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Krüppel-like factor 12 negatively regulates human endometrial stromal cell decidualization

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ABSTRACT

Members of the KLFs family of transcription factors play roles in maternal endometrium development during embryo implantation. However, the specific role of KLF12 in endometrium development has not yet been described. In this study, we showed that KLF12 expression in human endometrial stromal cells (HESCs) was significantly decreased after decidualization stimulated by 8-Br-cAMP and medroxyprogesterone acetate (MPA). The adenovirus-mediated overexpression of KLF12 in HESCs significantly repressed the expression and secretion of decidualization biomarker genes and their products decidual prolactin (PRL) and insulin-like growth factor binding protein-1 (IGFBP-1) induced by 8-Br-cAMP and MPA. Moreover, CHIP and luciferase reporter assays demonstrated that KLF12 bound to a CAGTGGG element within the decidual prolactin promoter and decreased decidual PRL promoter (dPRL/-2000Luc) activation in a sequence-specific manner. Taken together, these findings suggest KLF12 is a negative regulator of human endometrial stromal cell decidualization.

1. Introduction

Successful implantation depends on the quality of the blastocyst, a receptive endometrium, and synchronization between the developmental stages of the embryo itself and the maternal endometrium [1–3]. In facilitating blastocyst attachment and invasion, the endometrium promotes transformation of the elongated fibroblast-like endometrial stromal cells (ESCs) into more rounded, enlarged secretory cells in a process termed decidualization [4,5]. Decidualization is critical for successful uterine implantation of the blastocyst, coordinated trophoblast invasion, placenta formation, and establishment of the maternofetal immunomicroenvironment [6]. Abnormalities in decidualization result in many pregnancy disorders, such as recurrent miscarriages, infertility, and preeclampsia [6]. The process of decidualization is poorly understood at the molecular level; a key stimulus is progesterone action on estrogen-primed endometrial stromal cells, which leads to dramatic transcriptional reprogramming [5]. Accumulating evidence suggests that the progesterone receptor, CEBP/β, homeobox A10 (HOXA10), and HOXA11 physically associate with transcriptional factor FOXO1A, which induces the decidual marker genes prolactin and insulin-like growth factor binding protein-1 (IGFBP-1) [7–11].

Members of the Krüppel-like Factor (KLF) family of zinc-finger transcription factors are critical regulators of cell differentiation, phenotypic modulation, and physiologic function [12–14]. Emerging evidence has begun to elucidate the role of KLF family in the embryo implantation process. KLF5 is critical for the development of uterine receptivity to implantation by regulating COX2 expression [15]. KLF9 and KLF13 participate in cross-regulation with BMP2 to maintain progesterone sensitivity in differentiating stromal cells [16]. KLF12, together with KLF3 and KLF8, belongs to the KLF/BKLF subfamily and has been shown to repress transcription through the recruitment of a CtBP family member. These proteins repress targeted gene expression through an N-terminal PVDLS sequence (Pro-Xaa-Asp-Leu-Ser) that promotes physical interaction with the co-repressor CtBPs [17]. In contrast with KLF3 and KLF8, which recognize the CACCC sequence, KLF12 interacts with CAGTGGG, which is similar to the high-affinity binding sites of the tumor suppressor WT-1 [17–19]. In addition, the

Abbreviations: HESCs, human endometrial stromal cells; CEBP/β, CCAAT/enhancer-binding protein-b; FOXO1A, forkhead box O1A; HOXA10, homeobox A10; ETS1, v-ets erythroblastosis virus E26 oncogene homolog 1; PRL, prolactin; IGFBP-1, insulin-like growth factor binding protein-1; KLF, Krüppel-like Factor; CtBPs, C-terminal binding proteins; MPA, medroxyprogesterone acetate; 8-Br-cAMP, 8-bromoadenosine-cAMP; ChIP, chromatin immunoprecipitation; ABCD, avidin-biotin conjugate DNA precipitation.

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DNA-binding domain of KLF12 also shows significant similarity to the equivalent region of WT-1/egr proteins, suggesting that KLF12 regulates cell proliferation, differentiation, embryonic development, and tumor genesis and progress [20–22]. Microarray data have shown that KLF12 expression is down-regulated in first trimester decidua cultures [23]. However, recent data have revealed that KLF12 is expressed in the uterus and raise the question of whether KLF12 is important for the regulation of gene expression during decidualization.

In the present study, we confirmed that KLF12 is expressed in the human uterus and endometrial stromal cells. We further demonstrated that KLF12 acts to negatively regulate the expression of the decidual marker genes decidual prolactin and IGFBP-1 in human endometrial stromal cells.

2. Materials and methods

2.1. Isolation of human endometrial stromal cells and artificial induction of decidualization in vitro

Human endometrial stromal cells (HESCs) were isolated from normal endometrial tissue obtained by endometrial biopsy from normal cycling women who had not been treated with hormones for at least three months. The Drum Tower Hospital Research Committee approved this study, and patient consent was obtained prior to the biopsies. Endometrial stromal cells were isolated as described previously [24].

To induce decidualization, HESCs were cultured in phenol red-free DMEM/F12 medium containing 2.5% charcoal/dextran-treated FBS, 100 IU/ml penicillin, and 100 μg/ml streptomycin with added 0.5 mM 8-bromoadenosine-cAMP (8-Br-cAMP) and 1 μM MPA (Sigma, St. Louis, USA).

2.2. Immunohistochemistry

Formalin-fixed paraffin-embedded uterine endometria were serially sectioned, dewaxed with xylene and rehydrated through a graded alcohol series. Sections were then treated with 3% hydrogen peroxide to quench endogenous peroxidase activity, microwaved sequentially to retrieve antigen, and incubated in blocking solution for 1 h. Sections were then incubated with rabbit anti-human KLF12 (1:50 dilution, Proteintech Group, Inc. Chicago, USA) antibody overnight at 4 °C. The next day, the sections were incubated with goat anti-rabbit (rabbit ABC detect kit, ZSBio, Beijing, China) secondary antibodies at 37 °C for 30 min, and then, the sections were stained with 3,3′-diaminobenzidine (DAB) and counterstained with hematoxylin. Control sections were processed concurrently using PBS and similarly pre-treated. Non-specific staining was not detected with PBS.

2.3. Immunofluorescence staining

HESCs plated onto 18 mm micro-cover glasses (Matsunami, Osaka, Japan) were subjected to a decidualization stimulus of 8-Br-cAMP plus MPA for four days. Treated and untreated HESCs were fixed using 4% paraformaldehyde in PBS for 30 min at room temperature. After washing with PBS, the cells were permeabilized by incubation in 0.5% Triton X-100 in PBS for 15 min at room temperature. After blocking with 3% bovine serum albumin in PBS, the cells were incubated with an anti-Flag monoclonal antibody (AllBio Science, Inc. Taiwan) or Alexa Fluor 488-conjugated phalloidin to label actin filaments (Sigma, St. Louis, USA) at 4 °C overnight. Alexa Fluor®594-conjugated goat anti-mouse IgG (Life Technologies, New York, USA) was applied on the second day. Nuclei were stained with 4′,6-diamidine-2′-phenylindole (DAPI) contained in the Vectashield mounting medium for fluorescence with a DAPI kit (Vector Laboratories, Inc. Burlingame, USA). The images were visualized using an FV10i-LIV/FV10i-DOC confocal laser scanning biological microscope (Olympus Corporation, Shinjuku, Tokyo, Japan).

2.4. Western blotting

HESCs were lysed using whole cell lysis buffer containing 50.0 mmol/l Tris pH 7.6, 150.0 mmol/l NaCl, 0.1% SDS, 1.0% NP-40, and a protease-inhibitor cocktail (Sigma, St. Louis, USA), and the protein concentrations were quantified using the Bradford assay (Bio-Rad Laboratories, Hercules, USA). The proteins (20 μg) were separated using 10% SDS–PAGE and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, USA). The membranes were then blocked for 1 h in 5% nonfat milk. Subsequently, the membranes were exposed to primary antibodies against KLF12 (1:2000 dilution; Proteintech Group, Inc. Chicago, USA) and Flag-HRP (1:5000 dilution; Sigma, St. Louis, USA). β-actin (1:5000 dilution; Bioworld Technology, St. Louis, USA) was measured as an internal control. Immunodetection was accomplished using a goat anti-rabbit secondary antibody and an enhanced chemiluminescence kit (Millipore, Billerica, USA).

2.5. RNA isolation and real-time quantitative PCR

Total RNA was isolated from cells using TRIzol (Life Technologies, New York, USA) according to the manufacturer's instructions. RNA (1 μg) was reverse transcribed to cDNA and diluted 1:5 before use; 2 μl of RNA were used in a total reaction volume of 20 μl containing the SYBR-Green mixture (Bio-Rad Laboratories, Hercules, USA). The specific primers used for 18S rRNA detection were 5′-CGGCTACCACATCCAAGGAA-3′ and 5′-CTGGAATTACCGCGGCT-3′. The PRL primers were 5′-CACTACATCCATAACCTCTC-3′ and 5′-ATGCTGACTATCAAGCTCAG-3′. The IGFBP-1 primers were 5′-TATGATGGCTCGAAGGCTCTC-3′ and 5′-GTAGACGCACCAGCAGAGTC-3′. The KLF12 primers were 5′-CCTTTCCATAGCCAGAGCAG-3′ and 5′-TTGCATCCCTCAAAATCACA -3′. Reactions were carried out using a MyiQ Single-Color Real-time PCR Detection System (Bio-Rad Laboratories, Hercules, USA) for 40 cycles (95 °C for 10 s, 60 °C for 30 min) after an initial 3 min incubation at 95 °C. The fold change in expression of each gene was calculated using the $2^{-\triangle\triangle CT}$ method with 18S rRNA as an internal control.

2.6. Construction of adenovirus

Adenoviruses harboring human full-length KLF12 (NCBI Reference Sequence: NM_007249.4) were generated using the AdMax (Microbix) system according to the manufacturer's recommendations. Viruses were packaged and amplified in HEK293A cells and purified using CsCl banding, followed by dialysis against 10 mmol/l Tris-buffered saline with 10% glycerol. Titering was performed on HEK293A cells using the Adeno-X Rapid Titer kit (BD Biosciences Clontech, Palo Alto, USA) according to the manufacturer's instructions.

2.7. Prolactin and IGFBP-1 Assays

Prolactin and IGFBP-1 levels in supernatant were measured using the Vidas Prolactin kit (bioMérieux, France) and Human IGFBP1 ELISA kit (BOSTER, Wuhan, China). The limit of detection of the Vidas Prolactin kit was 0.5 ng/ml, and the detection range of the human IGFBP1 ELISA kit was 31.2–2000 pg/ml. DMEM/F12 supplemented with charcoal/dextran-treated FBS did not have measurable prolactin or IGFBP-1 concentrations.

2.8. Chromatin immunoprecipitation (ChIP) assay

HESCs (70% confluence) were infected with Ad-Flag-KLF12 for 24 h and then maintained in phenol red-free DMEM/F12 medium containing 2.5% charcoal/dextran-treated FBS with 0.5 mM 8-Br-cAMP plus 1 μM MPA. After 48 h, HESCs were prepared for ChIP using Flag-beads as described previously [25]. The recovered DNA was submitted for PCR and real-time PCR. Fold enrichments were calculated as $2^{-(Ctinput\ Cttest)}$ and expressed relative to the control without Flag-beads. The PCR reactions contained 2 μl of DNA, standard PCR reagents, and 50 pmol of both dPRL 5′-CCTTGGCATTTTTCCTTGAA-3′ and 5′-TTTGTTTCCCTTGCATCACA-3′ (spanning 530 bp), which are specific for dPRL promoter DNA fragments. The primers used for real-time PCR were 5′-TACCTTGGCATTTTTCCTTGA-3′ and 5′-GGCACTGGAACTCCAATTCTT-3′.

2.9. Avidin–biotin conjugate DNA precipitation (ABCD) assay

The following double-stranded oligonucleotides were used, all of which were biotinylated at the 3′-end of the sense strand: dPRL wild: 5′-CATAAGAAGCTCAGTGGGCACATAAAAAGG-3′, dPRL reverse: 5′-CCTTTTTATGTGCCCACTGAGCTTCTTATG-3′, and dPRL mutation: 5′-CATAAGAAGCTCACAAAGCACATAAAAAGG-3′. An ABCD assay was performed by incubating 500 μg of cell extracts derived from HESCs with 500 pmol of each double-stranded DNA immobilized on streptavidin agarose in binding buffer (10 mM Tris, pH 7.4, 150 mM NaCl, 0.5% Triton X-100, 0.5 mM DTT, 0.5 mM EDTA, 10% glycerol, 20 μg/ml poly [dI-dC], and protease inhibitors). After 4 h of incubation at 4 °C, the beads were washed four times with the same buffer, and proteins were resolved by SDS–PAGE, electrotransferred onto PVDF membranes, and probed with the anti-Flag antibody. Chemiluminescence was accomplished using the enhanced chemiluminescence kit.

2.10. Transient transfection and luciferase reporter assay

A pGL3-basic luciferase reporter plasmid (Promega, Madison, USA) harboring the dPRL promoter sequence (−2000 to +65) was constructed using the primers 5′-GCTAGGTACCTAGAAGTGACAAGTGAAAAAGT-3′ and 5′-TAGCAGATCTAGGATTCTTTTATGGCC TGCTT-3′; mutagenesis of the putative KLF12 site on the decidual prolactin promoter was performed by overlap-extension PCR using the primers 5′-CATAAGAAGCTCACAAAGCACATAAAAAGG-3′ and 5′-CCTTTTTATGTGCTTTGTGAGCTTCTTATG-3′. At 70% confluence, HESCs in 12-well plates were infected with Ad-Flag-KLF12 for 24 h, and the cells were then transfected with 600 ng of the reporter construct and 6 h later, 10 ng of the Renilla luciferase reporter plasmid pRL-RSV (Promega, Madison, USA) using the Lip2000 transfection reagent (Life Technologies, New York, USA). After 48 h, the cell lysates were assayed for luciferase activity using the Luciferase Assay System (Promega, Madison, USA) and a Centro XS3 LB 960 luminescence counter (Berthold Technologies, GmbH Co. Germany) according to the manufacturers' instructions. Firefly luciferase activity was normalized for transfection efficiency according to the corresponding Renilla luciferase activity.

2.11. Statistical analysis

Unless stated otherwise, the numerical data presented are the mean ± SD of at least three experiments. For the comparison of two populations, a *t*-test was used. Differences were considered significant when *p*-values were less than 0.05.

3. Results

3.1. Expression of KLF12 in human endometrium stromal cells

To examine KLF12 expression in human endometrium stromal cells, we obtained secretory phase endometria by endometrial biopsy from normal cycling women who had not been treated with hormones in the previous three months and then used immunohistochemistry to assess expression patterns of KLF12 in the endometria. As shown in Fig. 1A, there was specific staining for KLF12 protein in human endometrium, specifically in the glandular epithelium and stromal cells, compared with negative control staining using PBS. In addition, western blot analyses of proteins from HESCs and human endometrium glandular epithelium cells (HEEC) revealed that the endometrium cells contained the KLF12 protein (Fig. 1B).

3.2. Expression of KLF12 decreased during HESC decidualization in vitro

Next, we used quantitative real-time PCR to investigate the dynamic changes in KLF12 during human endometrial stromal cell decidualization. We found that 8-Br-cAMP and MPA affected KLF12 expression in HESCs; at 96 h after treatment, KLF12 mRNA expression was decreased by approximately 70% (Fig. 2A). KLF12 mRNA expression was also decreased in human first trimester decidual tissue compared with secretory phase endometrium ($n = 8$) (Fig. 2B). The detection of KLF12 protein in whole-cell lysates also revealed noticeable changes in KLF12 protein concentrations in HESCs after in vitro decidualization using 8-Br-cAMP and MPA at 72 h after stimulation (Fig. 2C).

3.3. Overexpression of KLF12 inhibits HESC decidualization in vitro

To determine the role of KLF12 in the regulation of HESC decidualization in vitro, we constructed a KLF12 recombinant adenovirus and then treated the HESCs with adenovirus-mediated overexpression of KLF12 and 8-Br-cAMP plus MPA for three to nine days. dPRL and IGFBP-1 mRNA expression was decreased 72% and 71%, respectively, in three days by the adenovirus-mediated overexpression of KLF12 in HESCs induced for decidualization stimulation (Fig. 3B). In addition, Fig. 3C also demonstrated that KLF12 overexpression was able to significantly decrease dPRL and IGFBP1 secretion in a time-dependent manner.

Because the decidualization of HESCs is also characterized by phenotypic changes in cellular morphology, we further examined whether KLF12 affects the organization of the actin cytoskeleton. The decidualizing HESCs transduced with Ad-Flag-KLF12 showed an undifferentiated fibroblastic phenotype and displayed a poorly formed actin cytoskeleton (Fig. 3D).

3.4. KLF12. represses the transcriptional activity of the dPRL promoter

Previous research has identified the consensus motif CAGTGGG of KLF12 using an in vitro binding site selection assay [18]. We visually scanned the wild-type dPRL sequence (−2000 to +65 relative to the transcriptional start, Promoter Database ID: 37139) and IGFBP-1 (−3600 to +65, Promoter Database ID: 37680) and found the specific KLF12 binding site in the promoters of dPRL (−1472/−1466) and IGFBP-1 (−3113/−3107), suggesting the possibility of a direct role for KLF12 in regulating dPRL and IGFBP-1 expression. To confirm the physical interactions between KLF12 and the CAGTGGG elements in the dPRL and IGFBP-1 promoters, we assessed the presence of KLF12 protein on the genomic regions encoding dPRL and IGFBP-1 in HESCs using ChIP and ABCD assays.

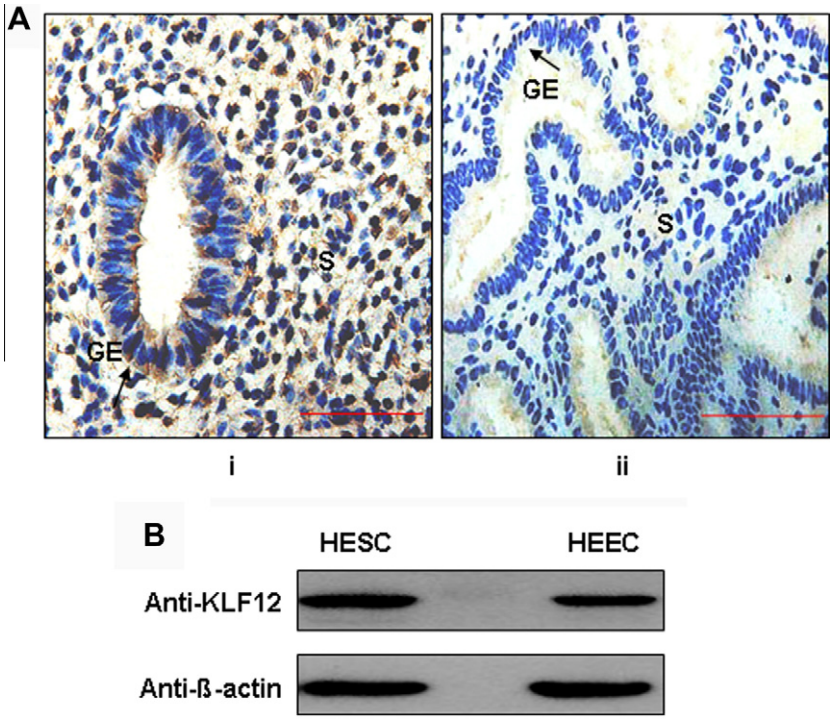

Fig. 1. Localization of KLF12 in human endometrium and human stromal cells .(A) Immunohistochemical detection of KLF12 in human endometrium during the secretory phases of the menstrual cycle by immunostaining of glandular epithelium (GE) and stromal cells (S), with KLF12-specific staining shown in brown. Hematoxylin nuclear costaining is shown in blue, with (Aii) showing staining with the nonspecific control PBS; all images are at ×400 magnification. Bar = 200 μm. (B) Western blot showing endogenous KLF12 expression in HESCs and HEECs.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

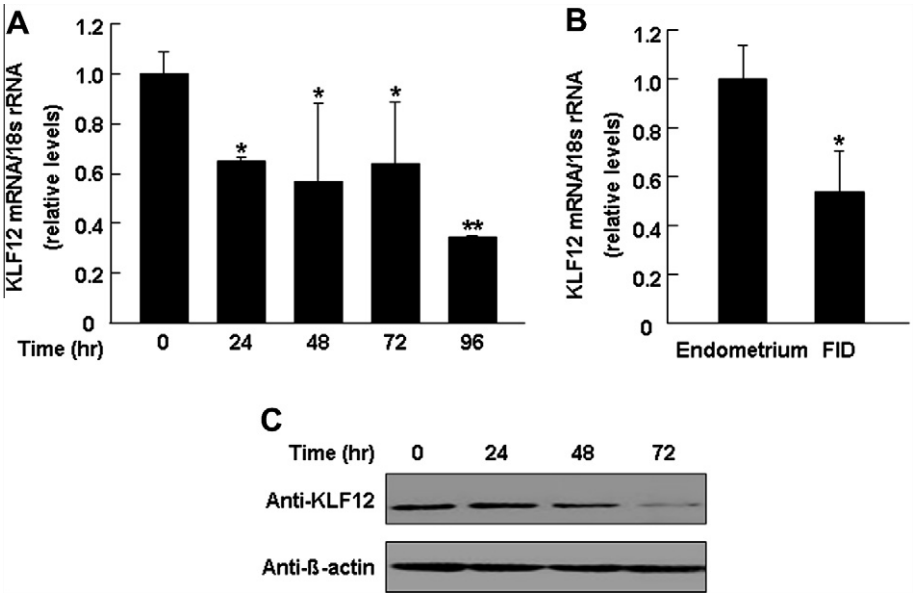

Fig. 2. KLF12 expression is decreased during HESC decidualization in vitro. Total RNA was isolated from HESCs treated with 8-Br-cAMP and MPA at the indicated concentrations for different periods, human first trimester decidual tissue ($n = 8$) and secretory phase endometrium ($n = 8$), (A). (B) Levels of KLF12 mRNA were measured using real-time PCR. (C) KLF12 protein expression during HESC decidualization in vitro was assessed by Western blot; $^{*}p < 0.05$, $^{**}p < 0.01$ compared with 0 h. The data represent the results of three independent experiments.

It was found that chromatin fragments encoding dPRL and including the CAGTGGG element specifically co-precipitated with KLF12 from lysates of HESCs but not fragments encoding IGFBP-1 (Fig. 4A and B). ABCD assays showed that KLF12 protein strongly bound to the PRL wild-type oligonucleotides, but not to the mutant sequence, suggested that the CAGTGGG motif of dPRL interacts with KLF12 but not IGFBP-1 in a sequence-specific manner.

Because KLF12 binds to the prolactin promoter, we next examined whether KLF12 inhibits decidual prolactin promoter activity. As shown in Fig. 4D, the reporter gene assay revealed a marked decrease in luciferase activity. When a mutant promoter with the KLF12 binding site mutated to CACAAAG was transfected into the HESCs, the results showed that the KLF12 binding site on the decidual prolactin promoter is essential for basal transcriptional

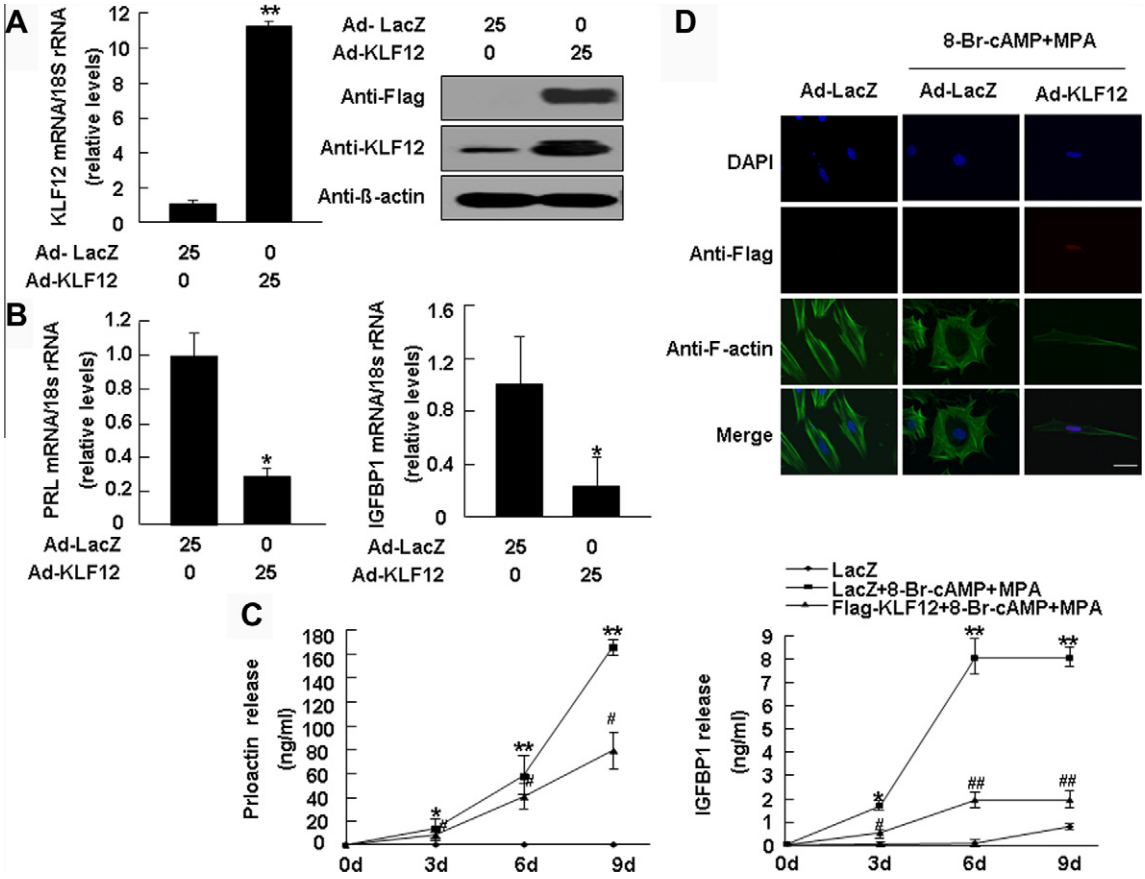

Fig. 3. Overexpression of KLF12 inhibited HESC decidualization in vitro. (A) HESCs were transduced for three days with Ad-LacZ or Ad-Flag-KLF12 at the MOI indicated. KLF12 protein expression was measured using a Western blot and real-time PCR. (B) HESCs were infected with Ad-LacZ or Ad-Flag-KLF12 at the MOI indicated and treated with 8-Br-cAMP plus MPA for three days, after which PRL and IGFBP-1 mRNA levels were measured using real-time PCR ($n = 3$). $^{*}p < 0.05$ compared with Ad-LacZ alone. (C) HESCs were infected with the indicated adenoviruses for 24 h, followed by treatment with or without 8-Br-cAMP and MPA for an additional three, six, or nine days; Prolactin and IGFBP-1 released into the medium were measured using ELISA ($n = 3$). $^{*}p < 0.05$, $^{**}p < 0.01$ compared with Ad-LacZ alone; $^{\#}p < 0.01$, $^{\#\#}p < 0.01$ compared with Ad-LacZ treated with 8-Br-cAMP and MPA. (D) Immunofluorescence using Alexa Fluor 488-conjugated phalloidin to label actin filaments (F-actin) to analyze adenovirus-treated HESCs.

activity, and the repression of prolactin promoter activity by KLF12 is not blocked (Fig. 4D). Therefore, the repression of prolactin promoter activity by KLF12 is not only mediated by the binding of KLF12 to the CAGTGGG motif in the prolactin promoter but also by interactions with other factors.

4. Discussions

In this study, we demonstrated that KLF12 is a critical repressor of the steroid hormone induction of decidualization. KLF12 encodes the third member of a subfamily of CtBP-mediated repressors and distinct from KLF3 and KLF8, shows a pattern of expression restricted to the kidney and brain [17–19]. In our present study, we found that KLF12 was predominantly expressed in human endometrial stromal cells and that its expression was decreased during the HESC decidualization process in vitro, suggesting that KLF12 has a role in stromal cell transformation.

Decidual prolactin is a major secretory product of the decidualized endometrium, which has many important functions in successful pregnancy [26]. In this study, we found that adenoviral-mediated overexpression of KLF12 markedly reduced decidual prolactin expression in decidualizing HESCs. Furthermore, ChIP and ABCD assays demonstrated that KLF12 binds to a CAGTGGG element within the decidual prolactin promoter in a sequence-specific manner. These results suggest that KLF12 is involved in the regulation of decidual PRL expression during 8-Br-cAMP- and MPA-induced decidualization. Although overexpression of KLF12 markedly inhibited prolactin promoter activity in decidualizing HESCs, in HEK293T cells, overexpression of KLF12 facilitated prolactin promoter activity (data not shown). These contradictory results suggest that during decidualization, KLF12 turns into a repressor when combined with other cofactors. Previous studies have provided evidence of a role for KLF12 in transcriptional repression that involves physical association with the corepressor C-terminal binding proteins (CtBPs) that in turn recruit a large repressor complex to mediate transcriptional silencing [27,28]. Some studies have suggested that a number of the KLFs are able to influence steroid hormone signaling through transcriptional networks involving steroid hormone receptors and members of the nuclear receptor family of transcription factors [29–31]. Therefore, it would be interesting to further examine the potential KLF12 cofactors that function cooperatively to regulate HESC decidualization.

Interestingly, the overexpression of KLF12 in HESCs similarly inhibited the transcriptional activity of a decidual prolactin promoter fragment lacking the KLF12 binding site (dPRL-−330/+65) (data not shown). This result indicated that KLF12 might repress decidual prolactin expression through physical association with other transcriptional factors because dPRL-−330/+65 harbors some regulatory elements of other transcription factors, such as FOXO1A, HOXA10, HOXA11, and ETS1 [11].

In this study, we also detected the regulatory role of KLF12 affecting another decidual marker molecule, IGFBP-1. Although there is a specific KLF12 binding site within the IGFBP-1 promoter

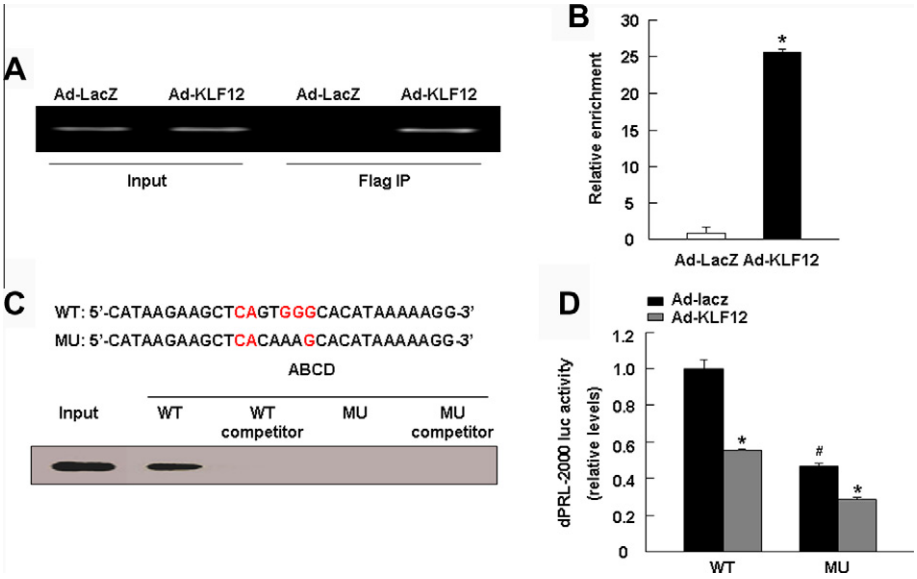

Fig. 4. KLF12 represses the transcriptional activity of the dPRL promoter. (A) Co-precipitating chromatin was amplified by PCR using primers specific for the region of the dPRL promoter. PCR products were separated by agarose gel electrophoresis. Input (non-precipitated) chromatin was a positive control for these studies. (B) Co-precipitating chromatin was analyzed using real-time PCR. The y-axis represents the relative fold enrichment. (C) The ABCD assays were performed using biotinylated or un-biotinylated (competitor) double-stranded dPRL wild-type (WT) and dPRL mutant (MU) oligonucleotides with whole cell extracts from HESCs. (D) HESCs were infected with the indicated adenoviruses for 24 h and then transfected with dPRL-2000/Luc or dPRL-2000 mut/Luc (600 ng/well) and treated with 8-Br-cAMP and MPA. After 72 h, luciferase assays were performed and normalized to a constitutive Renilla luciferase ($n = 3$). $^{*}p < 0.05$ compared with Ad-LacZ; $^{\#}p < 0.05$ compared with HESCs treated with Ad-LacZ and transfected with dPRL-2000/Luc.

and KLF12 can markedly decrease the expression and secretion of IGFBP-1, KLF12 does not directly bind to the IGFBP-1 promoter region, as demonstrated by the CHIP and ABCD assays. These results suggested that KLF12 might act through regulating other key factors, such as FOXO1A, during decidualization, thereby influencing IGFBP-1 expression [9,10].

HESC decidualization represents a complex cell transformation that requires morphological and functional changes in cell structure and involves the reorganization of the cytoskeleton. During decidualization, the actin cytoskeleton organizes to form short to intermediate filaments that lie parallel to the long axis of the stromal cells [32]. When we forced KLF12 expression, the decidualizing HESCs retained an undifferentiated fibroblastic phenotype and displayed a poorly formed actin cytoskeleton. All of these results demonstrated that KLF12 regulated pathways for biochemical and morphological processes associated with in vitro decidualization.

In summary, to our knowledge, our study provides the first evidence that KLF12 is a novel transcription factor that negatively regulates HESC decidualization. Abnormal KLF12 expression during stromal cell differentiation into decidual cells leads to implantation failure and abortion. Further studies are required to determine the other limiting factors in these cells required for the underlying mechanism of KLF12 activity in decidual differentiation of the human endometrium.

Acknowledgments

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