the negative control precursor miRNA.

**Statistical analysis**
All data were obtained from triplicate samples and are presented as percent values relative to the corresponding controls in the form of mean ± SD. Data were appropriately analyzed by the Student’s t-test using the Statistical Package for Social Science software (IBM SPSS Statistics 24; IBM, Armonk, NY, USA). P-values < 0.05 were considered statistically significant.

**Results**

**Expression of hsa-miR-100-5p**
To validate the miRNA microarray data [4], we evaluated the hsa-miR-100-5p expression levels in NESCs and ECSCs using quantitative RT-PCR. As shown in Fig. 2, the relative hsa-miR-100-5p levels in the ECSCs were significantly higher than those in the NESCs (p < 0.0005). Thus, the results of quantitative RT-PCR for hsa-miR-100-5p expression were consistent with our previous miRNA microarray data [4]. Age of the patients did not affect the expression of hsa-miR-100-5p (data not shown).

As shown in Fig. 3a, mature hsa-miR-100-5p expression in NESCs was significantly induced by hsa-miR-100-5p precursor transfection (p < 0.05). We thus considered this experimental model as appropriate for hsa-miR-100-5p functional analyses.

**Identification of hsa-miR-100-5p-regulated genes and predicted pathways in NESCs**
As shown in Table 1, gene expression microarray analyses detected 33 upregulated and 27 downregulated mRNAs using the criteria described above. Using the online public databases, we focused on SMARCD1 involved in the pathogenesis of endometriosis. The IPA software then identified MMP1 as a downstream target of SMARCD1 (Fig. 1). Regarding the known function of MMP1, we evaluated the cell motility of NESCs using the following experiments.

**Modulation of downstream target molecule expression by hsa-miR-100-5p transfection**
To investigate the underlying mechanisms of hsa-miR-100-5p functions, we investigated the expression levels of SMARCD1 and MMP1. As shown in Fig. 3b, SMARCD1 mRNA expression was significantly attenuated by hsa-miR-100-5p transfection (p < 0.05). In contrast, as shown in Fig. 3c and d, the expression levels of MMP1 mRNA, and active MMP1 protein were upregulated by hsa-miR-100-5p transfection (p < 0.005 and p < 0.0005, respectively).

**Fig. 1** Downstream signaling pathway of hsa-miR-100-5p in NESCs. A gene expression microarray and pathway analyses of hsa-miR-100-5p-transfected NESCs revealed that hsa-miR-100-5p upregulated the motility of NESCs by direct inhibition of SMARCD1 expression followed by MMP1 activation. SMARCD1, Switch/sucrose non-fermentable-related matrix-associated actin-dependent regulator of chromatin subfamily D member 1; MMP1, matrix metalloprotease 1; NESCs, normal endometrial stromal cells

**Fig. 2** hsa-miR-100-5p expression in NESCs and ECSCs. The relative hsa-miR-100-5p levels in ECSCs (n = 6) were significantly higher than those in the NESCs (n = 6). *p < 0.0005 vs. NESCs (Student’s t-test). Data are shown as the mean ± SD. ECSCs, endometriotic cyst stromal cells; NESCs, normal endometrial stromal cells
Modulation of MMP1 expression by SMARCD1 siRNA transfection

To confirm that the MMP1 expression is regulated by SMARCD1, we investigated the expression levels of MMP1 mRNA after SMARCD1 siRNA transfection. As shown in Fig. 4a, SMARCD1 mRNA expression was significantly suppressed by SMARCD1 siRNA transfection \((p < 0.005)\). As shown in Fig. 4b, the expression levels of MMP1 mRNA was significantly upregulated by SMARCD1 siRNA transfection \((p < 0.05)\).

Cell motility

As shown in Fig. 5a and b, the transwell invasion assay revealed that the number of invaded cells was significantly increased by hsa-miR-100-5p transfection \((p < 0.05)\).

We also investigated the effects of hsa-miR-100-5p on the motility of NESCs by an in vitro wound healing assay. As shown in Fig. 5c and d, the repaired area was significantly increased by hsa-miR-100-5p transfection \((p < 0.0005)\).

Discussion

To understand the role of hsa-miR-100-5p, which is upregulated in ECSCs, in the pathogenesis of endometriosis, we evaluated its expression in both ECSCs and NESCs. We also evaluated the hsa-miR-100-5p-mediated effects on the cellular functions of NESCs and sought to determine the underlying mechanisms of hsa-miR-100-5p action in those cells. With the present study, we found the following: (1) Expression of hsa-miR-100-5p in ECSCs was upregulated compared to that in NESCs. (2) hsa-miR-100-5p transfection enhanced the motility of NESCs. (3) hsa-miR-100-5p promoted these cellular functions through downregulation of SMARCD1 mRNA and induction of MMP1 expression. This suggests that hsa-miR-100-5p overexpression induces NESCs to acquire the highly motile characteristics of endometriosis and is involved in promoting the development and progression of this disease.

hsa-miR-100-5p can act as either a tumor suppressor gene or an oncogene, depending on the tumor type in different cancers [19, 20]. For example, hsa-miR-100-5p overexpression has been demonstrated in nasopharyngeal cancer [21], esophageal squamous cell carcinoma [22], colon cancer [19, 23], and gastric cancer [24]. In these tumors, this miRNA contributes to tumor progression. In contrast, hsa-miR-100-5p expression is suppressed in epithelial ovarian cancer [25], endometrial cancer [26], bladder carcinoma [27], renal cell carcinoma...
## Table 1 List of mRNAs aberrantly expressed in miR-100-5p-transfected NESCs

| Gene family          | Gene symbol | Control signal | miR-100 precursor signal | Z-score | Ratio |
|----------------------|-------------|----------------|--------------------------|---------|-------|
| **(A) Upregulated mRNAs** |             |                |                          |         |       |
| Cytokine             | CCL2        | 312.22         | 1041.65                  | 6.87    | 4.32  |
|                      | IL11        | 1034.43        | 2782.35                  | 5.98    | 3.11  |
|                      | LIF         | 128.77         | 261.89                   | 3.80    | 2.46  |
|                      | RP2         | 838.00         | 1737.49                  | 4.13    | 2.17  |
| Growth factor        | BMP2        | 817.37         | 461.26                   | -4.41   | 0.45  |
| Peptidase            | MMP1        | 49,499.07      | 74,041.18                | 7.47    | 2.40  |
|                      | CPXM1       | 70.96          | 120.43                   | 2.27    | 2.40  |
| Enzyme               | ASPH        | 1259.03        | 3722.52                  | 6.99    | 3.81  |
|                      | CYBRD1      | 1067.64        | 1867.57.94               | 4.18    | 2.08  |
|                      | MTAP        | 440.73         | 714.19                   | 3.88    | 2.18  |
| Transcription regulator | UBE2V1     | 2487.25        | 6026.87                  | 5.85    | 2.87  |
|                      | SCML1       | 48.29          | 98.99                    | 3.02    | 2.53  |
|                      | BATF3       | 147.71         | 291.50                   | 3.05    | 2.02  |
| Transmembrane receptor | ITGA6      | 1383.58        | 2680.82                  | 4.48    | 2.21  |
|                      | PVRL2       | 6699.21        | 13,211.87                | 4.72    | 2.04  |
| Transporter          | ABCA1       | 134.70         | 270.47                   | 3.29    | 2.12  |
|                      | BCAP29      | 1166.81        | 1994.48                  | 4.09    | 2.03  |
| Other                | TUBB2B      | 41.72          | 123.42                   | 4.64    | 3.54  |
|                      | PKA         | 192.01         | 593.66                   | 5.84    | 3.20  |
|                      | PALM3       | 260.83         | 736.98                   | 5.72    | 2.99  |
|                      | CEND1       | 196.72         | 412.79                   | 4.30    | 2.80  |
|                      | EMC10       | 4713.79        | 9455.68                  | 5.88    | 2.76  |
|                      | ERUN2       | 225.65         | 451.23                   | 3.82    | 2.41  |
|                      | TFP2        | 752.91         | 1839.06                  | 4.64    | 2.35  |
|                      | RDX         | 469.66         | 816.51                   | 4.11    | 2.27  |
|                      | FAM131B     | 47.52          | 91.03                    | 2.61    | 2.24  |
|                      | SWAP70      | 440.11         | 805.17                   | 4.01    | 2.18  |
|                      | LOC728392   | 1643.31        | 3433.27                  | 4.56    | 2.15  |
|                      | STBD1       | 104.93         | 162.95                   | 3.20    | 2.15  |
|                      | SNRPC       | 10,269.42      | 17,744.24                | 4.89    | 2.12  |
| Other                | CIDEC       | 488.37         | 852.48                   | 3.68    | 2.05  |
|                      | ANGPTL4     | 1403.18        | 2829.40                  | 4.28    | 2.01  |
| Null                 | LOC102723946| 38.79          | 110.21                   | 3.88    | 3.33  |
| **(B) Downregulated mRNAs** |             |                |                          |         |       |
| Enzyme               | HSDL17B2    | 286.50         | 128.94                   | -5.76   | 0.26  |
|                      | PLCH1       | 190.28         | 88.66                    | -4.24   | 0.38  |
|                      | INMT        | 1404.19        | 713.49                   | -5.10   | 0.40  |
| Ion channel          | KCNN2       | 118.10         | 32.10                    | -4.34   | 0.24  |
|                      | KCTD4       | 151.44         | 53.14                    | -5.41   | 0.28  |
|                      | KCTD10      | 1074.65        | 563.34                   | -4.14   | 0.47  |
| Kinase               | FGFR3       | 239.75         | 121.71                   | -3.31   | 0.49  |
| Peptidase            | ADAMTS5     | 3143.19        | 1023.39                  | -6.74   | 0.33  |
|                      | ADAM19      | 239.21         | 117.14                   | -3.26   | 0.47  |
To our knowledge, there is no report which evaluated the expression and function of SMARCD1 in endometriosis. Whereas, overexpression of MMP1 is reported in endometriotic tissues [14], however, the roles of MMP1 regarding the pathogenesis of endometriosis has not been elucidated yet. MMP1 gene polymorphisms may also affect the motility of EECSCs [13]. In the present study, we demonstrated that transfection with hsa-miR-100-5p induced MMP1 expression in NESC cells through downregulation of SMARCD1 and that MMP1 accelerated the migration of NESC cells.

A limitation of the present study is that the experiments were performed only with the stromal cells of...
Fig. 4 Effects of SMARCD1 siRNA transfection on the MMP1 mRNA expression in NESCs. (a) SMARCD1 mRNA expression after SMARCD1 siRNA transfection. (b) MMP1 mRNA expression. *p < 0.05, **p < 0.005 vs. controls (Student's t-test). MMP1, matrix metallopeptidase 1; NESCs, normal endometrial stromal cells; SMARCD1, SWItch/sucrose non-fermentable-related matrix-associated actin-dependent regulator of chromatin subfamily D member 1.

Fig. 5 Effects of hsa-miR-100-5p transfection on the motility of NESCs. (a) Results of transwell invasion assay. (b) Representative photographs of transwell invasion assay. (c) Results of in-vitro wound repair assay. (d) Representative photographs of in vitro wound repair assay. *p < 0.05, **p < 0.0005 vs. controls (Student's t-test). NESCs, normal endometrial stromal cells.
endometriosis and the eutopic endometrium of women without endometriosis. Due to difficulties in obtaining samples, the expression of hsa-miR-100-5p in the eutopic endometrium of women with endometriosis was not evaluated. Future study is necessary on this point.

Conclusions
In summary, we confirmed that hsa-miR-100-5p expression is upregulated in ECSCs. By transfecting hsa-miR-100-5p into NESCs, we observed that SMARCD1/MMP-1 is the downstream pathway of hsa-miR-100-5p. Inhibition of SMARCD1 mRNA expression, followed by MMP1 activation, enhanced the motility of NESCs. These findings suggest that enhanced expression of hsa-miR-100-5p in endometriosis is involved in a role in promoting the acquisition of endometriosis-specific characteristics during the development of endometriosis. Our present findings on the roles of hsa-miR-100-5p may thus contribute to understand the epigenetic mechanisms involved in the pathogenesis of endometriosis.

Abbreviations
ATM: Ataxia telangiectasia mutated; DMEM: Dulbecco’s modified Eagle medium; ECSCs: Endometrial cyst stromal cells; ELISA: Enzyme-linked immunosorbent assay; FBS: Fetal bovine serum; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; ICMT: Isoprenylcysteine carboxyl methyltransferase; IGF: Insulin-like growth factor; IPA: Ingenuity pathways analysis; IRB: Institutional Review Board; miRNA: microRNA; MMP1: Matrix metallopeptidase 1; mTOR: Mammalian target of rapamycin; NESCs: Normal endometrial stromal cells; Rac1: Ras-related C3 botulinum toxin substrate 1; RT-PCR: Reverse transcription-polymerase chain reaction; siRNA: Small interfering RNA; SMARCD1: SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D member 1; SWI/SNF: SWItch/sucrose non-fermentable

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Authors’ contributions
KN participated in the study design, data analysis and interpretation, literature search, generation of figures, and writing and editing the manuscript. KT, MO, YA, TH and HN executed the data/case collection, experiments, data analysis, and interpretation. All authors read and approved the final manuscript.

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Availability of data and materials
All the gene expression microarray data are available at the Gene Expression Omnibus through the NCBI under Accession No. GSE139954 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE139954). Other data in this study are available from the corresponding author.

Ethics approval and consent to participate
The present study was approved by the Institutional Review Board (IRB) of the Faculty of Medicine, Oita University (registration number: P-16-01). Written informed consent was obtained from all the patients.

Consent for publication
Not applicable.

Competing interests
There are no conflicts of interest to declare.

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