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Original Research

WD-repeat containing protein-61 regulates endometrial epithelial cell adhesion indicating an important role in receptivity

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ABSTRACT

Endometrial receptivity is crucial for successful embryo implantation during early pregnancy. The human endometrium undergoes remodeling within each menstrual cycle to prepare or become receptive to an implanting blastocyst in the mid-secretory phase. However, the mechanisms behind these changes are not fully understood. Recently, using hormone-treated endometrial organoids to model receptivity, we identified that the transcriptional regulator WD-repeat-containing protein-61 (WDR61) was reduced in organoids derived from infertile women. In this study, we aimed to determine the role of WDR61 in endometrial receptivity. Here, we demonstrated that WDR61 immunolocalizes in the nuclei and cytosol of endometrial glandular epithelium, luminal epithelium, and stroma. The staining intensity of WDR61 was significantly higher during the receptive mid-secretory phase compared to the non-receptive proliferative phase in fertile women. In a functional experiment to model blastocyst adhesion to the endometrial epithelium, we found that adhesion of cytotrophoblast progenitor spheroids was blocked when siRNA was used to knockdown *WDR61* in primary endometrial epithelial cells. Similarly, in Ishikawa cells (a receptive human endometrial epithelial cell line), siRNA knockdown of *WDR61* significantly reduced the cell adhesive and proliferative capacities. qPCR revealed that *WDR61* knockdown reduced expression of key genes involved in receptivity including *HOXD10*, *MMP2*, and *CD44*. Chromatin immunoprecipitation sequencing demonstrated that WDR61 directly targeted 2022 genes in Ishikawa cells, with functions including focal adhesion, intracellular signaling and epithelial-mesenchymal transition. Overall, these findings suggest that WDR61 promotes endometrial receptivity by modulating epithelial cell focal adhesions, proliferation, and epithelial–mesenchymal transition.

Keywords: embryo implantation failure / endometrial receptivity / endometrial epithelium / cell adhesion / WDR61 / Wnt pathway / Homeobox genes / chromatin immunoprecipitation sequencing

Introduction

Infertility affects approximately 17.5% of the adult population globally (Mascarenhas *et al.*, 2012; World Health Organization, 2023). Implantation failure is a significant barrier to live birth, with roughly 70% of embryos failing to implant (Coughlan *et al.*, 2014). Although endometrial factors contribute to two-thirds of implantation failures, there remains a significant lack of understanding regarding the specific endometrial factors that cause failed implantation (Ifenatuoha and Okewale, 2022).

The human endometrium undergoes structural and molecular remodeling in response to hormonal changes throughout the menstrual cycle. The luminal epithelial cell surface of the endometrium is refractory to blastocyst implantation for most of the menstrual cycle, except for a brief 2–3 day window in the mid-secretory phase when it becomes 'receptive' to implantation (Dimitriadis *et al.*, 2010). The mechanisms driving this functional change towards receptivity remain poorly understood. Recent single-cell transcriptomic analyses have suggested that the onset of the receptive window in humans is characterized by a sudden activation of transcriptomic activities in epithelial cells (Wang *et al.*, 2020). Dysregulation in these transcriptomic processes may be an underlying cause of impaired endometrial receptivity.

Our recent study demonstrated that WD-repeat-containing protein-61 (WDR61) was one of the top downregulated proteins in apical secretions of 'receptive phase' endometrial organoids derived from women with primary infertility (Zhou *et al.*, 2022). WDR61 is a component of the RNA polymerase II association factor 1 complex (Paf1c). Paf1c regulates transcription of Homeobox (Hox) genes which are vital in endometrial receptivity in both

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humans and mice (Taylor, 2000; Xu et al., 2014). WDR61 itself is known to regulate Hox gene expression. Knockdown of WDR61 in cervical cells reduces the levels of Hox proteins such as HoxA10 (Zhu et al., 2005), which is elevated in the endometrium during the receptive window (Xu et al., 2014). *HoxA10* and *HoxD10* genes are essential for endometrial receptivity, as demonstrated by reduced implantation rates following knockdown in murine uteri (Xu et al., 2014). Women experiencing recurrent implantation failure have impaired HoxA10 function in the endometrium, likely contributing to defective endometrial receptivity (Jiang et al., 2017). In addition, β-catenin binds to Paf1c subunits promoting RNA Pol II transcription of canonical Wnt pathway targets (Mosimann et al., 2006; Crisucci and Arndt, 2011) which have a number of reported roles in endometrial receptivity (Tulac et al., 2003; Chen et al., 2007, 2020).

The above studies suggest that WDR61 is a potential biomarker and treatment target for endometrial dysfunction during receptivity. In this study, we aimed to investigate the role of WDR61 in endometrial epithelial cells during receptivity. We characterized the spatial and temporal immunolocalization of WDR61 in the non-receptive proliferative phase and mid-secretory phase of the fertile human endometrium. We used Ishikawa cells (receptive endometrial cell-line) (Castelbaum et al., 1997) and a co-culture model using primary human endometrial epithelial cells (HEECs) and cytotrophoblast progenitor spheroids to determine the role of WDR61 on epithelial cell adhesion, which is a function required in the initiation of implantation. To identify how WDR61 may affect endometrial epithelial cell function, we used chromatin immunoprecipitation sequencing (ChIP-Seq) to identify WDR61 binding to specific gene targets in Ishikawa cells.

Materials and methods

Ethics statement

Ethical approval for this study was granted by the Human Research Ethics Committee at the Royal Women's Hospital (03066B) and Melbourne IVF (74/19-MIVF). Samples from patients were collected after gaining written informed consent. Women who were aged between 26–42 years who had regular menstrual cycles (28–32 days) and were not using intrauterine or hormonal contraceptives for a minimum of 3 months before surgery were included. The endometrium was collected by dilation and curettage, after inspection by gynecological pathologists confirmed no endometrial abnormalities or dysfunction.

Cell culture media

The Ishikawa cell line was cultured in DMEM (31053, Thermo, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS) (10091148, Thermo, Auckland, New Zealand), and 1% L-glutamine (25030, Thermo, USA). Human trophoblast progenitor cells (HTPCs), kindly provided by Professor Fisher and Dr Genbacev from the University of California, San Francisco, CA, USA, were maintained in DMEM/F-12, GlutaMAX (10565018, Thermo, USA) supplemented with 10% FBS, 10 ng/ml bFGF (233-FB, R&D, Minneapolis, MN, USA), and 10 μM SB431542 (#1614, Tocris, Bristol, UK), as described previously (Zdravkovic et al., 2015). HEECs were cultured in DMEM/F-12, GlutaMAX supplemented with 10% FBS and 1% penicillin/streptomycin (15070063, Thermo, USA).

Primary endometrial epithelial cell isolation and culture

Endometrial biopsy samples were thoroughly washed with Dulbecco's phosphate-buffered saline (DPBS)/1% penicillin/streptomycin. The tissue was transferred to a culture dish and finely minced with scissors before enzymatic digestion with 727 μg/ml Collagenase III (Worthington CLS-3, S8P18813, Lakewood, NJ, USA) and 25 μg/ml DNase I (#11284932001, Roche, Penzberg, Germany) in 0.36 mM calcium chloride/DPBS for 20 min in a shaking water bath at 37°C. Following this, four times the volume of Dulbecco's modified Eagle's medium (DMEM)/F12 was added to stop digestion. To separate stromal cells from the endometrial epithelial fragments, the digested sample was filtered using a 45-μm sieve. Epithelial fragments collected on the sieve were washed and resuspended in HEEC media (Zhou et al., 2021) and transferred to an appropriate cell culture plate. Epithelial fragments were incubated for 40 min and transferred into fresh wells by gently shaking the plate and collecting the supernatant to remove stromal cell contamination. Following this, HEECs were cultured until they were ready for transfection or other experiments.

In vitro siRNA transfection

HEECs or Ishikawa cells at approximately 70% confluency were transfected using Lipofectamine RNAi MAX (13778100, Thermo, USA) and Opti-MEM (11524456, Thermo, USA) following manufacturer's guidelines. The final concentration was 20 nM of *WDR61* or scramble control siRNA (s37273 and 4390843, Thermo, USA). Transfected cells were incubated at 37°C for 24 h, before removing transfection media and replacing it with respective cell culture media. Cells were cultured at 37°C for 48 h before functional assays were performed.

xCELLigence assay

The RTCA DP xCELLigence instrument (xCELLigence) (Roche ACEA, San Diego, CA, USA) was used to measure cell adhesion and proliferation *in vitro* in real-time. For this, 10 000 transfected Ishikawa cells were seeded into each well of a 96-well xCELLigence plate with 150 μl of DMEM/5% FBS (three wells per transfection condition). The cell index, determined in the xCELLigence, is a measurement of cells impeding the electrical flow transmission between the microelectrodes on each well as they adhere to the surface. Increasing the cell index correlates to an increase in cell adherence. The cell index was recorded every 15 min for 8 h to represent cell adhesive capacity and every 1 h following this for 80 h to represent cell proliferative capacity. Data were collected using RTCA software and exported for statistical analysis.

Spheroid adhesion assay

This *in vitro* assay mimics blastocyst adhesion to the endometrial luminal epithelial cell surface. Initially, HTPCs were used to generate human trophoblast progenitor spheroids. HTPCs are developed from blastomeres of cleavage-stage human embryos (Zdravkovic et al., 2015). Then, 2500 cells in 150 μl HTPC culture media were transferred into each well of a 96-well round bottom, ultra-low attachment plate (#7007, Corning, New York, NY, USA). The cells were then incubated at 37°C for 48 h to form spheroids, before 18–22 spheroids were collected and gently added onto the transfected endometrial epithelial cell monolayer. The total number of spheroids on the monolayer were counted. After incubating the spheroids on the monolayer for 2 h (HEECs) or 4 h (Ishikawa) at 37°C, unadhered spheroids were carefully washed

away using DPBS. The remaining attached spheroids were then counted, and the percentage attachment was calculated by expressing the number of attached spheroids as a percentage of the original number of spheroids.

RNA isolation and RT-qPCR

Cell pellets were lysed using TRI reagent (T9424, Sigma, St Louis, MO, USA) and total RNA was isolated following the manufacturer's instructions. DNA contamination was removed using the TURBO DNase Kit (AM1907, Thermo, USA). Nanodrop 2000 was used to calculate total RNA concentration. To perform reverse transcription, 300 ng of total RNA was collected in 8 µl of RNase-free water. The collected RNA was converted into cDNA using the Superscript III first strand synthesis kit (18080051, Thermo, USA). qPCR was performed using SYBR Green PCR master mix (4367659, Thermo, USA). Forward and reverse primers are outlined in Supplementary Table S1. RNase free water was included as a negative control and 18S was included for normalization. qPCR was conducted on the Applied Biosystems ViiA7 system for 10 min at 95°C and 40 cycles of 15 s at 95°C followed by 1 min at 60°C. Gene expression levels were calculated using the comparative cycle threshold method.

Chromatin immunoprecipitation sequencing

For each reaction, 10 million cultured Ishikawa cells were fixed for 10 min in 1% formaldehyde. Chromatin immunoprecipitation was performed using the MAGnify Chromatin Immunoprecipitation System (49-2024, Thermo, USA). Cells were sonicated in Qubit 0.5 ml thin-walled tubes using Q-Sonica at 70% amplitude for 20 min with intervals of 15 s on and 45 s off. Three reactions were immunoprecipitated using WDR61 polyclonal antibody (PA5-40079, Invitrogen, Carlsbad, CA, USA), and one Rabbit IgG control and input control was also included. Final DNA samples were collected in 150 µl elution buffer for library preparation. Preparation and sequencing were performed by the Australian Genomics Research Facility. Integrated DNA Technologies xGen cfDNA & FFPE DNA Library Preparation Kit was employed and samples were sequenced using Illumina NextSeq2000 on a P1 flow cell for up to 100 M reads.

Immunohistochemistry and immunocytochemistry

Human endometrial tissues were fixed in 10% formalin for 20–24 h, followed by washing with Tris-buffered saline (TBS) and embedding in paraffin. A series of 4 µm-thick endometrial sections were dewaxed and rehydrated using xylene and ethanol. Antigen retrieval was performed by intermittently microwaving for a total of 6 min in 10 mM sodium citrate buffer (pH 6.0, for WDR61) or 10 mM Tris-EDTA (pH 9.0, for acetylated α-tubulin) followed by cooling for 30 min at room temperature. Subsequently, endogenous peroxidase activity was quenched by incubating slides with 3% hydrogen peroxide in methanol at room temperature for 15 min. After additional washes, sections were incubated at room temperature for 30 min in non-immune blocking buffer consisting of 10% normal goat serum and 2% normal human serum in TBS. Sections were incubated at 4°C overnight with the WDR61 primary antibody (0.5 µg/ml, #039932, Sigma, Buchs, Switzerland), Acetylated α-tubulin primary antibody (0.12 µg/ml, #12152, Cell Signaling Technology, Danvers, MA, USA) or respective IgG negative controls (Dako, Carpinteria, CA, USA). Sections were then washed with TBS–Tween 0.1% and then incubated with Goat anti-Rabbit-biotinylated antibody (0.5 µg/ml, ZF0425, Vector Labs, Burlingame, CA, USA) or Goat anti-Mouse-biotinylated antibody (0.5 µg/ml, BA-9200, Vector Labs, USA) for 30 min at room temperature. After additional washing with 0.1% TBS–T, sections were incubated with Horseradish peroxidase conjugated antibody against Rabbit (20010775, Dako) for 30 min at room temperature. Sections were then treated with diaminobenzidine for 90 s to visualize positive signaling and counterstained with hematoxylin to highlight cell nuclei. Blinded quantitative staining analysis involved measuring the stain intensity and frequency in tissue structures: endometrial stroma, glands and luminal epithelium. The average score between proliferative and mid-secretory phases was calculated and analyzed statistically.

For immunocytochemistry, cells were cultured in an 8-well chamber slide, then fixed for 15 min using 4% paraformaldehyde. Following PBS washing, cells were incubated for 10 min in 0.1% Triton X-100 in PBS and treated as outlined for endometrial sections. E-cadherin (#3195, Cell Signaling Technology) was used at a concentration of 0.26 µg/ml to determine the purity of epithelial cells.

Statistical analysis

Statistical analysis was carried out using PRISM 8.0. Data were checked for normal distribution using the Shapiro Wilk test and all passed normality test. Repeated measures ANOVA was used to analyze xCELLigence data. Data were presented as mean ± SD. $P < 0.05$ was considered statistically significant.

Results

Immunolocalization of WDR61 in fertile human endometrium

Immunohistochemistry was used to determine the temporal and spatial localization of WDR61 in proliferative phase and mid-secretory phase endometrial tissue from women with normal fertility. Both phases showed nuclear and cytosolic WDR61 expression in endometrial stroma, endometrial glands, and luminal epithelium (Fig. 1A). Apical staining of WDR61 was exclusively observed in the endometrial glands during the mid-secretory phase (Fig. 1A). The staining score was highest in the glands during the mid-secretory phase and significantly reduced in the proliferative phase (Fig. 1B, $P < 0.0001$). Similarly, endometrial stromal cells and luminal epithelium had significantly higher staining scores in the mid-secretory phase compared to that in the proliferative phase, respectively (Fig. 1B, $P < 0.05$). Overall, the staining intensity scores demonstrate sparse and weak WDR61 staining throughout the non-receptive proliferative phase endometrium compared with the receptive mid-secretory phase. To investigate WDR61 immunolocalization in ciliated epithelial cells, endometrial sections were stained for acetylated α-tubulin. WDR61 staining was detected in both acetylated α-tubulin positive and negative epithelial cells during the proliferative and mid-secretory phases, except in the glands during the proliferative phase, where WDR61-positive cells were restricted to a subpopulation of acetylated α-tubulin-positive cells (Fig. 1C). Overall, the WDR61 staining was present in both acetylated α-tubulin-positive and -negative epithelial cells. The specificity of antibody staining (Fig. 1A and C) was confirmed by the negative IgG isotype control which showed no staining in the tissue (Fig. 1C).

Effect of WDR61 knockdown on adhesive capacity of endometrial epithelial cells *in vitro*

A spheroid adhesion assay using HTPCs and primary HEECs was employed to mimic blastocyst adhesion to the endometrial surface *in vitro*. Primary HEEC monolayers were derived from endometrial samples collected from fertile women. Prior to their use,

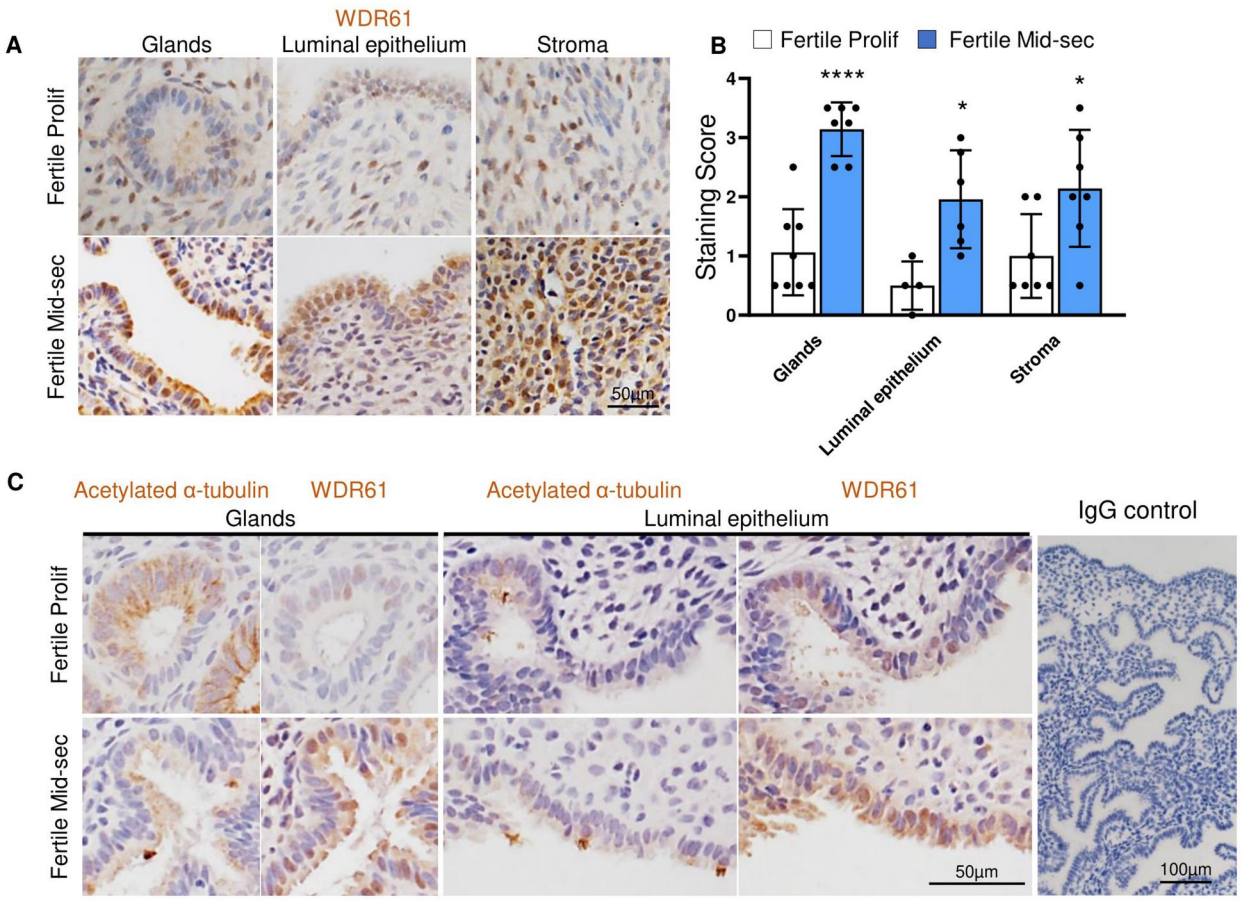


Figure 1. Spatial and temporal localization of WDR61 in fertile human endometrial tissue. (**A**) Immunohistochemistry staining of WDR61 in endometrial tissue from fertile women during the mid-secretory phase (n = 7) and proliferative phase (n = 8). Sections were co-stained with hematoxylin (blue) to denote nuclear components. (**B**) WDR61 expression in luminal epithelium, glands and stroma was semi-quantified by giving stain frequency and intensity a score out of 4. (**C**) Localization of WDR61 and acetylated α-tubulin (ciliated epithelial cells) was determined by immunohistochemistry staining on a series of endometrial sections. An isotype negative control was included, consisting of the same IgG isotype and concentration with a non-immune antibody in place of the primary antibody. Data are presented as mean ± SD. *$P < 0.05$, ****$P < 0.0001$. Prolif, proliferative phase; Mid-sec, mid-secretory phase.

immunocytochemistry was performed to determine the cellular localization of WDR61 in primary HEECs. Primary HEECs had maximal WDR61 staining in the nuclei and weak staining in the cytoplasm (Fig. 2A), similar to endometrial luminal epithelial cells in fertile women (Fig. 1A). Based on these consistent expression patterns, the primary HEECs were considered a suitable model for investigating the function of WDR61 in luminal epithelium. The epithelial purity of HEECs was confirmed by staining for E-cadherin (Fig. 2A). The HEEC monolayers were treated with *WDR61* siRNA and scramble control siRNA. *WDR61* mRNA expression was significantly reduced by approximately 40% compared to control ($P < 0.05$, Fig. 2B). HTPC spheroid (embryo surrogate) adhesion to the HEEC monolayer was also significantly reduced, from 46.29% in control monolayers to 19.17% ($P < 0.01$) following *WDR61* knockdown (Fig. 2B).

xCELLigence was utilized to measure the real-time impact of *WDR61* knockdown on Ishikawa cell adhesion and proliferation. Ishikawa cells were used as HEECs do not culture well on the xCELLigence plates. Immunocytochemistry confirmed the cellular localization of WDR61 in Ishikawa cells and determined its suitability for functional analysis. WDR61 showed intense expression in the nuclei and weak cytosolic staining in cells, which was consistent with fertile mid-secretory luminal epithelium (Fig. 2C). The knockdown of *WDR61* in siRNA transfected Ishikawa cells was confirmed by qPCR ($P < 0.001$) where *WDR61* was reduced by approximately 60% (Fig. 2D). Cell adhesion and spreading on the xCELLigence plate was measured between 0 and 8 h, and proliferation was measured from 8 to 85 h (Fig. 2E and F). Cell adhesion and spreading was significantly reduced at 7 and 8 h post-seeding in *WDR61* knockdown cells compared to controls (Fig. 2E). A similar reduction in Ishikawa cell adhesion after *WDR61* knockdown was confirmed by a spheroid adhesion assay (Fig. 2F, $P < 0.01$). For cell proliferation, control Ishikawa cells showed a steep increase in the average cell index from 1.66 to 5.03 between 55 and 85 h, indicating rapid proliferation (Fig. 2G). Conversely, the population of *WDR61* knockdown Ishikawa cells remained steady with an average cell index under 0.6, which was significantly lower compared to controls at 85 h post-seeding (Fig. 2G, $P < 0.01$). The *WDR61* siRNA transfected Ishikawa cells had similar numbers of viable cells from 8 to 85 h (all above 85%) demonstrating that they lost their ability to proliferate within this time window. qPCR analysis demonstrated that *WDR61* knockdown in Ishikawa cells significantly reduced the genes cyclin-dependent kinase inhibitor 1A (*CDKN1A*), indicating cell cycle arrest, and telomerase reverse transcriptase (*TERT*), indicating cellular senescence (Fig. 2H). Therefore, *WDR61* knockdown in Ishikawa cells was associated with reduced proliferation likely due to cell cycle arrest and cellular senescence following impaired cell adhesion.

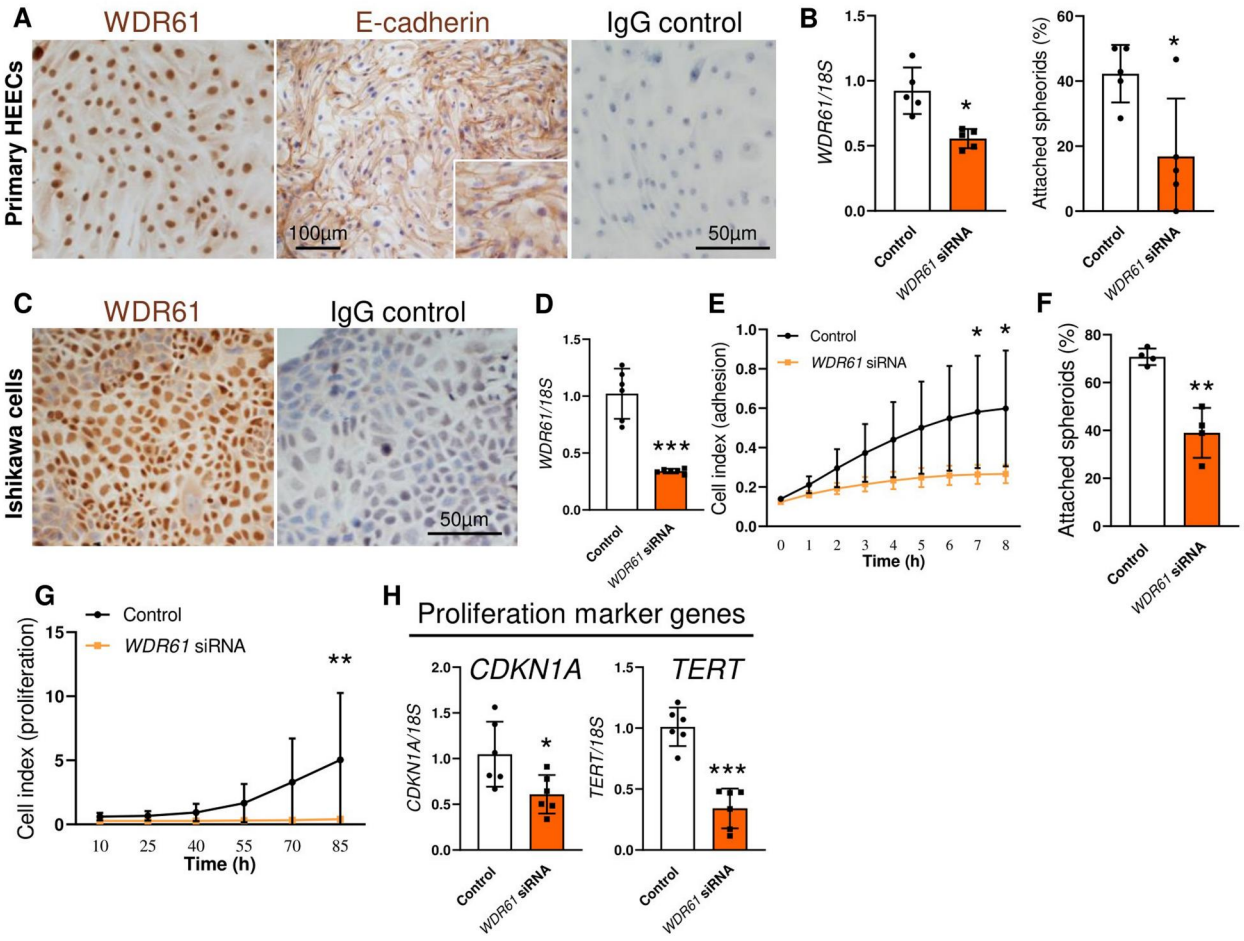


Figure 2. Functional effect on endometrial epithelial cell adhesion following *WDR61* knockdown. (**A**) Immunocytochemistry was performed on primary HEEC monolayers to determine WDR61 localization and confirm the epithelial purity of HEECs via E-cadherin staining. Cells were co-stained with hematoxylin to denote nuclear components. (**B**) The effect of *WDR61* knockdown in primary HEECs on the adhesion of trophoblast spheroids was measured 72 h post-transfection. The percentage of trophoblast spheroids adhering to primary HEECs was significantly reduced following *WDR61* knockdown (n = 5). (**C–G**) Ishikawa cells were used as a receptive endometrial epithelial cell model to test WDR61 functions on cell adhesion and proliferation. (C) Immunocytochemistry was performed on Ishikawa monolayers to determine WDR61 localization. (D–F) Adhesive capacity of Ishikawa cells was measured via xCELLigence and spheroid adhesion assay following treatment with *WDR61* or scramble control siRNA (n = 4). (G) Proliferation of Ishikawa cells was determined by xCELLigence. (**H**) Levels of expression of two proliferation genes following siRNA treatment was measured at 72 h post transfection in Ishikawa cells and normalized to *18S* (n = 6; qPCR). Data are presented as mean ± SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. HEEC, primary human endometrial epithelial cells.

Reduced WDR61 alters Wnt pathway and Homeobox gene expression in endometrial epithelial cells *in vitro*

The mechanisms by which reduced WDR61 influenced endometrial epithelial function *in vitro* were examined by qPCR of Ishikawa cells. We examined Hox and Wnt pathway genes due to their involvement in receptivity and known associations with WDR61 (Zhu *et al.*, 2005; Xu *et al.*, 2014; Van Oss *et al.*, 2017; Chen *et al.*, 2020). Loss of *WDR61* in Ishikawa cells significantly reduced expression of *HOXD10* ($P < 0.05$); however, increased expression of *HOXA9* and *HOXA11* compared to controls was detected ($P < 0.05$, Fig. 3A). Wnt pathway targets and endometrial receptivity markers, cluster domain-44 (*CD44*), and matrix metalloproteinase (*MMP*)2, were also downregulated following *WDR61* reduction ($P < 0.05$), however, *MMP7* remained unchanged compared to control (Fig. 3B).

WDR61 target genes identified by chromatin immunoprecipitation sequencing

ChIP-seq was used to examine the specific binding targets of WDR61 in Ishikawa cells. ChIP-Seq is a method that allows direct protein–DNA interactions to be identified. WDR61 is a component of PAF1c which activates gene transcription by RNA Polymerase II (Chen *et al.*, 2009b). Therefore, WDR61 binding to specific DNA regions in the receptive endometrial epithelium indicates that transcription of these genes is actively promoted. Ishikawa cells were sonicated, and gel electrophoresis was used to confirm DNA fragments were between 300 and 500 bp (Fig. 4A), an ideal size for chromatin immunoprecipitation. Following immunoprecipitation with anti-WDR61 antibody, qPCR was first used to confirm binding of positive control genes C-X-C motif chemokine 12 (*CXCL12*) and cellular Myc (*c-MYC*), which are known to bind to PAF1c subunits (Yu *et al.*, 2015; Zeng and Xu, 2015). Data were normalized to *18S*, and gene expression was determined by calculating fold change compared to negative IgG control. The input control contained sheared chromatin before immunoprecipitation to remove background binding during subsequent bioinformatic analyses. As expected, the input control demonstrated the highest fold change for both positive control genes, while three Ishikawa cell replicates showed an approximately 3-fold increase in expression (Fig. 4B). Downstream WDR61 target genes were examined

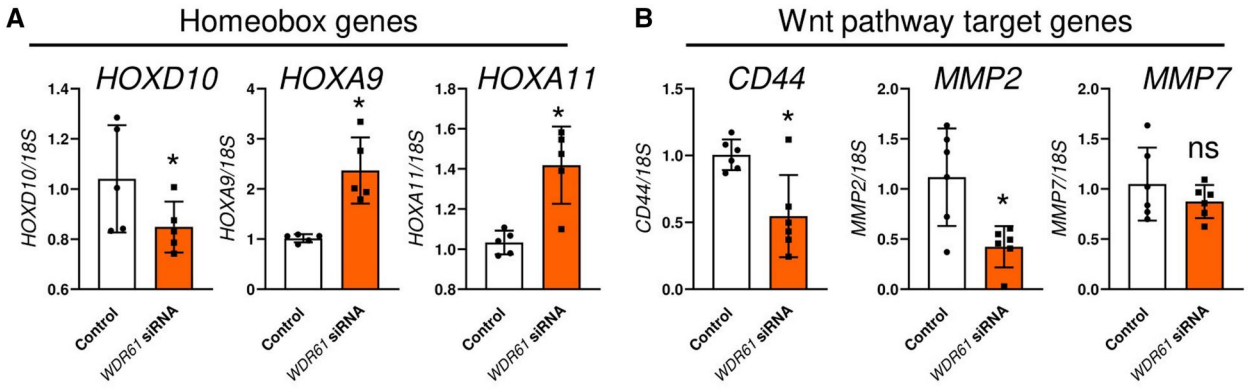
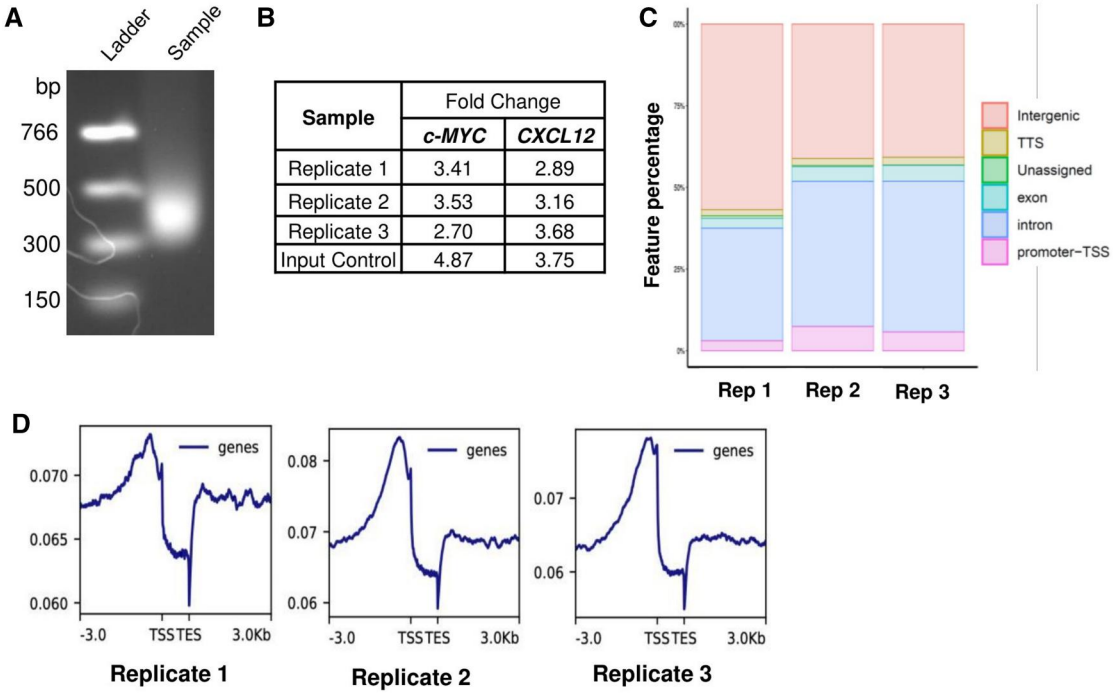


Figure 3. qPCR analysis of Homeobox genes and Wnt pathway target genes following *WDR61* knockdown in Ishikawa cells. Levels of gene expression were normalized to *18S* (n = 5). Data are presented as mean ± SD. *$P < 0.05$, ns, not significant.



Sample	Fold Change	
	c-MYC	*CXCL12*
Replicate 1	3.41	2.89
Replicate 2	3.53	3.16
Replicate 3	2.70	3.68
Input Control	4.87	3.75

Figure 4. ChIP-Seq analysis to determine genome-wide binding of WDR61 in Ishikawa cells. (**A**) Gel electrophoresis of sonicated Ishikawa cells following the protocol outlined in methods. DNA fragments were dominantly between 300 and 500 bp which was adequate to continue immunoprecipitation. (**B**) Immunoprecipitation was coupled with qPCR using primers *c-MYC* and *CXCL12* as positive controls for WDR61 binding. Gene expression was normalized to *18S* and fold change was calculated compared to negative IgG control. Ishikawa cell replicates demonstrated enrichment of positive controls compared to negative control, thus the samples were used for Illumina sequencing. (**C**) Distribution of genomic regions that WDR61 bound to in Ishikawa cells (n = 3) were evaluated relative to the total gene body using fastQC. Approximately half of the peaks were intergenic, followed by intronic, promoter, exonic, and TTS regions. (**D**) Heatmap of genome wide occupancy of WDR61 in three Ishikawa cell ChIP-Seq replicates measured as reads per million mapped reads (RPM) per bp. TSS, transcription start site; TES, transcription end site; TTS, transcription termination site.

using ChIP-seq (Illumina NextSeq 2000) to investigate WDR61 activity in Ishikawa cells.

Narrow peak calling was performed bioinformatically and genes were annotated using the GRCh38 reference genome. Each peak represents enrichment of a particular sequence of the reference genome indicating WDR61 binding to the gene region. Feature characterization of the binding sites among three replicates revealed major binding to intergenic and intronic regions (Fig. 4C). Less than 10% of peaks occurred in promoter regions (Fig. 4C). This is reflected in the number of reads per million mapped reads, indicating that the majority of WDR61 binding occurred up to 3 kb before the transcription start site, followed by up to 3 kb after the transcription end site (Fig. 4D).

For each sample, peaks in intergenic and unassigned regions were not used for downstream analysis. The replicates were then collated together, identifying a total of 2022 unique genes, with 1827 of these existing in the human protein database DAVID. To determine how these WDR61 target genes are associated with endometrial receptivity, we strictly narrowed our analysis down to the promoter regions (Supplementary Table S2). The reduction of two genes, *CTNNA1* and *PDLIM2*, chosen randomly after *WDR61* knockdown was confirmed by qPCR (Fig. 5A). DAVID was used to examine enriched KEGG pathways in these genes with WDR61 targeting their promoter region (Fig. 5B). The six most enriched pathways involved in endometrial receptivity were: Wnt signaling, adherens junctions, extracellular matrix (ECM)-receptor

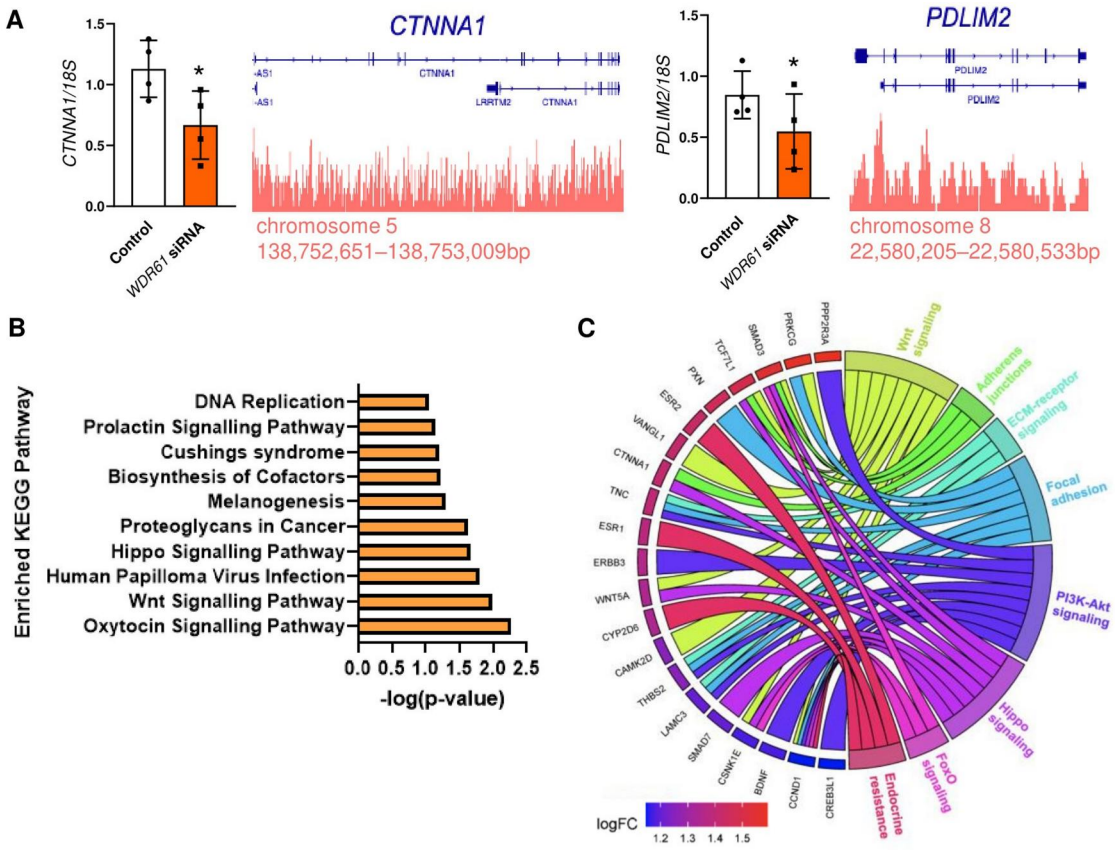


Figure 5. WDR61 target gene pathway enrichment analysis. (**A**) Ishikawa cells treated with scramble control or *WDR61* siRNA (n = 4) were analyzed by qPCR to confirm ChIP-seq data. Data are presented as mean ± SD. WDR61 was found to bind to promoter regions of *CTNNA1* and *PDLIM2*. Both *CTNNA1* and *PDLIM2* exhibited significantly downregulated expression following *WDR61* siRNA treatment (*$P < 0.05$). Visualization of WDR61 binding regions on *CTNNA1* and *PDLIM2* gene using Interactive Genomic Viewer. Binding peaks were calculated as significantly enriched binding of WDR61 compared to input control. (**B**) Top enriched KEGG pathways (adjusted *P*-value <0.05) for genes (Supplementary Table S2) identified to be targets for WDR61 binding at their promotor region. (**C**) Chord plot with identified pathways and representative genes involved in endometrial receptivity and implantation following the literature search. Genes listed in these pathways were identified as promoter binding sites in ChIP-seq data.

signaling, focal adhesion, PI3K-Akt, and MAPK signaling (Hayashi *et al.*, 2009;Whitby *et al.*, 2018; Lou *et al.*, 2023) (Fig. 5C).

To represent the proposed mechanisms whereby WDR61 promotes endometrial epithelial cell adhesion, findings from qPCR, ChIP-seq data, and DAVID analyses were integrated to generate Fig. 6. It is clear from KEGG pathway analysis that single genes are involved in multiple pathways, and these pathways have overlapping functions in the endometrial epithelium, particularly in adhesion and epithelial–mesenchymal transition (EMT). The stars indicate a possible role of WDR61 from target gene binding in ChIP-seq, such as *HOXA10*, *ERBB3* and several integrins. Alternatively, stars along pathway arrows indicate that WDR61 targeted several genes involved in these pathways such as Wnt, MAPK, and PI3K–Akt signaling. *CD44* and *CXCR4* were downregulated in Ishikawa cells following WDR61 knockdown; however, they were not identified in ChIP-seq data suggesting that WDR61 may indirectly regulate these genes, as indicated in the schematic. Overall, these data suggests WDR61 regulates endometrial receptivity by modulating adhesion, proliferation or the EMT in Ishikawa cells.

Discussion

This is the first study to explore the function of WDR61 in the human endometrium. WDR61 was significantly elevated in mid-secretory phase endometrial tissue compared with the proliferative phase in fertile women. We found that loss of WDR61 significantly impaired the adhesive capacity of both primary HEECs and Ishikawa cells and reduced Ishikawa cell proliferation, likely by driving cell cycle arrest and senescence. WDR61 knockdown in Ishikawa cells altered Hox genes and Wnt pathway target genes. ChIP-seq analysis revealed that WDR61 had enriched binding to transcription start sites of genes involved in focal adhesion, intracellular signaling and cytoskeletal structure in Ishikawa cells, indicating its transcriptional activity and potential mechanisms of action. Overall this study highlights the importance of WDR61 in promoting endometrial epithelial cell receptivity.

We demonstrated that in the endometrium, WDR61 immunostaining was elevated in the secretory progesterone dominated phase compared to the estrogen dominant proliferative phase. Recent bulk RNA sequencing (Fitzgerald *et al.*, 2019) has shown that in human endometrial organoids, estrogen reduces WDR61 expression compared to untreated controls. In contrast, the combination of estrogen, progesterone, and cyclic adenosine monophosphate, which mimics the receptive phase, increases WDR61 expression. This suggests that WDR61 is regulated by these steroid hormones. In our study, the localization of WDR61 and its increased intensity in the mid-secretory phase endometrium compared to the proliferative phase suggests it may facilitate the establishment of endometrial receptivity. In luminal epithelial cells where embryos initially adhere, we recorded an intense

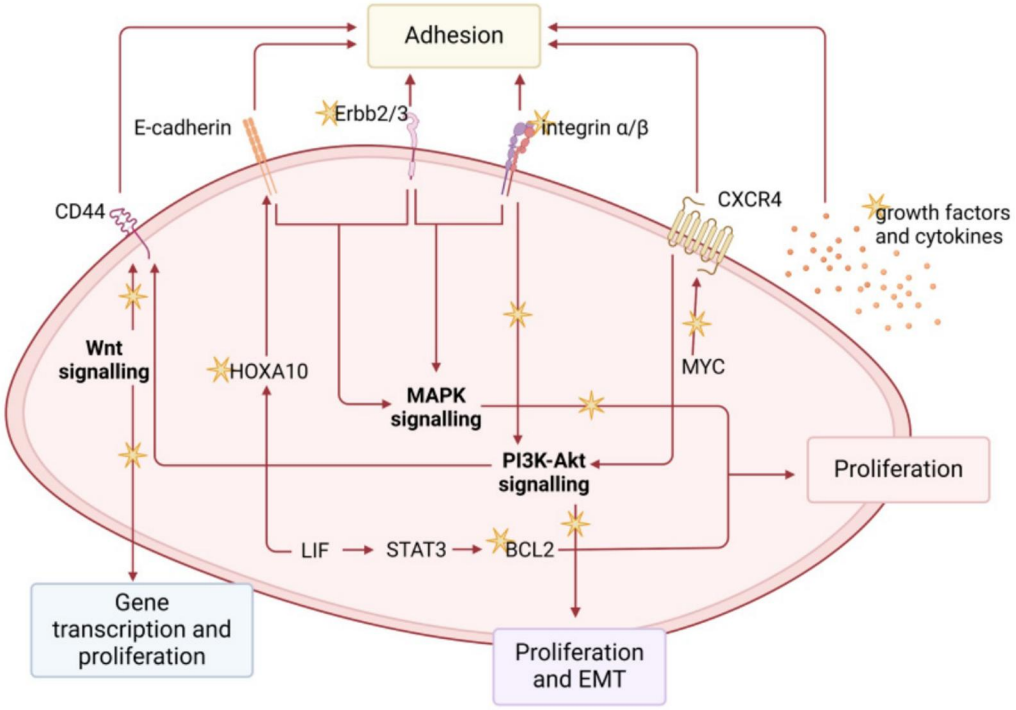


Figure 6. Pathways enriched following functional analyses of WDR61 target genes in Ishikawa cells, and their role in endometrial epithelial cell adhesion. Schematic of an endometrial epithelial cell and the identified receptivity pathways enriched in WDR61 ChIP-seq targets. Wingless (Wnt), mitogen-associated protein kinase (MAPK), phosphoinositide 3-OH kinase (PI3K)/protein kinase (Akt) have complex, interrelated roles in proliferation, adhesion molecule expression, and the epithelial mesenchymal transition in preparation for embryo implantation. Stars indicate WDR61 involvement in individual gene expression or multiple genes involved in a pathway extrapolated from ChIP-seq and qPCR data.

staining of WDR61 in the nuclei. WDR61 likely influenced luminal epithelial cell adhesion by regulating gene transcription of adhesion molecules. Consequently, loss of *WDR61* impaired endometrial epithelial cell adhesion. The finding that loss of WDR61 decreased Ishikawa cell proliferation contrasts with the well-known observation that endometrial epithelial cells typically cease to proliferate during the receptive phase. This discrepancy is likely attributable to the fact that Ishikawa cells are derived from endometrial adenocarcinoma. In cancer contexts, loss of WDR61 generally leads to reduced proliferation, as seen in breast cancer cells, where WDR61 knockdown decreases proliferation both *in vitro* and *in vivo* (Hou *et al.*, 2024).

The number of ciliated endometrial epithelial cells peaks during the proliferative phase and declines as progesterone levels increase during the secretory phase (Ferenczy *et al.*, 1972; Schueller, 1968). The Wnt pathway is known to play a role in cilia biogenesis in epithelial cells (Seidl *et al.*, 2023). While WDR61 typically regulates the Wnt pathway, this study identified WDR61-negative ciliated epithelial cells in the glands in the proliferative phase, suggesting that non-WDR61 mediated mechanisms drive the Wnt pathway's involvement in ciliation in these cells.

We aimed to identify the complete set of genes directly regulated by WDR61 in Ishikawa cells using ChIP-seq analysis. WDR61 is one of the six subunits that form the PAF1c complex, which regulates gene transcription (Jaehning, 2010). If the levels of WDR61 alone are altered in the PAF1c complex, it can lead to significant disruptions in transcription regulation. Previous studies have used ChIP-seq for other PAF1c subunits (Strikoudis *et al.*, 2016); however, they exhibited additional roles independent of the complex, and cannot be confidently extrapolated to the functions of WDR61 in the human endometrial epithelium. Our WDR61 ChIP-seq data demonstrated enriched binding to the transcription start site of *HOXA10* and *HOXA1* in Ishikawa cells, illustrating a direct relationship between WDR61 activity and Hox gene regulation. This is consistent with previous findings that PAF1c, the complex in which WDR61 functions in humans, is a known regulator of *Hox* genes (Zhu *et al.*, 2005). *Hox* genes are essential for endometrial receptivity (Xu *et al.*, 2014; Jiang *et al.*, 2017). Targeted mutation of *Hoxa10* in mice lead to uterine factor infertility (Satokata *et al.*, 1995). Our ChIP-seq analysis did not detect a large number of Hox genes. We demonstrated an increase in *HOXA9* and *HOXA11* by qPCR after *WDR61* knockdown in Ishikawa cells. This suggests that there are Hox genes that can be indirectly regulated by WDR61 in Ishikawa cells.

Our ChIP-seq also showed enriched binding of WDR61 to several canonical and non-canonical *Wnt* genes, *WNT11*, *WNT7A*, *WNT5A*, *WNT7B*, and *WNT8B*. Additionally, various Wnt-dependent transcription factors were bound by WDR61, identifying a function in Wnt signaling in receptive endometrial epithelial cells. Wnt signaling is essential for embryo implantation (Chen *et al.*, 2009a). In mice, activation of Wnt/b-catenin signaling is specific to the luminal epithelium where embryos implant (Mohamed *et al.*, 2005). Absence of Wnt/b-catenin signaling significantly reduces embryo implantation, outlining that it is necessary for successful initiation of pregnancy (Mohamed *et al.*, 2005). In addition, non-canonical Wnt ligands Wnt7b and Wnt11 are abundantly expressed in luminal epithelium in the receptive window in mice (Hayashi *et al.*, 2009b). Wnt7b showed acute expression at implantation sites, while Wnt11 remained present in the luminal epithelium but absent adjacent to implantation sites (Hayashi *et al.*, 2009b). Wnt11 also regulated spheroid adhesion in Ishikawa cells; however, the pathway it modulates is unclear, while Wnt7b is believed to be involved in trophoblast development, explaining its expression at the site of implantation

(Fritz *et al.*, 2014; Elbaz *et al.*, 2018). These Wnt ligands are all targets for WDR61 binding in our ChIP-seq data. Therefore, WDR61 may be crucial for modulating the expression of Wnt genes during receptivity.

Our ChIP-seq data demonstrated that WDR61 targeted PI3K signaling related genes, *PPP2R3A*, *TNC*, *ERBB3*, *THBS2*, *LAMC3*, *BDNF*, *CREB3L1*, and *AKT1*. In mouse uterine luminal epithelium, PI3K and Akt levels are significantly higher at implantation sites compared to inter-implantation sites (Liu *et al.*, 2014). Conversely, inhibition of PI3K–Akt signaling significantly reduces the number of implantation sites (Liu *et al.*, 2014). In human endometrium, PI3K signaling is essential for the preparation of receptivity (Liu *et al.*, 2018). Among genes we identified in ChIP-seq, the expression of *TNC1* and *AKT1* is significantly lower in the endometrium of women with recurrent implantation failure compared to a fertile group (Albayrak *et al.*, 2021; Lai *et al.*, 2023). Similarly, BDNF has been detected in uterine flushings and its level is significantly lower in the recurrent implantation failure group (Ali *et al.*, 2009). As a transcriptional regulator, the reduction of WDR61 may be responsible for the low expression levels of these receptivity essential genes identified in women with recurrent implantation failure.

In our ChIP-seq data, the majority of WDR61 binding occurred within 3 kb upstream of the transcription start site, a region commonly involved in the activation of gene transcription. However, binding to this region does not necessarily activate gene transcription. WDR61 has other various functions, including monitoring genome integrity and flagging damaged DNA for repair (Tomson and Arndt, 2013). Therefore, additional experiments are required to extrapolate our ChIP-seq data and validate genes activated by WDR61.

WDR61 is present in the secretory phase (Day 16–21) human endometrial fluid (Azkargorta *et al.*, 2020). Our previous study also identified WDR61 in the apical secretions of endometrial organoids, with significantly lower levels observed in organoids derived from primary infertile women (Zhou *et al.*, 2022). This suggests that WDR61 could be released from the endometrial glands and/or luminal epithelial cells into the uterine lumen. In agreement, in the present study, we demonstrated that WDR61 immunostaining was present in the apical surface in endometrial glandular epithelial cells in the mid-secretory phase, suggesting it is released into the uterine cavity. In support, on the Human Protein Atlas, WDR61 is detected in the apical region of the endometrial glands in a 34-year-old human, with large, coiled glandular morphology suggesting the secretory phase. While it remains to be explored whether WDR61 is released by endometrial glands via microparticles, it is known that endometrial epithelial cells release exosomes, which are taken up by embryos (Liu *et al.*, 2020). Therefore, it is possible that WDR61, released by glands via exosomes, could act on embryos and/or endometrial luminal epithelial cells, potentially altering adhesion and impacting implantation.

In summary, our study sheds light on the role of WDR61 in promoting human endometrial receptivity, with its expression being maximal in the receptive phase. We demonstrated that *WDR61* knockdown in endometrial epithelial cells reduced adhesion of HTPC spheroids, suggesting that WDR61 regulates endometrial epithelial cell adhesion enabling embryo attachment. Our data suggests that WDR61 acts by regulating key pathways including ECM-receptor signaling, focal adhesion, PI3K–Akt and MAPK signaling, in addition to Hox and Wnt genes to alter the adhesive capacity and receptivity. These findings highlight that WDR61 has a diverse and critical role in endometrial receptivity and suggest potential therapeutic avenues for improving receptivity and fertility outcomes.

Supplementary data

Supplementary data are available at *Molecular Human Reproduction* online.

Data availability

The ChIP-seq data were uploaded to Zenodo with the DOI: https://doi.org/10.5281/zenodo.14053265.

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Authors' roles

PD conceived and designed the ChIP seq study and conducted the experiments. PD, WZ, EM, and ED drafted the manuscript. MH, EM, and LLS assisted with the immunohistochemistry and scoring. MS contributed to primary endometrial epithelial cell isolation. ED and WZ conceived and designed the study overall and contributed to data interpretation and analysis. WT and TL identified and curated the human sample collections. ED supervised the entire study. All authors approved the submitted version.

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Conflict of interest

This study was completed in the absence of any commercial or financial relationships. The authors have no conflict of interest to declare.

References

Albayrak İG, Azhari F, Çolak EN, Balcı BK, Ülgen E, Sezerman U, Baştu E, Günel T. Endometrial gene expression profiling of recurrent implantation failure after in vitro fertilization. *Mol Biol Rep* 2021;**48**:5075–5082.

Ali AFM, Cosemi E, Kamel S, Mohammed S, Elhefnawy M, Farid L, Shaker S. ZAMZAM water stimulate BRAIN derived neurotrophic factor (BDNF) in uterine flushing of repeated implantation failure after (intracytoplasmic SPERUM injection)(ICSI). In: *Thirteenth International Water Technology Conference*. Citeseer, 2009. pp. 1549–1556.

Azkargorta M, Bregón-Villahoz M, Escobes I, Ibáñez-Pérez J, Iloro I, Iglesias M, Diez-Zapirain M, Rabanal A, Prieto B, Moragues M-D et al In-depth proteomics and natural peptidomics analyses

reveal antibacterial peptides in human endometrial fluid. *J Proteomics* 2020;**216**:103652.

Castelbaum AJ, Ying L, Somkuti SG, Sun J, Ilesanmi AO, Lessey BA. Characterization of integrin expression in a well differentiated endometrial adenocarcinoma cell line (Ishikawa). *J Clin Endocrinol Metab* 1997;**82**:136–142.

Chen L, Nakai M, Belton RJ, Nowak RA. Expression of extracellular matrix metalloproteinase inducer and matrix metalloproteinases during mouse embryonic development. *Reproduction* 2007; **133**:405–414.

Chen Q, Ni Y, Han M, Zhou W-J, Zhu X-B, Zhang A-J. Integrin-linked kinase improves uterine receptivity formation by activating Wnt/β-catenin signaling and up-regulating MMP-3/9 expression. *Am J Transl Res* 2020;**12**:3011–3022.

Chen Q, Zhang Y, Lu J, Wang Q, Wang S, Cao Y, Wang H, Duan E. Embryo–uterine cross-talk during implantation: the role of Wnt signaling. *Mol Hum Reprod* 2009a;**15**:215–221.

Chen Y, Yamaguchi Y, Tsugeno Y, Yamamoto J, Yamada T, Nakamura M, Hisatake K, Handa H. DSIF, the Paf1 complex, and Tat-SF1 have nonredundant, cooperative roles in RNA polymerase II elongation. *Genes Dev* 2009b;**23**:2765–2777.

Coughlan C, Ledger W, Wang Q, Liu F, Demirol A, Gurgan T, Cutting R, Ong K, Sallam H, Li T. Recurrent implantation failure: definition and management. *Reprod Biomed Online* 2014;**28**:14–38.

Crisucci EM, Arndt KM. The roles of the Paf1 complex and associated histone modifications in regulating gene expression. *Genet Res Int* 2011;**2011**:707641.

Dimitriadis E, Nie G, Hannan NJ, Paiva P, Salamonsen LA. Local regulation of implantation at the human fetal-maternal interface. *Int J Dev Biol* 2010;**54**:313–322.

Elbaz M, Hadas R, Bilezikjian LM, Gershon E. Uterine Foxl2 regulates the adherence of the Trophectoderm cells to the endometrial epithelium. *Reprod Biol Endocrinol* 2018;**16**:12.

Ferenczy A, Richart RM, Agate FJ Jr, Purkerson ML, Dempsey EW. Scanning electron microscopy of the human endometrial surface epithelium. *Fertil Steril* 1972;**23**:515–521.

Fitzgerald HC, Dhakal P, Behura SK, Schust DJ, Spencer TE. Self-renewing endometrial epithelial organoids of the human uterus. *Proc Natl Acad Sci USA* 2019;**116**:23132–23142.

Fritz R, Jain C, Armant DR. Cell signaling in trophoblast-uterine communication. *Int J Dev Biol* 2014;**58**:261–271.

Hayashi K, Erikson DW, Tilford SA, Bany BM, Maclean JA, Rucker IEB, Johnson GA, Spencer TE. Wnt genes in the mouse uterus: potential regulation of implantation. *Biol Reprod* 2009;**80**:989–1000.

Hou Y, Zhang C, Liu L, Yu Y, Shi LEI, Qin YAN. WDR61 ablation triggers R-loop accumulation and suppresses breast cancer progression. *FEBS J* 2024;**291**:3417–3431.

Ifenatuoha C, Okewale B. Zooming in on the endometrial factor of recurrent implantation failure. *Hum Fertil (Camb)* 2022; **25**:848–859.

Jaehning JA. The Paf1 complex: platform or player in RNA polymerase II transcription? *Biochim Biophys Acta* 2010;**1799**:379–388.

Jiang R, Ding L, Zhou J, Huang C, Zhang Q, Jiang Y, Liu J, Yan Q, Zhen X, Sun J *et al.* Enhanced HOXA10 sumoylation inhibits embryo implantation in women with recurrent implantation failure. *Cell Death Discov* 2017;**3**:17057–17058.

Lai Z-Z, Zhang J, Zhou W-J, Shi J-W, Yang H-L, Yang S-L, Wu J-N, Xie F, Zhang T, Li M-Q. Identification of potential biomarkers and immune infiltration characteristics in recurrent implantation failure using bioinformatics analysis. *Front Immunol* 2023;**14**:992765.

Liu C, Yao W, Yao J, Li L, Yang L, Zhang H, Sui C. Endometrial extracellular vesicles from women with recurrent implantation failure attenuate the growth and invasion of embryos. *Fertil Steril* 2020; **114**:416–425.

Liu L, Wang Y, Yu Q. The PI3K/Akt signaling pathway exerts effects on the implantation of mouse embryos by regulating the expression of RhoA. *Int J Mol Med* 2014;**33**:1089–1096.

Liu X, Zhang L, Liu Y, Cui J, Che S, An X, Song Y, Cao B. Circ-8073 regulates CEP55 by sponging miR-449a to promote caprine endometrial epithelial cells proliferation via the PI3K/AKT/mTOR pathway. *Biochim Biophys Acta Mol Cell Res* 2018;**1865**:1130–1147.

Lou Y, Pinel L, Dufort D. Uterine WNTS modulates fibronectin binding activity required for blastocyst attachment through the WNT/CA2+ signaling pathway in mice. *Reprod Biol Endocrinol* 2023;**21**:85.

Mascarenhas MN, Flaxman SR, Boerma T, Vanderpoel S, Stevens GA. National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys. *PLoS Med* 2012;**9**:e1001356.

Mohamed OA, Jonnaert M, Labelle-Dumais C, Kuroda K, Clarke HJ, Dufort D. Uterine Wnt/β-catenin signaling is required for implantation. *Proc Natl Acad Sci USA* 2005;**102**:8579–8584.

Mosimann C, Hausmann G, Basler K. Parafibromin/Hyrax activates Wnt/Wg target gene transcription by direct association with β-catenin/Armadillo. *Cell* 2006;**125**:327–341.

Satokata I, Benson G, Maas R. Sexually dimorphic sterility phenotypes in Hoxa lO-deficient mice. *Nature* 1995;**374**:460–463.

Schueller EF. Ciliated epithelia of the human uterine mucosa. *Obstet Gynecol* 1968;**31**:215–223.

Seidl C, Da Silva F, Zhang K, Wohlgemuth K, Omran H, Niehrs C. Mucociliary Wnt signaling promotes cilia biogenesis and beating. *Nat Commun* 2023;**14**:1259.

Strikoudis A, Lazaris C, Trimarchi T, Galvao Neto AL, Yang Y, Ntziachristos P, Rothbart S, Buckley S, Dolgalev I, Stadtfeld M *et al.* Regulation of transcriptional elongation in pluripotency and cell differentiation by the PHD-finger protein Phf5a. *Nat Cell Biol* 2016;**18**:1127–1138.

Taylor HS. The role of HOX genes in human implantation. *Hum Reprod Update* 2000;**6**:75–79.

Tomson BN, Arndt KM. The many roles of the conserved eukaryotic Paf1 complex in regulating transcription, histone modifications, and disease states. *Biochim Biophys Acta* 2013;**1829**:116–126.

Tulac S, Nayak NR, Kao LC, Van Waes M, Huang J, Lobo S, Germeyer A, Lessey BA, Taylor RN, Suchanek E *et al.* Identification, characterization, and regulation of the canonical Wnt signaling pathway in human endometrium. *J Clin Endocrinol Metab* 2003; **88**:3860–3866.

Van Oss SB, Cucinotta CE, Arndt KM. Emerging insights into the roles of the Paf1 complex in gene regulation. *Trends Biochem Sci* 2017; **42**:788–798.

Wang W, Vilella F, Alama P, Moreno I, Mignardi M, Isakova A, Pan W, Simon C, Quake SR. Single-cell transcriptomic atlas of the human endometrium during the menstrual cycle. *Nat Med* 2020; **26**:1644–1653.

Whitby S, Salamonsen LA, Evans J. The endometrial polarity paradox: differential regulation of polarity within secretory-phase human endometrium. *Endocrinology* 2018;**159**:506–518.

World Health Organization. *Infertility Prevalence Estimates: 1990–2021*. Geneva, Switzerland: World Health Organization, 2023.

Xu B, Geerts D, Bu Z, Ai J, Jin L, Li Y, Zhang H, Zhu G. Regulation of endometrial receptivity by the highly expressed HOXA9, HOXA11 and HOXD10 HOX-class homeobox genes. *Hum Reprod* 2014; **29**:781–790.

Yu M, Yang W, Ni T, Tang Z, Nakadai T, Zhu J, Roeder RG. RNA polymerase II–associated factor 1 regulates the release and phosphorylation of paused RNA polymerase II. *Science* 2015;**350**:1383–1386.

Zdravkovic T, Nazor KL, Larocque N, Gormley M, Donne M, Hunkapillar N, Giritharan G, Bernstein HS, Wei G, Hebrok M *et al.* Human stem cells from single blastomeres reveal pathways of embryonic or trophoblast fate specification. *Development* 2015; **142**:4010–4025.

Zeng H, Xu W. Ctr9, a key subunit of PAFc, affects global estrogen signaling and drives ERα-positive breast tumorigenesis. *Genes Dev* 2015;**29**:2153–2167.

Zhou W, Barton S, Cui J, Santos LL, Yang G, Stern C, Kieu V, Teh WT, Ang C, Lucky T *et al.* Infertile human endometrial organoid apical protein secretions are dysregulated and impair trophoblast progenitor cell adhesion. *Front Endocrinol* 2022; **13**:3276.

Zhou W, Menkhorst E, Dimitriadis E. Jagged1 regulates endometrial receptivity in both humans and mice. *Faseb J* 2021;**35**:e21784.

Zhu B, Zheng Y, Pham A-D, Mandal SS, Erdjument-Bromage H, Tempst P, Reinberg D. Monoubiquitination of human histone H2B: the factors involved and their roles in HOX gene regulation. *Mol Cell* 2005;**20**:601–611.

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