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Original Article

Hypoxia-induced upregulation of hsa-miR-584-3p suppresses endometrial glandular epithelial cell function by targeting DKK-1

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Abstract: Objective: To investigate the impact of hypoxia on microRNA (miRNA) expression profiles in endometrial glandular epithelial cells (EECs) and elucidate potential mechanisms underlying proliferation, migration, and invasion. Methods: EECs in the logarithmic growth phase were exposed to normoxic (21% oxygen) and hypoxic (1% oxygen) conditions. MiRNA expression profiles were analyzed using RNA sequencing, and differential expression of hsa-miR-584-3p was confirmed by real-time quantitative PCR (RT-qPCR). Target prediction through TargetScan identified Dickkopf-1 (DKK-1) as a target gene of hsa-miR-584-3p. The interaction between hsa-miR-584-3p and DKK-1 was validated through a double-luciferase reporter gene assay and Western blotting. Cell proliferation, migration, and invasion were assessed using the Cell Counting Kit-8 (CCK-8) assay, wound healing assay, and Transwell invasion assay, respectively. Results: Hypoxic conditions significantly upregulated the expression of hsa-miR-584-3p in EECs ($P<0.001$). TargetScan analysis predicted DKK-1 as a downstream target of hsa-miR-584-3p. The double-luciferase reporter gene assay confirmed the binding of hsa-miR-584-3p to the 3' untranslated region of the DKK-1 gene, leading to reduced DKK-1 protein expression ($P<0.001$). Functional assays demonstrated decreased proliferation and increased migration and invasion of EECs under hypoxia. Conclusion: Hypoxia-induced upregulation of hsa-miR-584-3p suppresses the function of EECs by targeting DKK-1 protein activity, thereby influencing their proliferation, migration, and invasion.

Keywords: Endometrial glandular epithelial cell, hypoxia, hsa-miR-584-3p, DKK-1

Introduction

Endometrial repair and angiogenesis are tightly regulated processes under hypoxic conditions. Various hypoxia-related markers, including hypoxia-inducible factor-1α (HIF-1α), vascular endothelial growth factor (VEGF), and interleukin-8 (IL-8, also known as CXCL8), have been identified in both preclinical models and human endometrial tissues [1]. These markers are associated with conditions such as endometriosis, endometrial cancer, thin endometrium, and repeated implantation failure [2]. Hypoxia triggers a physiological stress response that enhances cell proliferation, differentiation, migration, and proangiogenic capacity to facilitate repair at hypoxic injury sites. However, the hypoxic microenvironment can also lead to malignant transformation, activation of proto-oncogenes, and the development of cancerous lesions [2].

Despite extensive research, the molecular mechanisms underlying the involvement of endometrial glandular epithelial cells (EECs) in endometrial injury and repair within a hypoxic milieu remain elusive.

Noncoding microRNAs (miRNAs) are endogenous, highly conserved RNA molecules approximately 20-25 nucleotides in length. They serve as crucial gene regulators, maintaining intracellular gene and protein homeostasis. MiRNAs play diverse roles in cellular processes, includ-

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ing migration, proliferation, invasion, apoptosis, and differentiation, and are implicated in various diseases [3]. Research has shown that hypoxic conditions trigger cells to release numerous microRNAs (miRNAs) involved in signaling processes such as cell migration, apoptosis, proliferation, and invasion [4]. Specifically, miR-584-5p/-3p has been identified as regulators of migration and invasion in non-small cell lung cancer and liver cancer cells [5, 6]. Additionally, Shuilin et al. [7] described how MYC-regulated LINC00607 enhances proliferation, migration, and invasion of hepatocellular carcinoma cells through the miR-584-3p/ROCK1 axis. Conversely, Jun et al. [8] found that miR-584 inhibits invasion and migration in thyroid carcinoma by targeting the oncogene ROCK1, illustrating the diverse roles of miR-584 across different cell types.

Dickkopf-1 (DKK-1), an inhibitor of the Wnt/β-catenin signaling pathway, is primarily secreted by osteoblasts and osteocytes [9]. DKK-1 disrupts cell proliferation and differentiation by competitively binding to LRP5/6 proteins, thereby inhibiting their interaction with Wnt proteins, which affects cell growth, apoptosis, and invasion processes and plays a role in vertebrate developmental pathways related to the Wnt signaling [10]. Within the female reproductive system, DKK-1 is known to inhibit the self-renewal and proliferation of endometrial mesenchymal stem cells [11] and is linked to endometriosis [12]. Research suggests that DKK-1's effect on cell proliferation and invasion can vary significantly depending on the physiological environment [13, 14].

This study aims to determine if a hypoxic environment alters miRNA expression profiles in EECs, affects DKK-1 expression, and thereby influences the cytological functions of EECs, contributing to our understanding of gene-level responses in endometrial hypoxia injury.

Materials and methods

Cell lines

The EECs (CM-H058) and human embryonic kidney cells (HEK-293T cells; CL-0005) used in the experiment were purchased from Wuhan Procell Biologics.

Main reagents and instruments

This study employed a range of reagents including EEC primary cells and supporting medium (Wuhan Procell Biologics), Dulbecco's Modified Eagle Medium (Gibco, USA), fetal bovine serum (Gibco, USA), 0.25% trypsin, TRIzol and a TRIzol RNA Extraction Kit (Sigma, USA), Opti-MEM (Invitrogen, USA), 5× Reverse Transcriptase M-MLV, RNase inhibitor (TaKaRa Co., Ltd., Japan), 2× MItra Master Mix, dithiothreitol (DTT), RNase-free water, deoxynucleotide triphosphates (dNTPs; China Kangwei Century Co., Ltd.), primers (Shanghai Biotech Co., Ltd.), and JETPRIM Transfection Reagent (Polyplus). Antibodies used included rabbit anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and rabbit anti-β-catenin (Abcam, USA).

The laboratory was equipped with a BX19-HK830 inverted phase contrast microscope (Shenzhen Osmicro Optical Instrument Co., Ltd.), a 7500 Fast Real-Time PCR System (Applied Biosystems, USA), a CO_2 cell culture incubator, a VeritiProTM gradient thermal cycler, a Micro17R high-speed refrigerated centrifuge, a Multiskan™ FC microplate reader (Thermo Fisher Scientific, USA), a luciferase detector (Bio-Rad), and an SC-2546 laboratory desktop low-speed centrifuge (Zhongke Zhongjia Scientific Instrument Co., Ltd.).

Cell proliferation, migration, and invasion were quantified using 24-well Transwell inserts (Corning, USA) and a Cell Counting Kit-8 (CCK-8, Sigma, USA).

EEC culture

Primary EECs (Wuhan Procell Biologics, CM-H058) were isolated and cultured using specialized growth media, which included fetal bovine serum, growth additives, and penicillin-streptomycin. Upon reaching 80%-90% confluency, the cells were trypsinized with 0.25% trypsin and subcultured. The control group was maintained under normoxic conditions (21% oxygen), while the experimental group was subjected to hypoxic conditions (1% oxygen) [15].

RNA-sequence to analyze changes in miRNAs

For RNA sequencing, EECs in the logarithmic growth phase were harvested and seeded into six-well plates at a density of 3×10^5 cells/well,

Table 1. Real-time quantitative PCR primer sequences

Primer		Sequence (5'→3')
snoRNA202	Upstream primer:	ACACTCCAGCTGGGGCTGTACTGACTTGATG
	RT primer:	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCATCAGAT
miR-584-3p	Upstream primer:	TGGGATATGTCAGTTCCAGG
	RT primer:	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAGCCTG
URP-R		TGGTGTCGTGGAGTCG

with three replicates per group. After 24 hours under respective conditions, cells were collected into Eppendorf tubes (1×10^6 cells/tube). Total RNA was extracted using the TRIzol RNA Extraction Kit. BaseCare Co., Ltd. conducted the sequencing and generated a heatmap to illustrate the miRNA expression profiles.

Real-time quantitative PCR analysis of hsa-miR-584-3p expression

EECs in logarithmic growth were seeded into six-well plates at 3×10^5 cells per well and categorized into normoxia and hypoxia groups with three replicates each. After culturing for 12, 24, 36, or 48 hours, two sets of EECs were collected into Eppendorf tubes (1×10^6 cells per tube). Total RNA was extracted using the TRIzol RNA Extraction Kit, and 2 μg of RNA was reverse transcribed into complementary DNA (cDNA). The reverse transcription utilized two systems:

System A: Comprising miRNA-RT, Sno-202 RT, and dNTPs, incubated at 70°C for 10 minutes.

System B: Consisting of 4 μl of 5× M-MLV buffer, 1 μl of M-MLV (200 U/μl), 1 μl of RNase inhibitor (40 U/μl), and 2 μl of DTT (0.1 mmol/L), incubated at 42°C for 60 minutes followed by 70°C for 15 minutes.

The expression levels of hsa-miR-584-3p were quantified using real-time quantitative PCR (RT-qPCR). The RT-qPCR setup included:

Reaction Mix: 1 μl of cDNA, 1 μl each of upstream and downstream primers (10 μmol/L, as listed in **Table 1**), 10 μl of Master Mix, and 8 μl of RNase-free water.

Thermocycling Conditions: Initial denaturation at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds, 60°C for 1 minute, and a final extension of 95°C for 15 seconds; 60°C for 1 minute; and 95°C for 15 seconds.

Relative expression levels of hsa-miR-584-3p were calculated using the 2-ΔΔCt method, with snoRNA202 serving as the internal control.

Dual-luciferase reporter gene assay

293T cells in logarithmic growth phase were seeded in 24-well plates at a density of 4×10^4 cells per well, with three pairs of wells per condition. Cells were allowed to adhere for 18-24 hours until reaching 70%-90% confluence. Post-adherence, cells underwent transfection. For the luciferase assay, 100 μl of each sample was mixed with 100 μl of luciferase assay reagent to measure the relative light unit (RLU) of firefly luciferase. Subsequently, an equal volume of sea pansy (Renilla) luciferase assay reagent was added to each well to determine the RLU for the reference gene. The ratio of firefly to Renilla luciferase activities (reporter gene vs. reference gene) was calculated to assess transcriptional activity, and differences in these ratios were compared between the normoxic and hypoxic groups.

CCK-8 assay for analyzing cell proliferation

EECs were allocated into a control group and an experimental group, then seeded into 96-well plates at a density of 5×10^3 cells/well per well, with three replicates for each group. The control group was cultured under normoxic conditions, while the experimental group was subjected to hypoxic conditions. Cell viability was assessed at 0, 12, 24, 36, and 48 hours of incubation. For this purpose, each well received 10 μl of CCK-8 reagent and was incubated at 37°C for 2 hours. Subsequently, absorbance at 450 nm was measured using a microplate reader.

Scratch healing assay for determination of cell migration

EECs were randomly allocated into normoxia and hypoxia groups, seeded into 24-well plates

at a density of 5×10^4 cells/well with three replicates per group, and cultured until they reached 90% confluence. A scratch was then made horizontally across the cell monolayer using a 200-μl pipette tip. The cells were incubated further for 12, 24, 36, and 48 hours. Scratch healing was monitored using an inverted microscope. Healing was quantified by measuring the scratch distance at each time point, with the healing rate calculated using the formula: ([0-hour scratch distance/12-hour scratch distance]/0-hour scratch distance ×100%).

Transwell assay for assessing cell invasion

EECs were resuspended in serum-free medium and seeded into the upper chamber of a 24-well Transwell plate pre-coated with Matrigel, at a density of 2×10^4 cells/well per well, with three replicates per condition. A volume of 600 μl of serum-enriched medium was added to the lower chamber. The setup was then divided into normoxic and hypoxic groups. After incubation periods of 12, 24, 36, and 48 hours, the cells in the upper chamber were fixed with a fixative solution for 30 minutes. The chambers were washed with phosphate-buffered saline (PBS), and non-invading cells were gently removed using cotton swabs. The cells that migrated to the lower surface were stained with 0.1% crystal violet and washed with PBS. The stained cells were observed under a microscope, and images were captured from five randomly selected fields to quantify cell invasion. The average invasion rate was calculated and reported.

Western blotting analysis of DKK-1 protein expression

EECs were seeded into six-well plates at 3×10^5 cells/well with three replicates for each condition. The control group was maintained under normoxic conditions, while the experimental group was subjected to hypoxia for 2, 4, 6, and 8 hours. Cells were lysed and incubated for 30 minutes to extract proteins. The protein concentration was determined using the bicinchoninic acid method. Proteins (10 μg) were separated on a polyacrylamide gel and transferred to a polyvinylidene fluoride (PVDF) membrane using constant-pressure electrophoresis at 90 V. The membrane was blocked with 5% non-fat milk for 1 hour, followed by overnight incubation at 4°C with rabbit monoclonal antibodies against GAPDH and DKK-1. A goat anti-rabbit secondary antibody was applied for 2 hours, and protein bands were visualized using appropriate color development techniques. The expression levels of the target proteins were then analyzed using an imaging system.

Statistical analysis

Data were plotted and analyzed using GraphPad Prism software (version 7.00). Results were expressed as mean ± standard error of the mean. Differences between groups were evaluated using repeated-measures ANOVA or a t-test, with significance set at $P<0.05$.

Results

Differential expression of miRNAs in EECs in hypoxic environments

EECs were cultured under normoxic (21% oxygen) and hypoxic (1% oxygen) conditions. RNA sequencing was conducted to evaluate changes in miRNA profiles under hypoxic conditions. Notably, the expression of hsa-miR-584-3p increased during hypoxia (**Figure 1**), indicating that hypoxic stress alters miRNA expression in EECs.

Hypoxia upregulates hsa-miR-584-3p in EECs

To further investigate miRNA expression in hypoxic conditions, RT-qPCR was employed to measure hsa-miR-584-3p levels. We observed a significant increase in hsa-miR-584-3p expression in EECs subjected to hypoxia, which intensified over the culture duration of 12, 24, 36, and 48 hours (**Figure 2**). This suggests that the expression of hsa-miR-584-3p is influenced by the duration of hypoxic exposure.

EEC proliferation is inhibited under hypoxia

Hypoxia can induce cell growth arrest; thus, we utilized a CCK-8 kit to assess EEC proliferation under both normoxic and hypoxic conditions. The CCK-8 assay, based on the analysis of the 450-nm optical density, revealed that EEC proliferation increased over time under both conditions. However, no significant difference in proliferation was observed between the two groups at 24 hours. By 36-48 hours, the proliferative capacity of the EECs in the hypoxic group was significantly reduced compared to the control

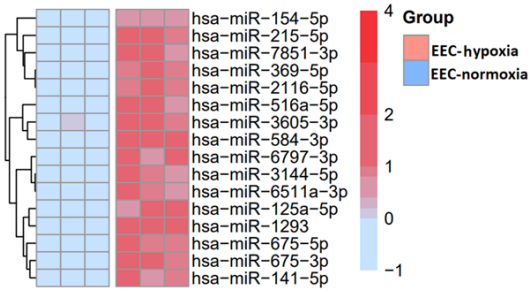

Figure 1. miRNA expression profiles in endometrial glandular epithelial cells (EECs) across varying oxygen concentrations.

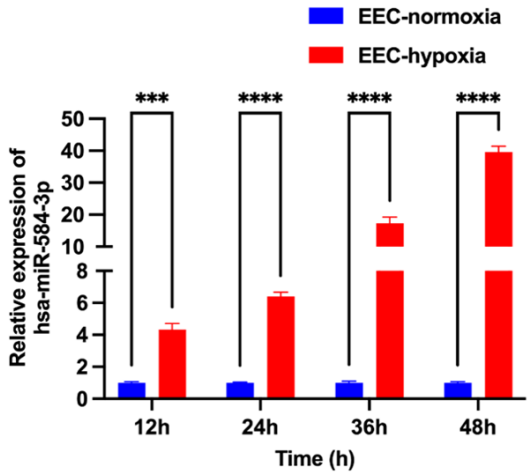

Figure 2. Differential expression of hsa-miR-584-3p in endometrial glandular epithelial cells (EECs) under normoxic and hypoxic conditions at multiple time points. ***P<0.001, ****P<0.0001.

group (P<0.05), indicating that prolonged hypoxic conditions inhibit the proliferative ability of EECs (**Figure 3**).

EEC migration is significantly enhanced under hypoxia

The scratch healing assay revealed no significant differences in EEC mobility between hypoxic and normoxic conditions before 24 hours. However, at 36 and 48 hours, EEC migration in the hypoxia group significantly exceeded that under normoxic conditions (P<0.05), indicating that hypoxia enhances EEC migration (**Figure 4**).

EEC invasion was significantly enhanced under hypoxia

The Transwell assay showed no significant difference in the number of invading EECs

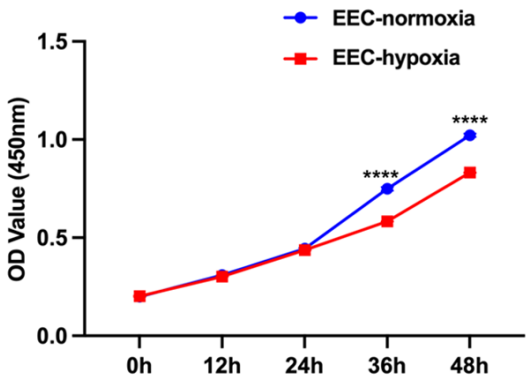

Figure 3. Cell viability data from the Cell Counting Kit-8 for endometrial glandular epithelial cells (EECs) under normoxic and hypoxic conditions at different time intervals. Significantly lower viability in the hypoxic compared to normoxic group, ****P<0.0001.

between the groups before 24 hours. Yet, at 36 and 48 hours, the invasion rates in the hypoxia group were significantly higher than in the normoxia group (P<0.05), suggesting that hypoxia increases EEC invasion (**Figure 5**).

A hypoxic environment causes a decrease in DKK-1 expression in EECs

Analysis using TargetScan (www.targetscan.org) revealed that hsa-miR-584-3p potentially targets the 3' untranslated region (UTR) of the DKK-1 gene, indicating that DKK-1 is a likely target of this miRNA (**Figure 6**). A dual-luciferase reporter assay further confirmed that hsa-miR-584-3p binds to six consecutive sites within the 3'UTR of DKK-1, and this interaction was abolished by mutational alterations (**Figure 6A**, **6B**). Western blot analysis demonstrated a decrease in DKK-1 protein levels in hypoxic EECs compared to normoxic controls, with a notable reduction in expression observed from 8 hours onwards, indicating continuous suppression over time (P<0.05) (**Figure 6C**, **6D**).

These results indicate that hypoxia induces the expression of hsa-miR-584-3p in EECs, leading to downregulation of DKK-1 protein, inhibition of EEC proliferation, and enhancement of EEC migration and invasion. The findings highlight the complex regulatory mechanisms triggered by hypoxic conditions in endometrial epithelial cells.

Discussion

The EECs, luminal epithelial cells, mesenchymal cells, and immune cells, form both the

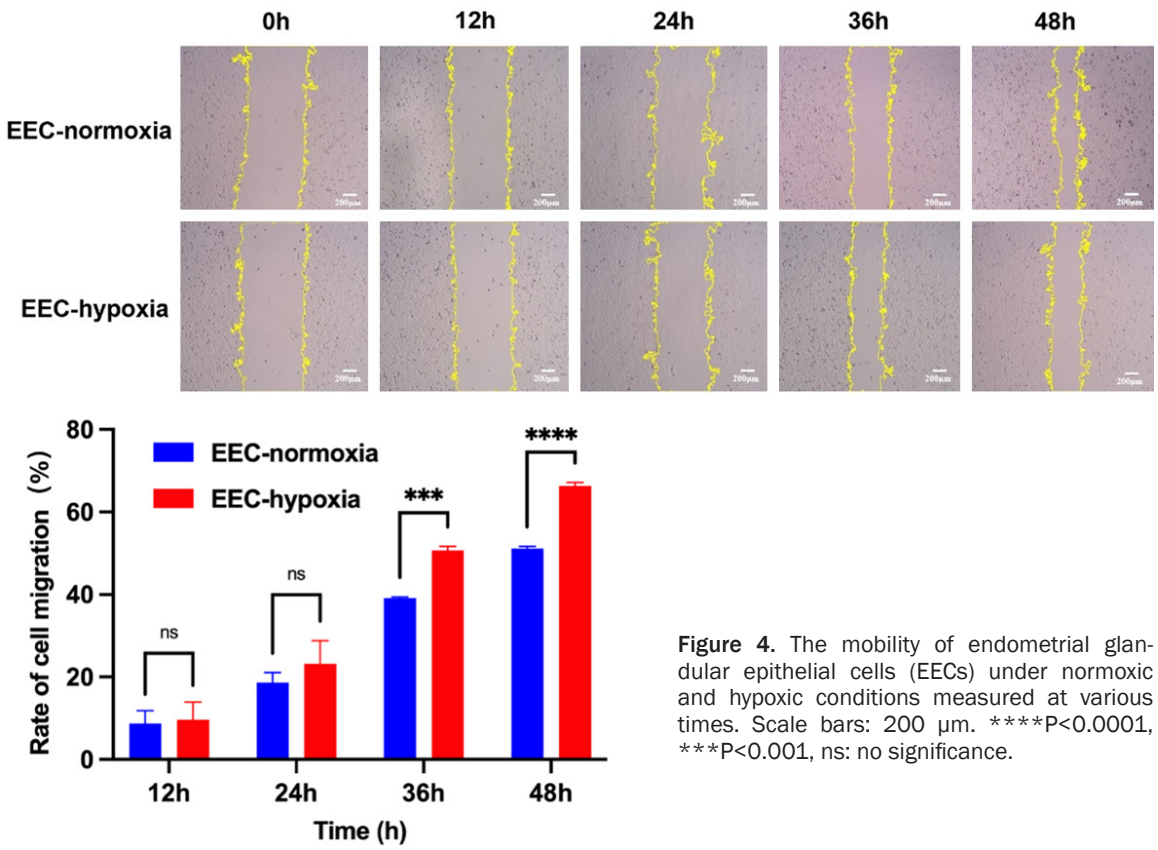

Figure 4. The mobility of endometrial glandular epithelial cells (EECs) under normoxic and hypoxic conditions measured at various times. Scale bars: 200 μm. ****P<0.0001, ***P<0.001, ns: no significance.

functional and basal layers. Prior to menstruation, the withdrawal of estrogen and progesterone induces rhythmic and increasing spasmodic contractions of the spiral arteries, leading to hypoxia, necrosis, and detachment of the functional layer. In the female reproductive cycle, a controlled hypoxic environment is crucial for processes such as endometrial repair, embryo implantation, trophoblast proliferation, differentiation, invasion, and fetal development [16, 17]. Conversely, dysfunctional hypoxic conditions are linked to reproductive pathologies including repeated implantation failure, embryonic termination, preeclampsia, endometriosis, and endometrial cancer [18]. However, the specific effects of hypoxia on EECs and the underlying mechanisms remain poorly understood.

In this study, RNA sequencing was employed to identify differentially expressed miRNAs in EECs under normoxic (21% oxygen) and hypoxic (1% oxygen) conditions. Further analyses using CCK-8, scratch healing, and Transwell invasion assays revealed that hypoxia significantly reduced EEC proliferation while enhancing their migration and invasion capabilities. This regulatory effect of hypoxia on cell biology mirrors findings in other cell types and diseases; for instance, hypoxia-induced upregulation of NDRG1 modulates the proliferation of pulmonary artery endothelial cells by targeting TAF15, contributing to pulmonary hypertension [19]. Additionally, dysregulation of migration in human vascular smooth muscle cells under hypoxic conditions [20], and HIF-1α-mediated regulation of invasion and migration in breast cancer cells [13], further supports the broad impact of hypoxia on cellular processes. These parallels underscore the potential universal mechanisms by which hypoxia influences cell behavior across different tissues and disease states.

MicroRNAs (miRNAs) are highly conserved noncoding RNAs that primarily silence gene expression by binding to the 3' untranslated regions (3'UTRs) of target genes. Extensive research has demonstrated that miRNAs orchestrate a variety of biological processes, including differentiation, proliferation, migration, invasion, metabolism, and apoptosis [21]. For instance, miR-584-3p has been shown to inhibit invasion

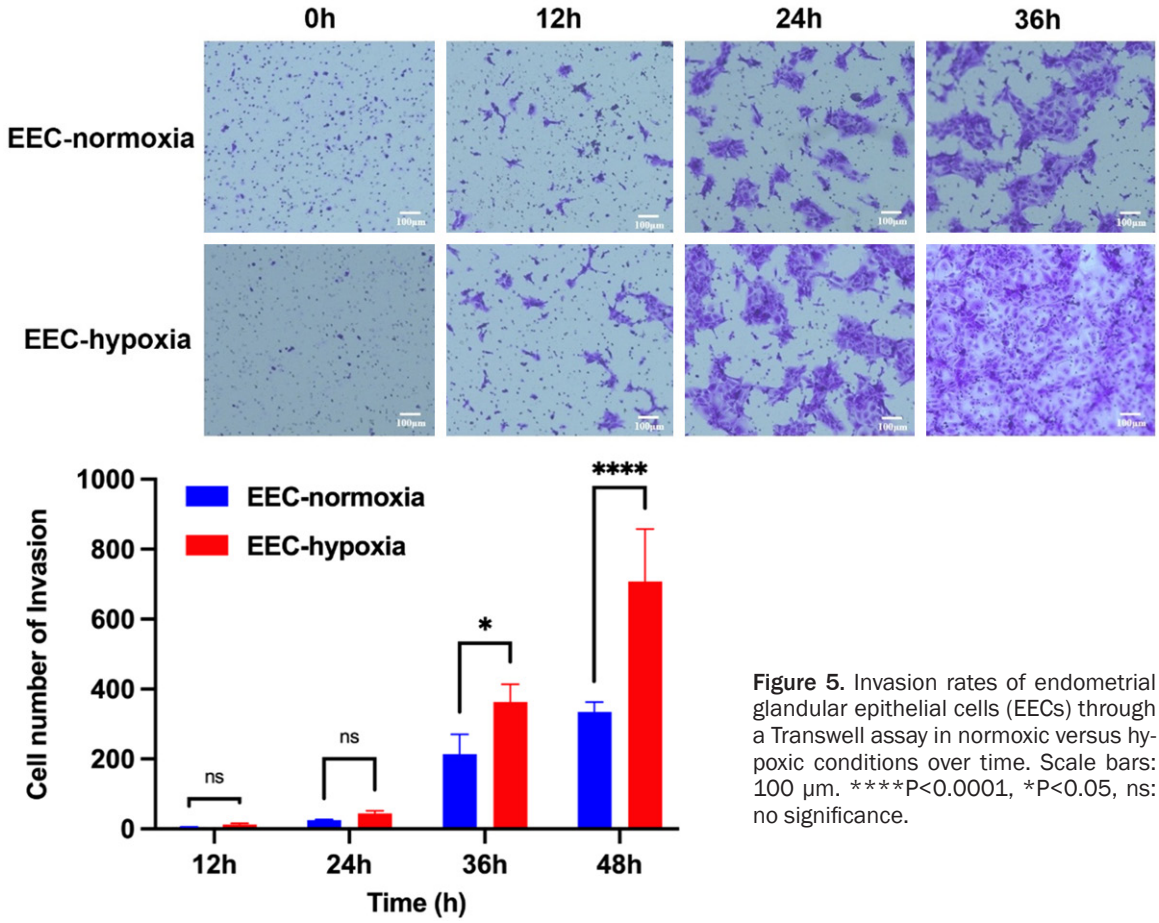

Figure 5. Invasion rates of endometrial glandular epithelial cells (EECs) through a Transwell assay in normoxic versus hypoxic conditions over time. Scale bars: 100 μm. ****P<0.0001, *P<0.05, ns: no significance.

in clear cell renal cell carcinoma by downregulating ROCK-1 [22] and to regulate migration in human thyroid cancer cells [23]. Additionally, in neuroblastoma, miR-584-3p suppresses migration, proliferation, invasion, and angiogenesis both in vivo and in vitro by targeting MMP-14 [24].

In the context of hypoxia, miR-584-3p plays a crucial role. Xue et al. [25] reported that miR-584-3p counteracts hypoxia-induced ROCK-1-dependent stress fiber formation, thereby inhibiting migration and invasion in glioma cells. Despite its known functions, the involvement of miR-584-3p in reproductive diseases remains understudied, particularly its effects on EECs under hypoxic conditions.

In this study, miRNA sequencing and RT-qPCR were utilized to assess miRNA expression at different oxygen levels. The findings reveal that miR-584-3p is upregulated in hypoxic conditions compared to normoxic environments. This upregulation suggests that miR-584-3p could be a key regulator of the functional changes observed in hypoxia-induced EECs.

Following the identification of differentially expressed miRNAs in EECs under hypoxic conditions, DKK-1 was identified as a target gene of hsa-miR-584-3p using the miRNA TargetScan database. This interaction was confirmed through double-luciferase reporter gene analysis, which demonstrated binding between hsa-miR-584-3p and the 3'UTR of DKK-1. Western blot analysis further revealed a significant reduction in DKK-1 expression in EECs under hypoxic conditions. DKK-1 is a well-known inhibitor of the Wnt/β-catenin signaling pathway, which is crucial for pluripotent differentiation, organ development, and stem cell regeneration [26], and plays roles in the development of the female reproductive tract, repair of endometrial damage, embryo implantation, and trophoblast proliferation and differentiation.

For instance, WNT5A, FZD5, and LRP5 have been shown to regulate the proliferation and

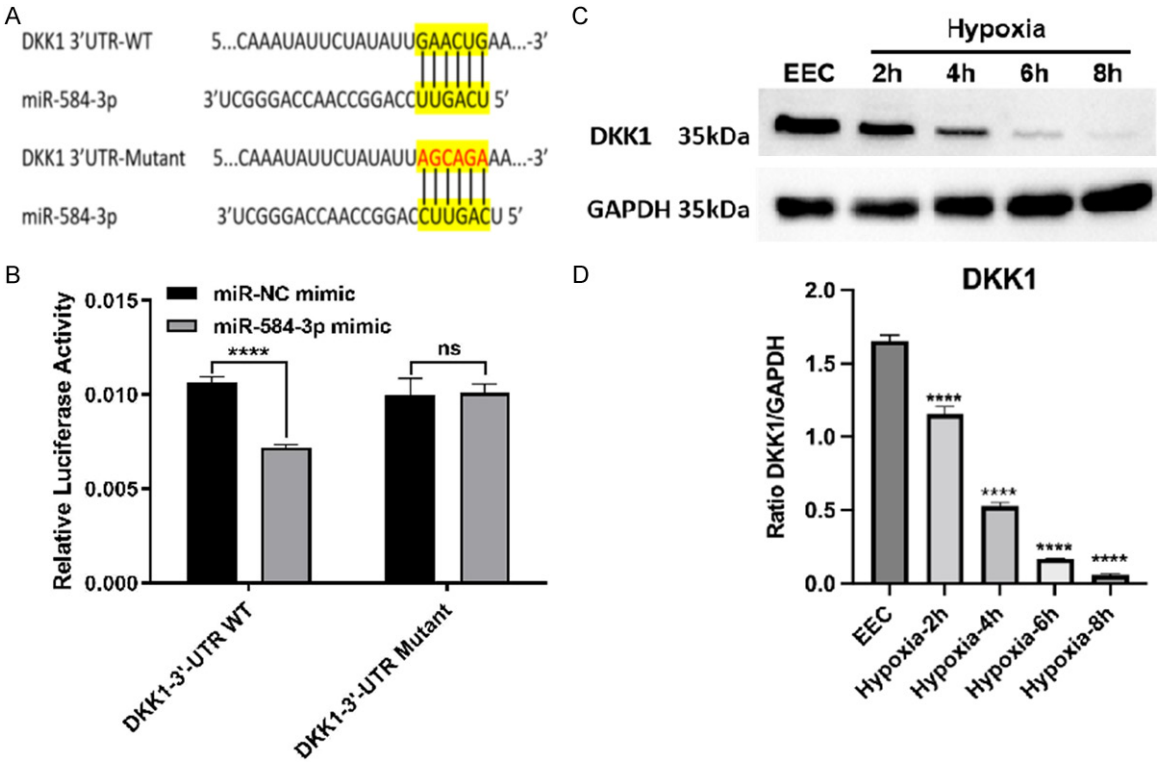

Figure 6. Interaction of hsa-miR-584-3p with the 3' untranslated region (UTR) of DKK-1: A, B. Dual-luciferase reporter assay results demonstrating regulation of DKK-1 by hsa-miR-584-3p. C, D. Western blot analysis showing DKK-1 protein levels in endometrial glandular epithelial cells (EECs) under normoxic and hypoxic conditions at various time points. ****P<0.0001, ns: no significance.

self-renewal of endomesenchymal stem cells through the Wnt/β-catenin pathway, which also modulates the activity of endometrial stem/progenitor cells during menstruation [27]. Animal studies indicate that the periodic activation of the Wnt/β-catenin pathway controls the cyclic regeneration of the endometrium in pigs [28]. Wnt4 is a crucial regulator of blastocyst implantation in mice [29]. Clinical studies have shown that bone marrow mesenchymal stem cells combined with estrogen can inhibit the epithelial-mesenchymal transition and promote the regeneration of damaged endometrium via the Wnt/β-catenin pathway [26]. In Asherman syndrome patients, activation of this pathway enhances angiogenesis, increases gland numbers, and facilitates repair of endometrial damage [30].

Despite these findings, the specific role of DKK-1 in regulating EEC function under hypoxic conditions remains unclear. Our study suggests that a hypoxic environment may modulate DKK-1 expression via the upregulation of miR-584-3p, thereby altering EEC function and potentially influencing endometrial injury and repair processes.

This study successfully constructed a cellular model of hypoxia-induced injury, elucidating the regulatory effects of hypoxia on the gene and functional phenotypes of EECs and establishing the targeted relationship between miR-584-3p and DKK-1. However, it did not include experiments on cell function regulation in hypoxia-damaged EECs or gene editing of miR-584-3p, such as through transfection with miRNA mimics or inhibitors to assess gene expression changes. Future studies will aim to explore these avenues, particularly focusing on the regulatory mechanisms of miR-584-3p in cell damage repair in hypoxia-injured EECs. Additionally, constructing an animal model to investigate the effects of miR-584-3p on endometrial repair and regeneration is also planned.

In conclusion, our findings suggest that the overexpression of hsa-miR-584-3p under hypoxic conditions may regulate the migration,

proliferation, and invasion of EECs by downregulating DKK-1 and activating the Wnt/β-catenin signaling pathway. This study lays the genetic groundwork for further research into diseases related to endometrial aplasia.

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Disclosure of conflict of interest

None.

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References

[1] Maybin JA, Hirani N, Brown P, Jabbour HN and Critchley HO. The regulation of vascular endothelial growth factor by hypoxia and prostaglandin $F_2\alpha$ during human endometrial repair. J Clin Endocrinol Metab 2011; 96: 2475-2483.

[2] Wang L, Liang J, Bi S, Li Y, Zhang W, Xiwen W, Liu Y and Liu H. Role of GLI1 in hypoxia-driven endometrial stromal cell migration and invasion in endometriosis. Comput Math Methods Med 2022; 2022: 6890790.

[3] Kimura M, Kothari S, Gohir W, Camargo J and Husain S. MicroRNAs in infectious diseases: potential diagnostic biomarkers and therapeutic targets. Clin Microbiol Rev 2023; 36: e0001523.

[4] Kazemi Shariat Panahi H, Dehhaghi M, Guillemin GJ, Peng W, Aghbashlo M and Tabatabaei M. Targeting microRNAs as a promising anticancer therapeutic strategy against traffic-related air pollution-mediated lung cancer. Cancer Metastasis Rev 2023; [Epub ahead of print].

[5] Guo T, Zheng C, Wang Z and Zheng X. miR-584-5p regulates migration and invasion in non-small cell lung cancer cell lines through regulation of MMP-14. Mol Med Rep 2019; 19: 1747-1752.

[6] Wei H, Wang J, Xu Z, Lu Y, Wu X, Zhuo C, Tan C, Tang Q and Pu J. miR-584-5p regulates hepatocellular carcinoma cell migration and invasion through targeting KCNE2. Mol Genet Genomic Med 2019; 7: e702.

[7] Dong S, Wang W, Liao Z, Fan Y, Wang Q and Zhang L. MYC-activated LINC00607 promotes hepatocellular carcinoma progression by regulating the miR-584-3p/ROCK1 axis. J Gene Med 2023; 25: e3477.

[8] Xiang J, Wu Y, Li DS, Wang ZY, Shen Q, Sun TQ, Guan Q and Wang YJ. miR-584 suppresses invasion and cell migration of thyroid carcinoma by regulating the target oncogene ROCK1. Oncol Res Treat 2015; 38: 436-40.

[9] Huang S, Fu D, Wan Z, Huang Z, Li M, Li H and Chong T. DKK1 ameliorates myofibroblast differentiation in urethral fibrosis in vivo and in vitro by regulating the canonical Wnt pathway. Int J Med Sci 2023; 20: 1631-1643.

[10] Bley IA, Zwick A, Hans MC, Thieser K, Wagner V, Ludwig N, Khalmurzaev O, Matveev VB, Loertzer P, Pryalukhin A, Hartmann A, Geppert CI, Loertzer H, Wunderlich H, Naumann CM, Kalthoff H, Junker K, Smola S and Lohse S. DKK1 inhibits canonical Wnt signaling in human papillomavirus-positive penile cancer cells. Transl Oncol 2022; 15: 101267.

[11] Li T, Chan RWS, Lee CL, Chiu PCN, Li RHW, Ng EHY and Yeung WSB. WNT5A interacts with FZD5 and LRP5 to regulate proliferation and self-renewal of endometrial mesenchymal stem-like cells. Front Cell Dev Biol 2022; 10: 837827.

[12] Pazhohan A, Amidi F, Akbari-Asbagh F, Seyedrezazadeh E, Farzadi L, Khodarahmin M, Mehdinejadiani S and Sobhani A. The Wnt/β-catenin signaling in endometriosis, the expression of total and active forms of β-catenin, total and inactive forms of glycogen synthase kinase-3β, WNT7a and DICKKOPF-1. Eur J Obstet Gynecol Reprod Biol 2018; 220: 1-5.

[13] Zheng PF, Rong JJ, Zheng ZF, Liu ZY, Pan HW and Liu P. Investigating the causal effect of Dickkopf-1 on coronary artery disease and ischemic stroke: a Mendelian randomization study. Aging (Albany NY) 2023; 15: 9797-9808.

[14] Liu H, Zhang S, Li Z, Zheng Z, Shi W, Hu M and Liu F. Promotion of hepatic stellate cell activation and liver fibrosis by microRNA-33a-5p through targeting the Dickkopf-1-mediated wingless-related integration site/beta-catenin pathway. J Physiol Pharmacol 2023; 74.

[15] Hashimoto Y, Tsuzuki-Nakao T, Kida N, Matsuo Y, Maruyama T, Okada H and Hirota K. Inflammatory cytokine-induced HIF-1 activation promotes epithelial-mesenchymal transition in endometrial epithelial cells. Biomedicines 2023; 11: 210.

[16] Gonzalez AC, Costa TF, Andrade ZA and Medrado AR. Wound healing - a literature review. An Bras Dermatol 2016; 91: 614-620.

[17] Biswas Shivhare S, Bulmer JN, Innes BA, Hapangama DK and Lash GR. Altered vascular smooth muscle cell differentiation in the endometrial vasculature in menorrhagia. Hum Reprod 2014; 29: 1884-1894.
[18] Wang XX, Hua T and Wang HB. Estrogen receptor-related receptor γ uppresses hypoxia-induced angiogenesis by regulating VEGFA in endometrial cancer. Gynecol Endocrinol 2023; 39: 2264411.
[19] Li C, Lv J, Wumaier G, Zhao Y, Dong L, Zeng Y, Zhu N, Zhang X, Wang J, Xia J and Li S. NDRG1 promotes endothelial dysfunction and hypoxia-induced pulmonary hypertension by targeting TAF15. Precis Clin Med 2023; 6: pbad024.
[20] Chu H, Qin Y, Qiu T, Zhou S, Na Z, Sun Y, Xu Y and Zhong Y. Phenotype and function of smooth muscle cells derived from the human normal great saphenous vein in response to hypoxia. Phlebology 2024; 39: 96-107.
[21] Li J, Gu X, Gao C and Zhang J. Six microRNA prognostic models for overall survival of lung adenocarcinoma. Genet Res (Camb) 2022; 2022: 5955052.
[22] Ueno K, Hirata H, Shahryari V, Chen Y, Zaman MS, Singh K, Tabatabai ZL, Hinoda Y and Dahiya R. Tumour suppressor microRNA-584 directly targets oncogene Rock-1 and decreases invasion ability in human clear cell renal cell carcinoma. Br J Cancer 2011; 104: 308-315.
[23] Lima CR, Gomes CC and Santos MF. Role of microRNAs in endocrine cancer metastasis. Mol Cell Endocrinol 2017; 456: 62-75.
[24] Xiang X, Mei H, Qu H, Zhao X, Li D, Song H, Jiao W, Pu J, Huang K, Zheng L and Tong Q. miRNA-584-5p exerts tumor suppressive functions in human neuroblastoma through repressing transcription of matrix metalloproteinase 14. Biochim Biophys Acta 2015; 1852: 1743-1754.
[25] Xue H, Guo X, Han X, Yan S, Zhang J, Xu S, Li T, Guo X, Zhang P, Gao X, Liu Q and Li G. MicroRNA-584-3p, a novel tumor suppressor and prognostic marker, reduces the migration and invasion of human glioma cells by targeting hypoxia-induced ROCK1. Oncotarget 2016; 7: 4785-4805.
[26] Yuan L, Cao J, Hu M, Xu D, Li Y, Zhao S, Yuan J, Zhang H, Huang Y, Jin H, Chen M and Liu D. Bone marrow mesenchymal stem cells combined with estrogen synergistically promote endometrial regeneration and reverse EMT via Wnt/β-catenin signaling pathway. Reprod Biol Endocrinol 2022; 20: 121.
[27] Xu S, Chan RWS, Li T, Ng EHY and Yeung WSB. Understanding the regulatory mechanisms of endometrial cells on activities of endometrial mesenchymal stem-like cells during menstruation. Stem Cell Res Ther 2020; 11: 239.
[28] Bukowska J, Ziecik AJ, Laguna J, Gawronska-Kozak B and Bodek G. The importance of the canonical Wnt signaling pathway in the porcine endometrial stromal stem/progenitor cells: implications for regeneration. Stem Cells Dev 2015; 24: 2873-2885.
[29] Hayashi K, Erikson DW, Tilford SA, Bany BM, Maclean JA 2nd, Rucker EB 3rd, Johnson GA and Spencer TE. Wnt genes in the mouse uterus: potential regulation of implantation. Biol Reprod 2009; 80: 989-1000.
[30] Xue X, Li X, Yao J, Zhang X, Ren X and Xu S. Transient and prolonged activation of wnt signaling contribute oppositely to the pathogenesis of Asherman's syndrome. Int J Mol Sci 2022; 23: 8808.