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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 (NESCs) and inhibited apoptosis of these cells [5]. Whereas, hsa-miR-196b induced the apoptosis of human endometriotic cyst stromal cells (ECSCs) 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-associatedactin-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 NESCs, 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 intramural 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 NESCs 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-strippedheat-inactivated fetal bovine serum (FBS) (Gibco-BRL) at 37 °C in 5% CO_2 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 NESCs ($n = 6$) 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 silener predesigned 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 mRNA 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 ≥ 2.0 and ratio ≥ 2.0-fold for upregulated genes, and Z-score ≤ − 2.0 and ratio ≤ 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://pictar.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 (2×10^5 cells) were cultured in DMEM supplemented with 10% charcoal-strippedheat-inactivated FBS on the growth factor-reducedMatrigel-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 × 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

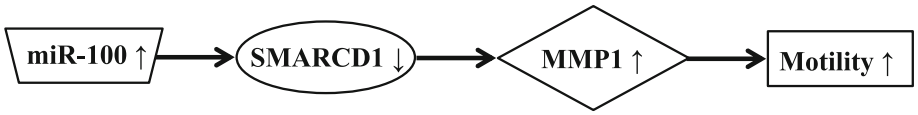

Fig. 1 Downstream signaling pathway of hsa-miR-100-5p in NESCs. A gene expression microarray and pathway analyses of hsa-miR-100-5p-transfected NESCs revealed that hsa-miR-100-5p upregulated the motility of NESCs by direct inhibition of SMARCD1 expression followed by MMP1 activation. SMARCD1, SWItch/sucrose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

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).

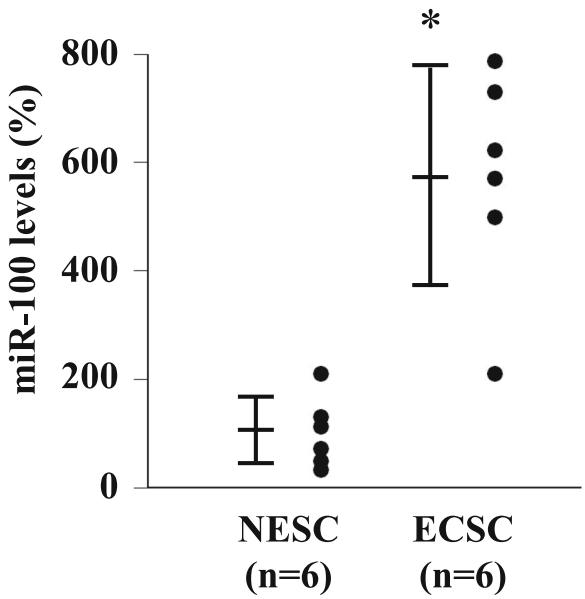

Fig. 2 hsa-miR-100-5p expression in NESCs and ECSCs. The relative hsa-miR-100-5p levels in ECSCs ($n = 6$) were significantly higher than those in the NESCs ($n = 6$). *$p < 0.0005$ vs. NESCs (Student's *t*-test). Data are shown as the mean ± SD. ECSCs, endometriotic cyst stromal cells; NESCs, normal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

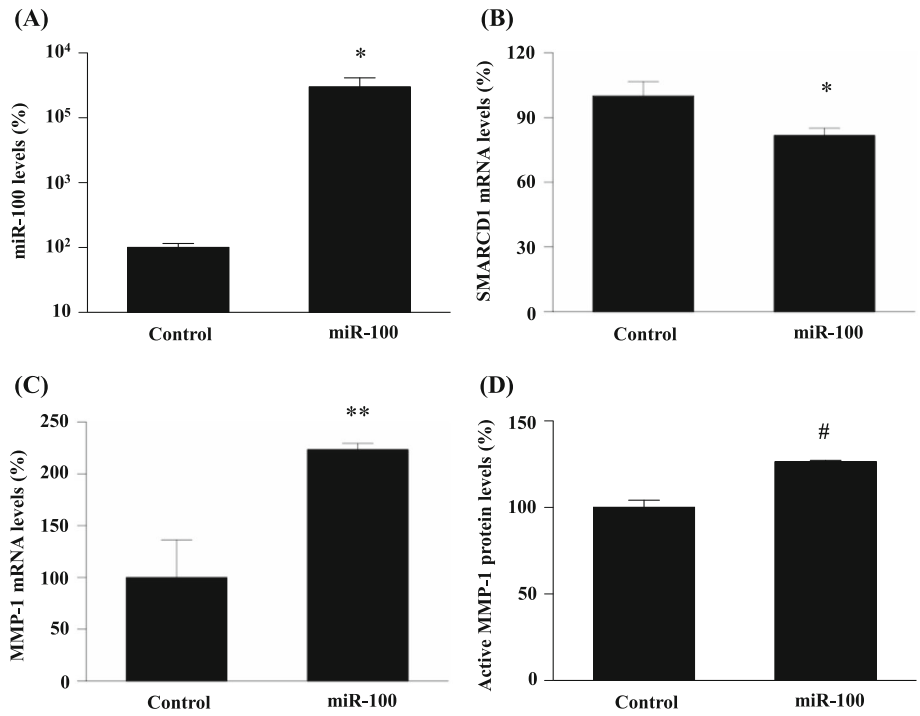

Fig. 3 Effects of hsa-miR-100-5p transfection on the downstream target molecule expression in NESCs. (**a**) hsa-miR-100-5p expression after precursor miRNA transfection. Note that the vertical axis is expressed as a logarithmic scale. (**b**) *SMARCD1* mRNA expression. (**c**) *MMP1* mRNA expression. (**d**) Active MMP1 protein expression. *$p < 0.05$, **$p < 0.005$, #$p < 0.0005$ 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

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
	PKIA	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
	ERLIN2	225.65	451.23	3.82	2.41
	TFPI2	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	*HSD17B2*	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

Table 1 List of mRNAs aberrantly expressed in miR-100-5p-transfected NESCs *(Continued)*

Gene family	Gene symbol	Control signal	miR-100 precursor signal	Z-score	Ratio
Phosphatase	*LPPR4*	184.80	73.50	−4.33	0.34
Transcription regulator	*SMARCD1*	2210.80	932.50	−5.34	0.43
	BAZ2A	288.00	135.67	−3.32	0.47
Transporter	*ATP6AP1*	9337.60	3682.26	−6.29	0.40
Other	*EPDR1*	3173.94	780.35	−8.19	0.27
	MPZL3	125.86	59.79	−3.82	0.43
	ZBED2	161.28	71.97	−3.74	0.43
	SUDS3	568.38	289.31	−4.34	0.46
	CGA	112.70	61.15	−3.01	0.46
	TMEM30A	3229.37	1492.02	−4.80	0.47
	DGCR2	4328.05	2074.04	−4.65	0.47
	CTDSPL	748.62	362.73	−4.11	0.48
	DEPTOR	398.87	187.27	−3.37	0.48
	DNAJC11	1560.93	839.74	−3.46	0.49
	CLDN11	1351.65	687.71	−3.46	0.49
Null	*SLC16A14*	144.45	54.61	−3.92	0.35
	PROSER2-AS1	35.66	16.12	−2.11	0.42
	AREG	120.26	61.28	−3.53	0.43

ABCA1 ATP-binding cassette, sub-family A, member 1; *ADAM19* ADAM metallopeptidase domain 19; *ADAMTS5* ADAM metallopeptidase with thrombospondin type 1 motif, 5; *ANGPTL4* angiopoietin-like 4 transcript variant 1; *AREG* amphiregulin; *ASPH* aspartate beta-hydroxylase, transcript variant 3; *ATP6AP1* ATPase, H+ transporting, lysosomal accessory protein 1; *BATF3* basic leucine zipper transcription factor, ATF-like 3; *BAZ2A* bromodomain adjacent to zinc finger domain, 2A; *BCAP29* B-cell receptor-associated protein 29, transcript variant 2; *BMP2* bone morphogenetic protein 2; *CCL2* chemokine ligand 2; *CEND1* cell cycle exit and neuronal differentiation 1; *CGA* glycoprotein hormones, alpha polypeptide, transcript variant 2; *CIDEC* cell death-inducing DFFA-like effector c, transcript variant 3; *CLDN11* claudin 11, transcript variant 1; *CPXM1* carboxypeptidase X, member 1, transcript variant 1; *CTDSPL* CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase-like, transcript variant 1; *CYBRD1* cytochrome b reductase 1, transcript variant 1; *DEPTOR* DEP domain containing MTOR-interacting protein, transcript variant 1; *DGCR2* DiGeorge syndrome critical region gene 2, transcript variant 1; *DNAJC11* DnaJ (Hsp40) homolog, subfamily C, member 11; *EMC10* ER membrane protein complex subunit 10, transcript variant 1; *EPDR1* ependymin related 1, transcript variant 1; *ERLIN2* ER lipid raft associated 2, transcript variant 1; *FAM131B* family with sequence similarity 131, member B, transcript variant a; *FGFR3* fibroblast growth factor receptor 3, transcript variant 1; *HSD17B2* hydroxysteroid (17-beta) dehydrogenase 2; *IL11* interleukin 11; *INMT* indolethylamine N-methyltransferase, transcript variant 2; *ITGA6* integrin, alpha 6, transcript variant 2; *KCNN2* potassium channel, calcium activated intermediate/small conductance subfamily N alpha, member 2, transcript variant 1; *KCTD10* potassium channel tetramerization domain containing 10; *KCTD4* potassium channel tetramerization domain containing 4; *LIF* leukemia inhibitory factor, transcript variant 1; LOC102723946, Zinc finger protein 695; LOC728392, uncharacterized LOC728392; *LPPR4* lipid phosphate phosphatase-related protein type 4, transcript variant 1; *MMP1* matrix metallopeptidase 1, transcript variant 1; *MPZL3* myelin protein zero-like 3, transcript variant 1: *MTAP* methylthioadenosine phosphorylase; *PALM3* paralemmin 3; *PKIA* protein kinase (cAMP-dependent, catalytic) inhibitor alpha, transcript variant 1; *PLCH1* phospholipase C, eta 1, transcript variant 2; *PROSER2-AS1* PROSER2 antisense RNA 1; *PVRL2* poliovirus receptor-related 2, transcript variant delta; *RDX* radixin, transcript variant 3; *RP2* retinitis pigmentosa 2; *SCML1* sex comb on midleg-like 1, transcript variant 1; *SLC16A14* solute carrier family 16, member 14; *SMARCD1* SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 1, transcript variant 2; *SNRPC* small nuclear ribonucleoprotein polypeptide C, transcript variant 1; *STBD1* starch binding domain 1; *SUDS3* suppressor of defective silencing 3 homolog; *SWAP70* SWAP switching B-cell complex 70 kDa subunit, transcript variant 1; *TFPI2* tissue factor pathway inhibitor 2, transcript variant 1; *TMEM30A* transmembrane protein 30A, transcript variant 1; *TUBB2B* tubulin, beta 2B class IIb; *UBE2V1* ubiquitin-conjugating enzyme E2 variant 1, transcript variant 4; *ZBED2* zinc finger, BED-type containing 2

[28], prostate cancer [29], breast carcinoma [30], hepatocellular carcinoma [31], and non-small cell lung cancer [32]. In these tumors, this miRNA behaves as a tumor suppressor.

The reported target genes of hsa-miR-100-5p include polo-like kinase 1 [21, 32], insulin-like growth factor (IGF) [33], IGF-1 receptor [34], mammallian target of rapamycin (mTOR) [34], fibroblast growth factor receptor 3 [35], ataxia telangiectasia mutated (ATM) [36], Argonaute 2 [37], isoprenylcysteine carboxyl methyltransferase (ICMT) [38], nuclear factor-κB3 [39], ras-related C3 botulinum toxin substrate 1 (Rac1) [38], and β-tubulin [40].

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 ECSCs [13]. In the present study, we demonstrated that transfection with hsa-miR-100-5p induced MMP1 expression in NESCs through downregulation of SMARCD1 and that MMP1 accelerated the migration of NESCs.

A limitation of the present study is that the experiments were performed only with the stromal cells of

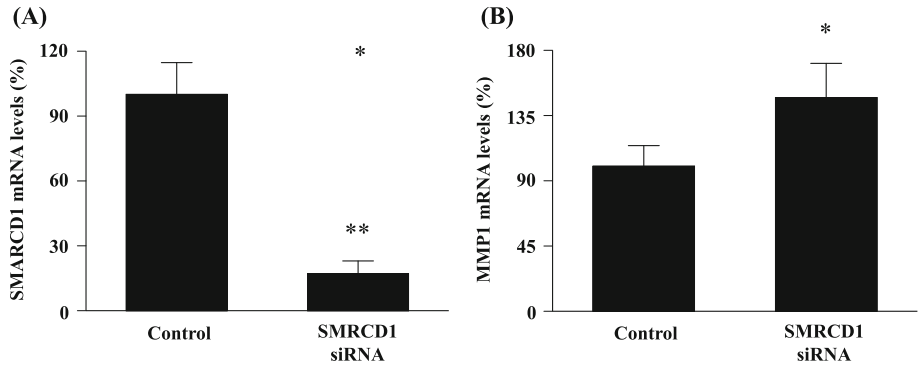

Fig. 4 Effects of SMARCD1 siRNA transfection on the MMP1 mRNA expression in NESCs. (**a**) *SMARCD1* mRNA expression after SMARCD1 siRNA transfection. (**b**) *MMP1* mRNA expression. *$p < 0.05$, **$p < 0.005$ vs. controls (Student's *t*-test). MMP1, matrix metallopeptidase 1; NESCs, normal endometrial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

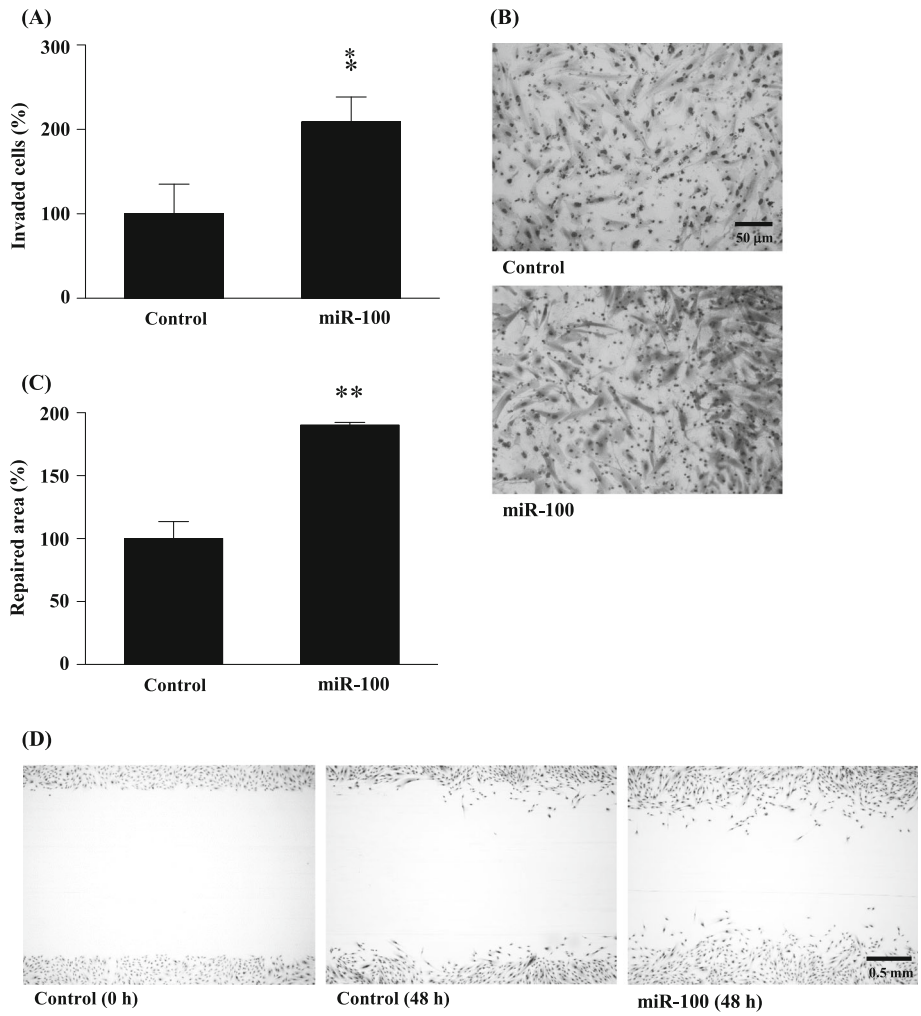

Fig. 5 Effects of hsa-miR-100-5p transfection on the motility of NESCs. (**a**) Results of transwell invasion assay. (**b**) Representative photographs of transwell invasion assay. (**c**) Results of in-vitro wound repair assay. (**d**) Representative photographs of in vitro wound repair assay. *$p < 0.05$, **$p < 0.0005$ 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 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: Endometriotic 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: Mammallian 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-associatedactin-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 asvailable 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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References

1. Giudice LC. Clinical practice. Endometriosis N Engl J Med. 2010;362:2389–98.
2. Nasu K, Yuge A, Tsuno A, Narahara H. Mevalonate-Ras homology (rho)/rho-associated coiled-coil-forming protein kinase (ROCK)-mediated signaling pathway as a therapeutic target for the treatment of endometriosis-associated fibrosis. Curr Signal Transduct Ther. 2010;5:141–8.
3. Nasu K, Nishida M, Kawano Y, Tsuno A, Abe W, Yuge A, Takai N, Narahara H. Aberrant expression of apoptosis-related molecules in endometriosis: a possible mechanism underlying the pathogenesis of endometriosis. Reprod Sci. 2011;18:206–18.
4. Abe W, Nasu K, Nakada C, Kawano Y, Moriyama M, Narahara H. miR-196b targets c-Myc and Bcl-2 expression, inhibits proliferation and induces apoptosis in endometriotic stromal cells. Hum Reprod. 2013;28:750–61.
5. Okamoto M, Nasu K, Abe W, Aoyagi Y, Kawano Y, Kai K, Moriyama M, Narahara H. Enhanced miR-210 expression promotes the pathogenesis of endometriosis through activation of signal transducer and activator of transcription 3. Hum Reprod. 2015;30:632–41.
6. Hirakawa T, Nasu K, Abe W, Aoyagi Y, Okamoto M, Kai K, Takebayashi K, Narahara H. miR-503, a microRNA epigenetically repressed in endometriosis, induces apoptosis and cell-cycle arrest and inhibits cell proliferation, angiogenesis, and contractility of human ovarian endometriotic stromal cells. Hum Reprod. 2016;31:2587–97.
7. Aoyagi Y, Nasu K, Kai K, Hirakawa T, Okamoto M, Kawano Y, Abe W, Tsukamoto Y, Moriyama M, Narahara H. Decidualization differentially regulates microRNA expression in eutopic and ectopic endometrial stromal cells. Reprod Sci. 2017;24:445–55.
8. Zhang P, Li L, Bao Z, Huang F. Role of BAF60a/BAF60c in chromatin remodeling and hepatic lipid metabolism. Nutr Metab. 2016;13:30.
9. Arts FA, Keogh L, Smyth P, O'Toole S, Ta R, Gleeson N, O'Leary JJ, Flavin R, Sheils O. miR-223 potentially targets SWI/SNF complex protein SMARCD1 in atypical proliferative serous tumor and high-grade ovarian serous carcinoma. Hum Pathol. 2017;70:98–104.
10. Ring HZ, Vameghi-Meyers V, Wang W, Crabtree GR, Francke U. Five SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin (SMARC) genes are dispersed in the human genome. Genomics. 1998;51:140–3.
11. Bultman S, Gebuhr T, Yee D, La Mantia C, Nicholson J, Gilliam A, Randazzo F, Metzger D, Chambon P, Crabtree G, Magnuson T. A Brg1 null mutation in the mouse reveals functional differences among mammalian SWI/SNF complexes. Mol Cell. 2000;6:1287–95.
12. Roberts CW, Orkin SH. The SWI/SNF complex--chromatin and cancer. Nat Rev Cancer. 2004;4:133–42.
13. Arakaki PA, Marques MR, Santos MCLG. MMP-1 polymorphism and its relationship to pathological processes. J Biosci. 2009;34:313–20.
14. Kokorine I, Nisolle M, Donnez J, Eeckhout Y, Courtoy PJ, Marbaix E. Expression of interstitial collagenase (matrix metalloproteinase-1) is related to the activity of human endometriotic lesions. Fertil Steril. 1997;68:246–51.
15. Nishida M, Nasu K, Fukuda J, Kawano Y, Narahara H, Miyakawa I. Down regulation of interleukin-1 receptor expression causes the dysregulated expression of CXC chemokines in endometriotic stromal cells: a possible mechanism for the altered immunological functions in endometriosis. J Clin Endocrinol Metab. 2004;89:5094–100.
16. Matsumoto H, Nasu K, Nishida M, Ito H, Bing S, Miyakawa I. Regulation of proliferation, motility, and contractility of human endometrial stromal cells by platelet-derived growth factor. J Clin Endocrinol Metab. 2005;90:3560–7.
17. Nasu K, Nishida M, Matsumoto H, Sun B, Inoue C, Kawano Y, Miyakawa I. Regulation of proliferation, motility, and contractivity of cultured human endometrial stromal cells by transforming growth factor-beta isoforms. Fertil Steril. 2005;84(Suppl):1114–23.
18. Hendricks KB, Shanahan F, Lees E. Role for BRG1 in cell cycle control and tumor suppression. Mol Cell Biol. 2004;24:362–76.

19. Chen P, Xi Q, Wang Q, Wei P. Downregulation of microRNA-100 correlates with tumor progression and poor prognosis in colorectal cancer. Med Oncol. 2014;31:235.
20. Wang H, Wang L, Wu Z, Sun R, Jin H, Ma J, Liu L, Ling R, Yi J, Wang L, Bian J, Chen J, Li N, Yuan S, Yun J. Three dysregulated microRNAs in serum as novel biomarkers for gastric cancer screening. Med Oncol. 2014;31:298.
21. Shi W, Alajez NM, Bastianutto C, Hui AB, Mocanu JD, Ito E, Busson P, Lo KW, Ng R, Waldron J, O'Sullivan B, Liu FF. Significance of Plk1 regulation by miR-100 in human nasopharyngeal cancer. Int J Cancer. 2010;126:2036–48.
22. Ko MA, Zehong G, Virtanen C, Guindi M, Waddell TK, Keshavjee S, Darling GE. MicroRNA expression profiling of esophageal cancer before and after induction chemoradiotherapy. Ann Thorac Surg. 2012;94:1094–102.
23. Rokavec M, Horst D, Hermeking H. Cellular model of colon cancer progression reveals signatures of mRNAs, miRNA, lncRNAs, and epigenetic modifications associated with metastasis. Cancer Res. 2017;77:1854–67.
24. Shi D-B, Wang Y-W, Xing A-Y, Gao J-W, Zhang H, Guo X-Y, Gao P. C/EBPα-induced miR-100 expression suppresses tumor metastasis and growth by targeting ZBTB7A in gastric cancer. Cancer Lett. 2015;369:376–85.
25. Peng DX, Luo M, Qiu LW, He YL, Wang XF. Prognostic implications of microRNA-100 and its functional roles in human epithelial ovarian cancer. Oncol Rep. 2012;27:1238–44.
26. Torres A, Torres K, Pesci A, Ceccaroni M, Paszkowski T, Cassandrini P, Zamboni G, Maciejewski R. Deregulation of miR-100, miR-99a and miR-199b in tissues and plasma coexists with increased expression of mTOR kinase in endometrioid endometrial carcinoma. BMC Cancer. 2012;12:369.
27. Wang S, Xue S, Dai Y, Yang J, Chen Z, Fang X, Zhou W, Wu W, Li Q. Reduced expression of microRNA-100 confers unfavorable prognosis in patients with bladder cancer. Diagn Pathol. 2012;7:159.
28. Wang G, Chen L, Meng J, Chen M, Zhuang L, Zhang L. Overexpression of microRNA-100 predicts an unfavorable prognosis in renal cell carcinoma. Int Urol Nephrol. 2013;45:373–9.
29. Leite KR, Tomiyama A, Reis ST, Sousa-Canavez JM, Sanudo A, Camara-Lopes LH, Srougi M. MicroRNA expression profiles in the progression of prostate cancer from high-grade prostate intraepithelial neoplasia to metastasis. Urol Oncol. 2013;31:796–801.
30. Gebeshuber CA, Martinez J. miR-100 suppresses IGF2 and inhibits breast tumorigenesis by interfering with proliferation and survival signaling. Oncogene. 2013;32:3306–10.
31. Chen P, Zhao X, Ma L. Downregulation of microRNA-100 correlates with tumor progression and poor prognosis in hepatocellular carcinoma. Mol Cell Biochem. 2013;383:49–58.
32. Liu J, Lu KH, Liu ZL, Sun M, De W, Wang ZX. MicroRNA-100 is a potential molecular marker of non-small cell lung cancer and functions as a tumor suppressor by targeting polo-like kinase 1. BMC Cancer. 2012;12:519.
33. Tovar V, Alsinet C, Villanueva A, Hoshida Y, Chiang DY, Solé M, Thung S, Moyano S, Toffanin S, Mínguez B, Cabellos L, Peix J, Schwartz M, Mazzaferro V, Bruix J, Llovet JM. IGF activation in a molecular subclass of hepatocellular carcinoma and pre-clinical efficacy of IGF-1R blockage. J Hepatol. 2010;52:550–9.
34. Ge YY, Shi Q, Zheng ZY, Gong J, Zeng C, Yang J, Zhuang SM. MicroRNA-100 promotes the autophagy of hepatocellular carcinoma cells by inhibiting the expression of mTOR and IGF-1R. Oncotarget. 2014;5:6218–28.
35. Luan Y, Zhang S, Zuo L, Zhou L. Overexpression of miR-100 inhibits cell proliferation, migration, and chemosensitivity in human glioblastoma through FGFR3. Onco Targets Ther. 2015;8:3391–400.
36. Ng WL, Yan D, Zhang X, Mo YY, Wang Y. Over-expression of miR-100 is responsible for the low-expression of ATM in the human glioma cell line: M059J. DNA Repair. 2010;9:1170–5.
37. Wang M, Ren D, Guo W, Wang Z, Huang S, Du H, Song L, Peng X. Loss of miR-100 enhances migration, invasion, epithelial-mesenchymal transition and stemness properties in prostate cancer cells through targeting Argonaute 2. Int J Oncol. 2014;45:362–72.
38. Zhou HC, Fang JH, Luo X, Zhang L, Yang J, Zhang C, Zhuang SM. Downregulation of microRNA-100 enhances the ICMT-Rac1 signaling and promotes metastasis of hepatocellular carcinoma cells. Oncotarget. 2014;5:12177–88.
39. Liu M, Han T, Shi S, Chen E. Long noncoding RNA HAGLROS regulates cell apoptosis and autophagy in lipopolysaccharides-induced WI-38 cells via modulating miR-100/NF-kB axis. Biochem Biophys Res Commun. 2018;500:589–96.
40. Lobert S, Jefferson B, Morris K. Regulation of β-tubulin isotypes by microRNA 100 in MCF7 breast cancer cells. Cytoskeleton. 2011;68:355–62.

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