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HOXA10 co-factor MEIS1 is required for the decidualization in human endometrial stromal cell

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Abstract

Decidualization is a critical process for embryo implantation and pregnancy maintenance in humans. The homeobox gene HOXA10 has been widely studied in endometrial receptivity establishment and decidualization. MEIS1, a three-amino-acid loop extension (TALE) family homeobox gene, has been proven to be a co-factor for HOXA10 in mouse uterus. However, the interaction between MEIS1 and HOXA10 in the human decidual cells remains to be elucidated. siRNA and CRISPR-Cas9 were employed to knockdown and knockout *MEIS1* in the cultured human endometrial stromal cells, and it was found that MEIS1 deficiency leads to impaired decidualization. The physical interaction between the MEIS1 and HOXA10 in human endometrial stromal cell was confirmed by immunoprecipitation. Moreover, *KAT2B* and *ETA* were proved to be downregulated in the absence of *MEIS1*, and luciferase reporter and ChIP assays demonstrated that MEIS1-HOXA10 complex binds to the promoters of *KAT2B* and *ETA* and regulates their activity. Overexpression of *KAT2B* and *ETA* can partially rescue the decidualization defects in *MEIS1*-knockout HESCs. Taken together, these data suggest that MEIS1 plays an indispensable role in decidualization in human endometrial stromal cells, and MEIS1 interacts with HOXA10 to regulate the downstream genes, such as *KAT2B* and *ETA*. These findings will contribute to our understanding about the regulatory network in the process of decidualization in humans.

Key Words

- ▶ decidualization
- ▶ decidual stromal cell
- ▶ MEIS1
- ▶ HOXA10

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Introduction

Decidualization is an indispensable process for successful pregnancy in both rodents and humans, which indicates the transformation of endometrial stromal fibroblasts into specialized secretory decidual cells (Gellersen & Brosens 2014). The decidualization process is regulated by endocrine, paracrine and autocrine factors, such as

estrogen, progesterone, prostaglandin and interleukins (Johansson & Wide 1969, Gravanis et al. 1999, Palejwala et al. 2002, Ivell et al. 2003, Strakova et al. 2005, Geisert et al. 2012), and multiple transcriptional factors, such as FOXO1 (Christian et al. 2002, Sekiya et al. 2004), C/EBPβ (Mantena et al. 2006, Wang et al. 2010) and PLZF

(Fahnenstich *et al.* 2003, Suliman *et al.* 2012). Although there is some understanding of decidualization, it has not been fully elucidated in regarding the regulatory network contributing to differentiation of uterine stromal cells into decidual cells.

Myeloid ecotropic virus insertion site 1 (*Meis1*) was first described in 1995 as a common viral integration site in the BXH-2 murine model of myeloid leukemogenesis (Moskow *et al.* 1995). *Meis1* contributes to endometrial development during the menstrual cycle and is necessary for implantation in mice (Xu *et al.* 2008). In humans, MEIS1 and HOXA10 proteins are both expressed in the endometrium (Sarno *et al.* 2005). However, it is unknown how MEIS1 works in the decidualization and whether it interacts with HOXA10 to target the same genes.

The *HOX* genes are developmental genes that cluster in 4 genomic loci. In the *HOX* family, HOXA10 is expressed both in the human and mouse uterus. *Hoxa10*-deficient female mice are sterile (Hsieh-Li *et al.* 1995, Satokata *et al.* 1995), due to implantation failure and a deficient decidual reaction (Gendron *et al.* 1997, Lim *et al.* 1999). In the human endometrium, decidualizing stromal cells show elevated HOXA10 levels and it demonstrates that HOXA10 relates to decidualization. However, the regulatory function of MEIS1 in the process of decidualization of endometrial stromal cells and whether it interacts with HOXA10 to regulate the downstream target genes still need further study.

In the present study, we demonstrate that the expression level of MEIS1 is gradually increased during the process of decidualization, and knockdown or knockout of *MEIS1* leads to impaired decidualization in the human endometrial stromal cells. MEIS1 and HOXA10 interact to regulate the target genes *ETA* and *KAT2B*, which play important roles in the decidualization process. These findings add a new line of evidence supporting the fact that the interaction between MEIS1 and HOXA10 is required for human endometrial stromal decidualization.

Materials and methods

Isolation of primary human endometrial stromal cells and cell culture

For culture of primary human endometrial stromal cells (pHESCs), histologically normal endometrial tissue samples from the proliferative phase were collected by biopsy from five subjects. The subjects were not on hormone therapy at the time of procedure. All of them have given informed consent. Samples were collected at room temperature and transported in iced HBSS containing 50 µg/mL Penicillin

Streptomycin (Gibco) and processed for the endometrial stromal cell isolation. Tissue samples were washed with DMEM-F12 (Sigma) containing 50 µg/mL Penicillin Streptomycin (Gibco) and minced to 1 mm³. Tissues were incubated in fresh DMEM-F12 medium (3 mL) containing 2.5 mg/mL collagenase (Sigma) for 90 min at 37°C. After enzymatic digestion, stromal cells were separated from epithelial aggregates by passing them through a 40-µm nylon cell strainer (BD Biosciences). Cells were plated in 60-mm dishes, containing DMEM-F12 medium with 10% charcoal-stripped fetal bovine serum (CS-FBS) (Biological Industries) and 50 µg/mL Penicillin Streptomycin (Gibco).

Cell culture and *in vitro* decidualization

The immortalized human endometrial stromal cells (HESCs) were purchased from the American Type Culture Collection (ATCC® CRL-4003TM) and cultured according to previous report (Krikun *et al.* 2004). HESCs were cultured in phenol red-free DMEM/F-12 (Sigma) containing 10% (vol/vol) charcoal-stripped fetal bovine serum (CS-FBS) (Biological Industries), 3.1 g/L glucose (Sigma) and 1 mM Sodium Pyruvate (Sigma), supplemented with 1.5 g/L sodium bicarbonate (Sigma), 50 µg/mL Penicillin Streptomycin (Gibco), 1% Insulin-Transferrin-Selenium (ITS) (Gibco), and 500 ng/mL puromycin (Sigma).

To induce stroma cell decidualization *in vitro*, the cells were treated with medium containing 2% CS-FBS, 1 µM Medroxy progesterone 17-acetate (MPA, Sigma), and 0.5 mM dibutyryl cAMP (db-cAMP, Sigma) as previously described (Liao *et al.* 2015). The media was changed every 48 h, and the cultures were maintained for up to 8 days.

Small interfering RNA transfection

MEIS1 siRNA were purchased from Ribo. Primary human endometrium stromal cells were transfected using a Lipofectamine™ RNAiMAX Transfection Reagent (Invitrogen). pHESCs were plated at approximately 5 × 10⁴ cells in six-well plates. Before transfecting, the culture medium of pHESCs was changed to serum-free Opti-MEM™ Medium. At 80% confluence, siRNA (25 pmol/µL) and RNAi MAX (2.5 µL/well) were diluted in Opti-MEM and transfected to pHESCs. The medium was changed 6 h later, and cells were subjected to a change of differential medium.

Deletion of MEIS1 by CRISPR/Cas9 system

To generate *MEIS1*-knockout immortalized human endometrial stromal cell, the sgRNA

(AGAGAGGCCGATGGCGCAAA) was designed according to the CRISPR design tool (<https://zlab.bio/guide-design-resources>). The HESCs cells were first infected with the *Cas9* lentivirus targeting *MEIS1*, and then single cells with *GFP* expression, which indicated the successful infection, were sorted by FACS, and plated into the 96-well plate. After the clone expansion from the single cell, the genome DNA was extracted. The *Cas9* cutting site was amplified by PCR and then ligated into the T vector for Sanger sequence. The absence of *MEIS1* was identified by DNA sequencing and western blot detection.

RNA extraction and quantitative polymerase chain reaction (q-PCR)

Total RNA was extracted from cultured pHESCs by using TRIzol RNA purification kit (Invitrogen) following the manufacturer's protocol. RT was performed with the Takara PrimeScript RT reagent kit using 1 µg of total RNA. The cDNA was sent for quantitative PCR reactions, which were performed using the ABI Q5 Real Time PCR System (Applied Biosystems) and the amplifications were conducted using the SYBR Green PCR Master Mix (Takara). All primers for the real-time PCR were listed in Table 1. The experiments were carried out in duplicate for each data point. The mRNA expression level of target gene was displayed as relative value to the treatment controls.

The value was calculated as $2^{-\Delta\Delta C_t}$ ($\Delta C_t = C_{t_Target\ gene} - C_{t_GAPDH}$, $\Delta\Delta C_t = \Delta C_t(test) - \Delta C_t(calibrator)$).

Western blotting

To extract whole-cell protein, cells were washed twice with PBS and lysed on ice in lysis buffer containing a cocktail of protease inhibitors (Roche). Solid cellular debris was removed by centrifugation at 13,800 g for 30 min and supernatant was collected. Protein concentration was measured by BCA methods. The protein samples (40 µg each) were subjected to 12% SDS-PAGE and transferred onto PVDF using a Bio-Rad apparatus. The membrane was blocked in 5% skimmed milk (BD, 232100) in PBS containing 0.05% Tween-20. Primary antibody was applied (*MEIS1* (ab19867, Abcam) at 1:1000, *IGFBP-1* (Abcam) at 1:500 and *GAPDH* (Abmart) at 1:1000) in 2% milk PBST at 4°C overnight. Bands were visualized using Thermo Supersignal West Pico Chemiluminescent substrate according to the manufacturer's instructions.

Immunofluorescence

pHESCs were cultured on glass chamber slides and treated with or without differential medium for 4 days. After *in vitro*-induced decidualization, pHESCs were fixed in methanol for 5 min, permeabilized with 0.1% Triton X-100 in PBS for 30 min and blocked in 0.5% BSA in

Table 1 Primers names and sequences.

Experiment	Primer names and sequences (5'–3')	
	Sense	Anti-sense
Real-time PCR		
<i>MEIS1</i>	CACGGGACTCACCATCCTTC	CCACTCATAGGTCCTGGTGC
<i>PRL</i>	CTACATCCATAACCTCTCCTCAG	GGGCTTGCTCCTTGCTTC
<i>IGFBP-1</i>	AGAGTCGTAGAGAGTTTAGC	ACACTGTCTGCTGTGATAA
<i>GAPDH</i>	ATTGGCTACAGCAACAGG	TTGAGCACAGGGTACTTTATT
<i>ETA</i>	CCTACCCAGCAATGGCTCAA	CCCACCATTCCCACGATGAA
<i>KAT2B</i>	AAAGAGCCCAGAGACCCTGA	TCACAGGTTCCATGAAGGGC
<i>ET-1</i>	GGAGAAACCCACTCCCAGTC	TCCAAGGCTCTCTTGGACCT
ETA ChIP PCR		
P1	AGCTGTCTCAATAGGTGGGTCATGA	GCCCGTGAAACATGTGCTGGAT
P2	CCTCTTTTGGGAGGAAGCAGTA	AGGAGGGGAGAATCAGCCAAC
P3	GTTGGCTGATTCTCCCTCCTAGAGA	GAGCCCAACCCTTAGTGTGAGGA
P4	GCAGAAATGACACGGTGCCAGT	TGGGCTTCTCAGCATGGT
P5	ACACCAAGCAAGTGAAGTGTCT	AGGTTGGAGCACTGGTCATGTT
P6	TGCACTGAAGGACTCCAAGG	AGCCGACCTTACAGAAAGTGG
P7	GTGTCCAGTTGTTCCGTCAC	TCCTGAAAGAAAAGGTGGGGG
KAT2B ChIP PCR		
P1	GTTGGAAACACTGAGTTGAGGG	CGTTTCTGGGTGGTGGTTCT
P2	CCACCCAGAAACGAATCCTGT	TGGGTCAAATCTGCCGGAGG
P3	TCCGGCAGATTGACCCAG	TTTGAAAAGTACCCGTCATGC
P4	GCATGGACGGGTACTTTTCAA	GGGCAGCCTCTCAATCATTG

PBS for 1 h. Proteins were stained with rabbit anti-MEIS1 (Abcam, 1:500) overnight at 4°C. Fluorescence (cyanine3)-conjugated secondary antibodies were used to visualize the signal and nuclei were stained with DAPI (Sigma, 1 µg/mL), respectively.

Chromatin immunoprecipitation (ChIP)

pHESCs were treated with MPA+db cAMP for 4 days to induce decidualization. Thereafter, pHESCs were suspended in 1% formaldehyde PBS solution for 10 min at room temperature. The cross-linking was terminated by adding glycine to a final concentration 0.125 M. Cells were then collected by centrifugation at 700 g for 5 min at 4°C. Cell pellets were lysed in lysis buffer containing a protease inhibitor. Chromatin was sheared by sonication until the average length of DNA was ~500 bp as evaluated by agarose gel electrophoresis. 1% of the chromatin fragments were stored at -20°C to be used later for non-precipitated total chromatin (input) for normalization. The rest of chromatin fragments were equally divided, and incubated overnight at 4°C with 4 µg anti-MEIS1 antibody or 4 µg anti-rabbit IgG, as a negative control for nonspecific immunoprecipitation. The chromatin-antibody complex was incubated with protein A beads for 1 h at 4°C. The beads were washed in the order of low salt immune complex buffer, high salt immune complex buffer, LiCl immune complex buffer and TE buffer. The beads were suspended in elution buffer and the precipitated protein/DNA complexes were eluted from the antibodies/beads. The cross-linking was reversed, and then proteins were digested using proteinase K. DNA was purified by phenol/chloroform extraction and ethanol precipitation. Specific primers (Table 1) were used to detect immunoprecipitated chromatin fragments, as well as input chromatin. For the calculation of the ChIP qPCR: the first step is to adjust the Ct of the input: in this case 1% of the starting chromatin has been used. The dilution factor (100 times) must be converted in (x) quantification cycles. As for each PCR cycle the number of amplicons is doubling $2^{(x)} = 100 \rightarrow x \ln 2 = \ln 100 \rightarrow x = \ln 100 / \ln 2 \rightarrow x = 6.644$ cycles. This value is subtracted from the values of the Ct inputs (Ct input-6.644) and gives the 'Adjusted Ct'. The second step is the calculation of the ratio in 'percent of the input' as follows: $100 \times 2^{-(\text{Ct IP})} / 2^{-(\text{Adjusted Ct input})} \rightarrow 100 \times 2^{-(\text{Ct IP} - \text{Adjusted Ct input})}$ (Lacazette 2017).

Luciferase reporter assay

HEK293T cells were cultured in DMEM (Hyclone) supplemented with 10% FBS (Hyclone) and applied to

luciferase reporter assay. Luciferase reporter assay was carried out using the Dual-Luciferase Reporter Assay System (PGL3-Basic vector, Promega). The two fragments (F1: -63~-1577 of the ETA promoter and F2: -217~-836 of the KAT2B promoter) containing the predicted binding sites for MEIS1 or HOXA10 were cloned into PGL3-Basic vector, respectively. The pCMV-HA-N vector expressing HOXA10 or MEIS1 was constructed by cloning the full-length PCR product of human HOXA10 or MEIS1. All constructs were transiently transfected into HEK293T cells using Lipofectamine 2000. pRL-TK, internal control plasmid expressing Renilla (Promega), was co-transfected into the cells to normalize firefly luciferase activity of the reporter plasmids. Renilla Luciferase acts as an internal reference to eliminate differences in cell number or transfection efficiency. Assays were performed at least three times with each in duplicate.

Co-IP

The proteins were extracted from decidualized pHESCs by using IP lysis buffer. Protein A magnetic beads (Thermo) were washed and incubated with protein lysates for 2 h at 4°C. Protein lysates were incubated with 4 µg of anti-MEIS1 antibody (Abcam) or rabbit IgG (CST) rotated overnight at 4°C. Immunoprecipitated proteins were separated by SDS-PAGE and detected by immunoblotting using antibody specific to MEIS1, HOXA10.

Construction of recombinant lentiviral vectors and generation of virus particles

KAT2B over-expression plasmid was kindly provided by Professor Guijun Yan. PCR was used to generate the full-length coding region of human ETA from cDNA templates. PCR primers carrying linkers for SpeI sequence at 5'-ends were as follows: 5'-GGACTAGTATGGATTAC AAGGATGACGACGATAAGATGGAACCCCTTTCCT CAGGGC-3' (forward) and NotI at 3'-ends were as follows: 5'-ATGCGGCCGCTCAGTTCATGCTGTCCTTATGG CTGCTCCG-3' (reverse). The amplified DNA fragment was cloned into lentiviral transfer vector. The constructs were sequenced to confirm identity. Empty vector was performed as control. The production and purification of lentiviral vectors was carried out according to previous descriptions (Tiscornia *et al.* 2006, Kutner *et al.* 2009).

Statistical analysis

All values were shown as mean ± S.E.M. of three independent experiments. Statistical analyses were performed by

Figure 1

MEIS1 expression gradually increased during decidualization in hESCs. (A) Relative MEIS1 expression in hESCs treated with cAMP and MPA from day 0 to day 6 were assayed by real-time PCR. $n = 3$. (B) The protein levels of MEIS1 were evaluated by western blot during decidualization. $n = 3$. (C) Immunofluorescence staining of MEIS1 in hESCs treated with proliferation (PRO) or differentiation (DIF) medium for 3 days. Scale bar, 100 µm. (D) Relative MEIS1 expression in hESCs treated with MPA or MPA and RU486 in the indicated times were assayed by real-time PCR. $n = 3$. Results are means $\pm$ s.e.m. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

two-tailed Student's *t*-tests, one-way ANOVA or two-way ANOVA. Figures 2A, B, 4E, F and 5A, B use *t*-test. Figures 1A, B, D, 2B, C, 3C, D, 4B, C, G, H and 5C, D, E, F use one-way ANOVA. A multiple pairwise comparison method is performed after using one-way ANOVA. Figures 2D and 3E use two-way ANOVA and after the two-way ANOVA calculations had statistical differences, multiple comparisons were performed. Statistical analysis was performed using SPSS 19.0 program. The difference was regarded statistically significant if the two-tailed *P* value was < 0.05 .

Results

MEIS1 expression is increased during decidualization in pHESCs

To explore the physiological functions of MEIS1 in human endometrial decidualization, we firstly examined the expression levels of MEIS1 mRNA and protein in endometrial stroma cell during decidualization *in vitro*. As shown in Fig. 1A, B and C, both MEIS1 mRNA and protein levels gradually increased during the process of decidualization and MEIS1 was specifically expressed in the nucleus before and after decidualization. Based on previous report (Rytönen *et al.* 2019) that MEIS1 is regulated by PGR, we detected the expression of MEIS1 in response to P4 stimulation and found the expression of MEIS1 was increased when only added MPA (progesterone

Figure 2

MEIS1 knockdown impaired hESCs decidualization. (A) Primary cultured human endometrium stromal cells were treated with or without MEIS1/siRNA during decidualization and cultured for 4 days. Relative MEIS1 expression in cells were assayed by real-time PCR. $n = 3$. (B and C) Relative expressions of PRL and IGFBP-1 in hESCs transfected with si-ctr or si-MEIS1 and then treated with differentiation medium from day 0 to day 6 assayed by real-time PCR. $n = 3$. (D) Western blot of MEIS1, IGFBP-1 and GAPDH in hESCs transfected with si-ctr or si-MEIS1 and then treated with differentiation medium from day 0 to day 6. $n = 3$. Results are means $\pm$ s.e.m. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. The arrow indicates that there is data here, although the value is low. A full colour version of this figure is available at <https://doi.org/10.1530/JME-19-0100>.

Figure 3

MEIS1 deletion impaired hESCs decidualization. (A) Schematic diagram of the structure of human *MEIS1* gene and its mutant form (KO) targeted by CRISPR/Cas9. (B) Western blot of MEIS1 in WT and KO hESCs cultured in differentiation medium. $n = 3$. (C and D) Relative expressions of *PRL* and *IGFBP-1* in WT and KO hESCs treated with differentiation medium from day 0 to day 6 were assayed by real-time PCR. $n = 3$. (E) Western blot of *IGFBP-1* and MEIS1 in WT and KO hESCs. $n = 3$. Results are means $\pm$ S.E.M. * $P < 0.05$, ** $P < 0.01$. The arrow indicates that there is data here, although the value is low. A full colour version of this figure is available at <https://doi.org/10.1530/JME-19-0100>.

analogue) and this upregulation response was abrogated when co-treated with progesterone receptor (PR) inhibitor (RU486), indicating that MEIS1 is regulated by P4-PR signaling (Fig. 1D). The results suggest that the expression level of MEIS1 is increased during the process of decidualization, which is regulated by MPA, suggesting that MEIS1 may be a regulatory factor on the occurrence and development of decidualization.

Deficiency of MEIS1 leads to impaired decidualization

To further explore the role of MEIS1 in human endometrial decidualization, we knocked down MEIS1 with siRNA, and qRT-PCR results revealed that the expression of MEIS1 was efficiently reduced in the knockdown cells (Fig. 2A). As shown in Fig. 2B and C, the mRNA expression levels of *PRL* and *IGFBP-1* in MEIS1-knockdown decidual cells were significantly lower than that of the control group. Consistently, the protein level of *IGFBP-1* was also decreased with the knockdown of MEIS1 during decidualization. These results demonstrated that MEIS1 is essential for the differentiation of endometrial stromal cells.

To further confirm the role of MEIS1 in decidualization, we knocked out MEIS1 by using CRISPR-Cas9 system (Fig. 3A). The two alleles are both successfully edited as demonstrated by the Sanger sequence flanking the target site. One allele missed 3 bases and inserted 1 base; while the other allele missed 30 bases, in which the missing base includes the initiation codon ATG, leading to the totally loss of MEIS1 (Fig. 3B). With knockout of MEIS1, the mRNA levels of *PRL* and *IGFBP-1* were significantly decreased, compared to the control group (Fig. 3C and D). As shown in Fig. 3E, the *IGFBP-1* protein was also decreased during decidualization in MEIS1^{-/-} cells. The above results were consistent with that of MEIS1 knockdown, providing a solid proof that MEIS1 is necessary for the decidualization process.

MEIS1 interacted with HOXA10 to regulate target genes

As an interacting protein of MEIS1, HOXA10 plays an important role during decidualization. To reveal the potential mechanism of MEIS1 in differentiated pHESCs, we examined the relationship between MEIS1 and HOXA10. As shown in Fig. 4A, there was an interaction between HOXA10 and MEIS1 in human decidual cells. Since KAT2B and ETA are the reported target genes of HOXA10, we want to investigate whether KAT2B and ETA are regulated by MEIS1 in pHESCs. The results showed that the basal expression levels of ETA and KAT2B were not decreased in MEIS1^{-/-} cells compared to the controls in D0. However, significantly decreased expressions of ETA and KAT2B were detected in MEIS1 knockout cells during induced decidualization (Fig. 4B and C). These results suggest that MEIS1 participates in the regulation of KAT2B

Figure 4

MEIS1 interacts with HOXA10 to regulate target genes. (A) Co-immunoprecipitation analysis of MEIS1 and HOXA10 in hESCs during decidualization. (B and C) Relative expressions of KAT2B and ETA in WT and KO in hESCs treated with differentiation medium from day 0 to day 6 were assayed by real-time PCR. $n = 3$. (D) Schematic diagram of ETA and KAT2B promoters with MEIS1 and HOXA10-binding sites. ETA ChIP-qPCR primers, P1-P7. KAT2B ChIP-qPCR primers, P1-P4. (E) ChIP-qPCR analysis for enrichment of MEIS1 to the promoter region of ETA in hESCs treated with differentiation medium for 3 days. (F) ChIP-qPCR analysis for enrichment of MEIS1 to the promoter region of KAT2B in hESCs treated with differentiation medium for 3 days. (G) Relative luminescence intensity assay of Cis-activation potential of the region containing ETA P1-P7 in the presence of the negative control plasmid, MEIS1 over-expression plasmid, HOXA10 over-expression plasmid, or both MEIS1 and HOXA10 over-expression plasmids. (H) Relative luminescence intensity assay of Cis-activation potential of the region containing KAT2B P1-P4 in the presence of the negative control plasmid, MEIS1 over-expression plasmid, HOXA10 over-expression plasmid, or both MEIS1 and HOXA10 over-expression plasmids. Results are means $\pm$ s.e.m. $n = 3$. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$. The arrow indicates that there is data here, although the value is low. A full colour version of this figure is available at <https://doi.org/10.1530/JME-19-0100>.

and ETA expressions during decidualization. ETA is the receptor of endothelin 1 (ET-1) protein, and ET-1 has been proved to play an indispensable role in decidualization (Chao *et al.* 1993). So we also examined the expression level of endothelin (ET-1) in MEIS1-knockout cells. The ET-1 level in MEIS1-knockout cells was not different from the control group, suggested that MEIS1 regulates the expression of ETA but not ET-1 (Supplementary Fig. 1; see section on [supplementary materials](#) given at the end of this article).

To investigate whether KAT2B and ETA are direct target genes regulated by MEIS1, we performed ChIP-qPCR experiments. Because of the spatial interaction between MEIS1 and HOXA10 proteins, the transcription of MEIS1 regulatory target genes may contain the binding motifs (TGACA) of MEIS1, or possibly HOXA10-binding motifs (TTAT) flank the promoter. Through bioinformatics analysis, we found MEIS1 and HOXA10 binding motifs in the promoter regions of ETA and KAT2B and designed primers as shown in Fig. 4D (ETA is P1-P7 and KAT2B is P1-P4). As shown in Fig. 4E and F, by ChIP-qPCR analysis, there were significant enrichment of MEIS1

in the promoter regions of ETA and KAT2B compared to IgG. To further confirm that MEIS1 can regulate the expression of ETA and KAT2B, we performed a dual-luciferase assay using reporter constructs containing the promoters of ETA (regions covering P1-P7) and KAT2B (regions covering P1-P4), respectively. When MEIS1 or HOXA10 was overexpressed alone, the fluorescein intensity of the reporter plasmid was statistically different from that of the control group, but the luciferase activity was significantly enhanced when the MEIS1 and HOXA10 were simultaneously overexpressed (Fig. 4G and H). These results demonstrated that MEIS1 and HOXA10 form a complex that collectively regulated the expression of the target genes KAT2B and ETA.

Overexpression of KAT2B and ETA can at least partially rescue decidualization defects caused by MEIS1 knockout

To further explore the physiological functions of downstream effector KAT2B and ETA in MEIS1-knockout cells, we conducted the rescue experiment through

overexpression of KAT2B and ETA (Fig. 5A and B). We examined the expression of PRL and IGFBP-1 after overexpression of KAT2B and ETA in MEIS1-knockout cells (Fig. 5C and D). After overexpression of KAT2B, the expressions of PRL and IGFBP-1 were upregulated. However, after overexpression of ETA, only PRL expression was increased. The expressions of PRL and IGFBP-1 were significantly increased when KAT2B and ETA were both overexpressed, indicating that KAT2B and ETA may have some synergistic effects during the decidualization. Overexpression of either KAT2B or ETA in WT cells lead to increased expressions of both PRL and IGFBP1, though the KAT2B was more effective for the induction of IGFBP1 (Fig. 5E and F), suggested that KAT2B and ETA have a positive effect on decidualization.

Discussion

Our study demonstrated that MEIS1 is expressed in pHESCs, and its expression level is gradually increased during decidualization induced by cAMP and MPA. The protein levels of MEIS1 were not obviously increased on D2 of differentiation though the mRNA levels of MEIS1 were increased. This may due to the post-transcriptional regulation or the delay changes between the mRNA and

protein levels. The further *in vitro* results demonstrated that MEIS1 exerted its regulatory effect on decidualization by acting as a coactivator of HOXA10. In supporting this, ChIP and luciferase assay showed that MEIS1 synergized with HOXA10 to directly bind to the promoters of the ETA and KAT2B. ETA and KAT2B promoted the expressions of PRL and IGFBP1 to facilitate decidualization of pHESCs (Fig. 6). These findings demonstrated that MEIS1 is a novel coactivator of HOXA10 during human decidualization.

Previous study reported that estrogen and progesterone increased MEIS1 expression in primary cultured endometrial stromal cells, Ishikawa cells, and endometrium of ovariectomized mice (Xu *et al.* 2008). However, the expression pattern of MEIS1 during human decidualization is not still clear. In the present study, we demonstrated that MEIS1 is not only expressed in decidual cells, but also upregulated in pHESCs by MPA treatment (Fig. 1D), whereas the mechanism by which MPA regulated MEIS1 expression remained further investigation.

In previous studies, the researchers found that HOXA10, PBX2, and MEIS1 were expressed in the stroma throughout the menstrual cycle. In addition, PBX2, but not MEIS1, interacted with HOXA10 as a heterodimer, which acted as an enhancer of EMX2 (Sarno *et al.* 2005). In our study, we demonstrated that MEIS1 also acts as

Figure 5

Overexpression of KAT2B and ETA can rescue decidualization defects caused by MEIS1 knockout. (A) Relative expression of ETA in ETA over-expression hESCs and control hESCs. (B) Relative expression of KAT2B in KAT2B over-expression hESCs and control hESCs. (C and D) Relative expressions of PRL and IGFBP-1 in control hESCs or MEIS1-knockout hESCs transfected with the negative control plasmid, ETA over-expression plasmid, KAT2B over-expression plasmid, or both ETA and KAT2B over-expression plasmids. (E and F) Relative expressions of PRL and IGFBP-1 in hESCs transfected with the negative control plasmid, ETA over-expression plasmid, or KAT2B over-expression plasmid. Results are means $\pm$ s.e.m., $n = 3$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. A full colour version of this figure is available at <https://doi.org/10.1530/JME-19-0100>.

Figure 6

Schematic illustration of MEIS1 interacts with HOXA10 regulation on KAT2B and ETA. When MEIS1 absent, the expressions of KAT2B and ETA are low and decidualization markers upregulate insignificantly. When with MEIS1, MEIS1 interacts with HOXA10 to regulate the transcription of KAT2B and ETA, ensuring normal decidualization. A full colour version of this figure is available at <https://doi.org/10.1530/JME-19-0100>.

a coactivator interacting with HOXA10 directly and regulates the expression of ETA and KAT2B, which were beneficial for human stromal cell decidualization.

ETA belongs to the G protein-coupled receptor family and is a receptor for the Endothelin-1 (ET-1) protein (Lappano & Maggiolini 2011). The ligand ET-1 is synthesized and released by human decidual cells (Kubota *et al.* 1992) and it plays an indispensable role in decidualization (Chao *et al.* 1993). In the present study, we found that the expression level of ET-1 was dramatically increased during decidualization, which was consistent with ETA expression. However, there was no significant difference in the expression of ET-1 between MEIS1-knockout and control HESCs, suggested that ET-1 is not a downstream target of MEIS1. But the expression of ETA was significantly decreased in MEIS1-knockout decidual cells. These results suggest that ET-1 and ETA are regulated by different signaling pathways and transcription factors during human decidualization. Over-expression of ETA rescued the expression of PRL but not IGFBP-1 in MEIS1-knockout decidual cells, indicated that the ET-1/ETA axis may mediate the regulation of MEIS1 on PRL expression during decidualization.

KAT2B is a lysine acetyltransferase that directly interacts with and acetylates HOXA10 in Ishikawa cells (Zhu *et al.* 2013). In our study, we demonstrated that KAT2B is a target gene of MEIS1, which is a coactivator of HOXA10. It means that KAT2B may be both a target gene and a cofactor of HOXA10. KAT2B, HOXA10 and MEIS1 may form as a complex to regulate the expressions of PRL and IGFBP-1. Over-expression of KAT2B rescued both PRL and IGFBP-1 expressions in MEIS1-knockout decidual cells, suggested KAT2B is one of the most important downstream-molecule of MEIS1, participating in decidual process.

In summary, we provide the first genetic evidence that MEIS1 is essential for human decidualization, potentially by interacting with HOXA10 to target the promoters of ETA and KAT2B and regulate their expressions. Our study substantiates MEIS1 is a novel regulator during human decidualization.

Supplementary materials

This is linked to the online version of the paper at <https://doi.org/10.1530/JME-19-0100>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

Y Xu, H Wang, S Kong and S Wang were involved in the study design. All authors were involved in experimental data collection. Y Xu, S Kong and J Lu prepared the manuscript.

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