Biochemical and Biophysical Research Communications 445 (2014) 622–628

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Activation of matrix metalloproteinase-26 by HOXA10 promotes embryo adhesion *in vitro*

Yue Jiang [a,1], Guijun Yan [b,1], Hui Zhang [a], Huizhi Shan [a], Chengcai Kong [b], Qiang Yan [b], Bai Xue [a], Zhenyu Diao [b], Yali Hu [a,*], Haixiang Sun [a,*]

[a] Reproductive Medicine Center, Drum Tower Clinic Medical College of Nanjing Medical University, Nanjing 210029, Jiangsu, China
[b] Reproductive Medicine Center, The Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing 210008, Jiangsu, China

ARTICLE INFO

Article history:
Received 3 February 2014
Available online 22 February 2014

Keywords:
MMP-26
HOXA10
Embryo adhesion

ABSTRACT

Successful embryonic implantation requires an effective maternal–embryonic molecular dialogue. However, the detailed mechanisms of epithelial-embryo adhesion remain poorly understood. Here, we report that matrix metalloproteinase-26 (MMP-26) is a novel downstream target gene of homeobox a 10 (HOXA10) in human endometrial cells. HOXA10 binds directly to a conserved TTAT unit (−442 to −439) located within the 5′ regulatory region of the MMP-26 gene and regulates the expression and secretion of MMP-26 in a concentration-dependent manner. Moreover, the adenovirus-mediated overexpression of MMP-26 in Ishikawa cells markedly increased BeWo spheroid adhesion. An antibody blocking assay further demonstrated that the promotion of BeWo spheroid adhesion by HOXA10 and MMP-26 was significantly inhibited by pre-treatment with a specific antibody against MMP-26. These results demonstrate that the HOXA10-mediated expression of MMP-26 promotes embryo adhesion during the process of embryonic implantation.

1. Introduction

Embryo implantation is a dynamic process that includes the apposition and attachment of the blastocyst to the receptive endometrium and the invasion of the trophoblast cells of the conceptus into the endometrium [1], all of which is ensured by sex steroids, growth factors, adhesion molecules and extracellular matrix proteins [2]. Indeed, any aberration in this process can result in infertility, which is currently considered the most important limiting factor for the establishment of viable pregnancy during assisted reproduction [3].

Several key transcription factors, such as liver receptor homolog-1 (LRH-1) [4], signal transducer and activator of transcription 3 (STAT3) [5] and Kruppel-like factor 5 (KLF5) [6], have been implicated in the control of embryo implantation. HOXA10 is a homeobox-containing transcription factor that is necessary for implantation [7–10]. Proper HOXA10 expression is essential for endometrial cell differentiation and receptivity, which facilitate the development of the conditions that are necessary for embryo implantation [11]. HOXA10 null mice are infertile primarily due to defective implantation (a failed attachment reaction) as well as aberrant decidualization [7,9]. These mice produce viable embryos that can implant and develop normally in a wild-type surrogate. In contrast, wild-type embryos fail to implant in HOXA10-deficient mice. HOXA10 is expressed cyclically during the menstrual cycle in the endometrium under the influence of estrogen and progesterone, with maximal expression in the human endometrial epithelium and stromal cells during the implantation window [8,12]. Furthermore, HOXA10 regulates the expression of various downstream target genes that are also involved in implantation, such as β3 integrin (ITGB3), empty spiracles homeobox 2 (EMX2) and p300/CBP-associated factor (p/CAF) [13–15]. Deficiencies in the expression of HOXA10 have been described in association with endometriosis, polycystic ovary syndrome (PCOS) and hydrosalpinges [12,16–17], all of which are conditions associated with abnormal implantation. The role of HOXA10 in the human endometrium, however, is less clear.

Here, we report that MMP-26 is a novel HOXA10 downstream target gene in a human endometrial adenocarcinoma cell line (Ishikawa cells). Our studies have shown that HOXA10 specifically binds to the MMP-26 promoter and regulates the expression of MMP-26 to promote embryo attachment. The results of this study provide us with a better understanding of the role, regulation and function of HOXA10 during the embryo adhesion phase.

* Corresponding authors. Fax: +86 25 8310 7188.
E-mail addresses: yali_hu@hotmail.com (Y. Hu), stevensunz@163.com (H. Sun).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2014.02.057
0006-291X/

2. Materials and methods

2.1. Cell culture

The Ishikawa cells, HEK293T cells and BeWo cells used in this study were cultured in Dulbecco's modified minimum essential medium (HyClone, Thermo Scientific, South Logan, UT, USA) containing 10% fetal bovine serum (FBS, Gibco BRL/Invitrogen, Carlsbad, CA, USA) and 1% penicillin/streptomycin (HyClone, Thermo Scientific, South Logan, UT, USA). The Ishikawa cells were cultured in the presence or absence of 10 nM 17β-estradiol (E2) and 1 μM progesterone (P4) (Sigma, St. Louis, MO, USA) for the indicated time periods.

2.2. Construction of adenovirus vectors

Adenovirus vectors harboring the full-length HOXA10 (Ad-HOXA10-Myc) and MMP-26 genes (Ad-MMP26-Flag) were generated using AdMax (Microbix) systems according to the manufacturer's recommendations. An adenovirus bearing LacZ (Ad-LacZ) was obtained from Clontech (Mountain View, CA, USA).

2.3. RNA isolation and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from Ishikawa cells using TRIzol reagent (Life Technologies, New York, NY, USA) according to the manufacturer's instructions. A 2 μg aliquot of total RNA was reverse transcribed into cDNA using the M-MLV RT system (Promega, Madison, WI, USA). Quantitative real-time PCR was performed using SYBR green (Bio-Rad Laboratories, Hercules, CA, USA). The specific primers used for human MMP-26 primers were 5′-GTGCAGAATGGAGATGCAGA-3′ and 5′-TGAGTTGCAACCAGGAACAG-3′. The reactions were carried out using a MyiQ Single Color Real-time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA) and were run for 40 cycles (95 °C for 10 s, 60 °C/63.6 °C for 30 s) after an initial 3 min incubation at 95 °C. The fold change in expression of each gene was calculated using the $2^{-\Delta\Delta CT}$ method; 18S rRNA served as an internal control.

2.4. Transient transfection and luciferase reporter assay

A pGL3-basic luciferase reporter vector (Promega, Madison, WI, USA) harboring the MMP-26 promoter sequence (−550 to −200, Promoter ID: 6224) was constructed with the following primers: 5′-TATAGGTACCTCCTGACTTGTGATCTGCCCT-3′ and 5′-TATAGCTAGCCACCTGATGGTCCTCA-3′. Mutagenesis of the putative HOXA10 binding site in the MMP-26 promoter was performed using overlapping extension PCR. At 60% confluence, Ishikawa cells in 12-well plates were infected with Ad-HOXA10-Myc for 24 h. The cells were then co-transfected with 300 ng of the MMP-26 reporter construct and 10 ng of the Renilla luciferase reporter plasmid pRL-RSV using Lipofectamine™ 2000 transfection reagent (Life Technologies, Grand Island, NY, USA). Luciferase activity was measured after 48 h of transfection using the Dual-Luciferase reporter assay system (Promega, Madison, WI, USA) with a Centro XS3 LB 960 luminescence counter (Berthold Technologies, GmbH Co., Bad Wildbad, Germany) according to the manufacturer's instructions. Firefly luciferase activity was normalized for transfection efficiency using the corresponding Renilla luciferase activity. The experiments were performed in triplicate.

2.5. Chromatin immunoprecipitation (ChIP) assay

The protocol for the ChIP assay was based on the method of Sun et al. as previously described [15]. Ishikawa cells were prepared for ChIP using anti-c-Myc affinity gel (Sigma, St. Louis, MO, USA). The purified DNA fragments were subjected to PCR using the following set of primers designed to amplify the human MMP-26 promoter: 5′-ATCTCCTGACTTGTGATCTG-3′ and 5′-ACTGCTTGAATTCAGGTACA-3′.

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

ABCD assay was performed as described by Shen et al. [18]. The following double-stranded oligonucleotides were used, all of which were biotinylated at the 3′-end of the sense strand: MMP-26 forward: 5′-TGGATCTTTATTACAGCAGACATTATTTATTTCCC-3′, MMP-26 reverse: 5′-GGGAAATAAATAATGTCTGCTGTAATAAAGATCCA-3′.

2.7. Western blotting

Immunoblotting was performed with primary antibodies against HOXA10 and MMP-26 (1:500; Santa Cruz Biotechnology, Santa Cruz, CA, USA), Myc-HRP (1:5000; Invitrogen, San Diego, CA, USA), Flag-HRP (1:5000; Sigma, St. Louis, MO, USA), FAK and p-FAK(Y925) (1:1000; Bioworld, Nanjing, China), GADPH and β-actin (1:10000; Bioworld, Nanjing, China). Immunodetection was accomplished using rabbit anti-mouse or goat anti-mouse secondary antibodies and an enhanced chemiluminescence kit.

2.8. Attachment assay of BeWo spheroids to Ishikawa cells

According to our standard laboratory protocol, we used multicellular spheroids of human choriocarcinoma BeWo cells with endometrial Ishikawa cells as an *in vitro* model of attachment [10]. Ishikawa cells grown in 12-well plates to 50% confluency were infected with Ad-HOXA10-Myc, Ad-LacZ or Ad-MMP-26-Flag at the indicated MOI for 24 h. A functional blocking antibody against MMP-26 was also used in the attachment assay, as the adenovirus-treated cells were cultured in the presence of a mouse antibody against MMP-26 or mouse preimmune IgG (Abcam, Cambridge, MA, USA) at a concentration of 0.5 μg/mL for 1 h before the transfer of the BeWo spheroids onto the surface of a confluent monolayer of Ishikawa cells. After incubation at 37 °C for 2 h, the unattached spheroids were removed by washing the cells with PBS containing Ca^{2+} (0.1 mg/L) and Mg^{2+} (0.1 mg/L). We then counted the attached spheroids under a light microscope and expressed the attachment rate as a percentage of the total number of spheroids added to the Ishikawa monolayer. All attachment assays were performed in triplicate.

2.9. MMP-26 enzyme-linked immunosorbent assay

To detect the secretion of MMP-26, Ishikawa cells were cultured in DMEM supplemented with 10% charcoal/dextran-treated FBS (HyClone, Thermo Scientific, South Logan, UT, USA). MMP-26 secretion levels were measured using MMP-26 ELISA kits (Uscn Life Science Inc., Wuhan, China). The detection range of the human MMP-26 ELISA kit was 0.156–10 ng/mL. DMEM supplemented with charcoal/dextran-treated FBS did not contain measurable amounts of MMP-26.

2.10. Statistical analysis

The data are expressed as the means ± SEM from at least three independent experiments. Student's *t*-test and ANOVA were performed to detect differences between two groups and among more than two groups, respectively. The values were determined to be significant when $P < 0.05$.

3. Results

3.1. HOXA10 transactivates the human MMP-26 promoter in Ishikawa cells

Previous studies have shown that the transcription factor HOXA10 plays an important role in endometrial physiological functions [7–11]. However, the targets of HOXA10 in human endometrial cells are less well known. We employed a gain-of-function approach using adenovirus-mediated overexpression of HOXA10 (Ad-HOXA10-Myc) to identify potential target genes for HOXA10 in Ishikawa cells. The purified DNA fragments from the HOXA10–chromatin complexes were subsequently cloned and sequenced. Among the sequenced clones, we obtained five that appeared to be from promoter regions and corresponded to bases −650 to −76 of the MMP-26 promoter.

Fig. 1A shows that the human MMP-26 promoter was efficiently amplified by PCR from the HOXA10–Myc chromatin complexes, but not from the controls, suggesting that HOXA10 could bind directly to the MMP-26 promoter. To explore the functional role of HOXA10 binding to the MMP-26 promoter, we constructed a luciferase reporter plasmid containing approximately 350 bp of the human MMP-26 promoter with the TTAT-rich sequence (Fig. 1B, −550 to −200; promoter ID: 6224). As depicted in Fig. 1C, overexpression of HOXA10 in Ishikawa cells enhanced human MMP-26 promoter activity in a concentration-dependent manner. In addition, the transfection of HOXA10 siRNA resulted in an 80% inhibition of endogenous HOXA10 protein expression in human endometrial cells [15], which was reflected by a 70% decrease in MMP-26 promoter activity in Ishikawa cells (Fig. 1D). Taken together, these results demonstrated that HOXA10 bound directly to the human MMP-26 promoter and activated MMP-26 promoter activity.

3.2. HOXA10 is required for hormone-induced MMP-26 expression in Ishikawa cells

HOXA10 binds to the MMP-26 promoter and activates MMP-26 promoter activity in Ishikawa cells, which prompted us to investigate whether MMP-26 expression was regulated by HOXA10. We found that the overexpression of HOXA10 (Fig. 2A) in Ishikawa cells markedly increased the expression of MMP-26 mRNA by 5-fold (Fig. 2B) and led to the secretion of MMP-26 protein into the culture medium in a concentration-dependent manner (Fig. 2C).

Moreover, the knockdown of endogenous HOXA10 mRNA expression by 70% (Fig. 2D) in Ishikawa cells substantially decreased the expression of MMP-26 mRNA expression to half of that observed in the siCTL group (Fig. 2E). The release of MMP-26 protein into the medium was also monitored by ELISA, as illustrated in Fig. 2F. Treatment with HOXA10 siRNA markedly inhibited MMP-26 protein secretion compared with control siRNA at 50 nM (3.34 ± 0.23 vs. 4.74 ± 0.40; $P < 0.05$ vs. control siRNA group, $n = 3$) and at 100 nM (2.91 ± 0.33 vs. 4.74 ± 0.40, $P < 0.05$ vs. control siRNA group, $n = 3$).

Consistent with previous reports, we found that estrogen and progesterone induced the expression of HOXA10 and MMP-26 (Fig. 2G). Importantly, HOXA10 knockdown in Ishikawa cells significantly attenuated the expression of MMP-26 mRNA induced by estrogen and progesterone compared to the control siRNA group (Fig. 2H).

Taken together, these data suggest that MMP-26 is a novel target gene of HOXA10 in human endometrial epithelial cell lines.

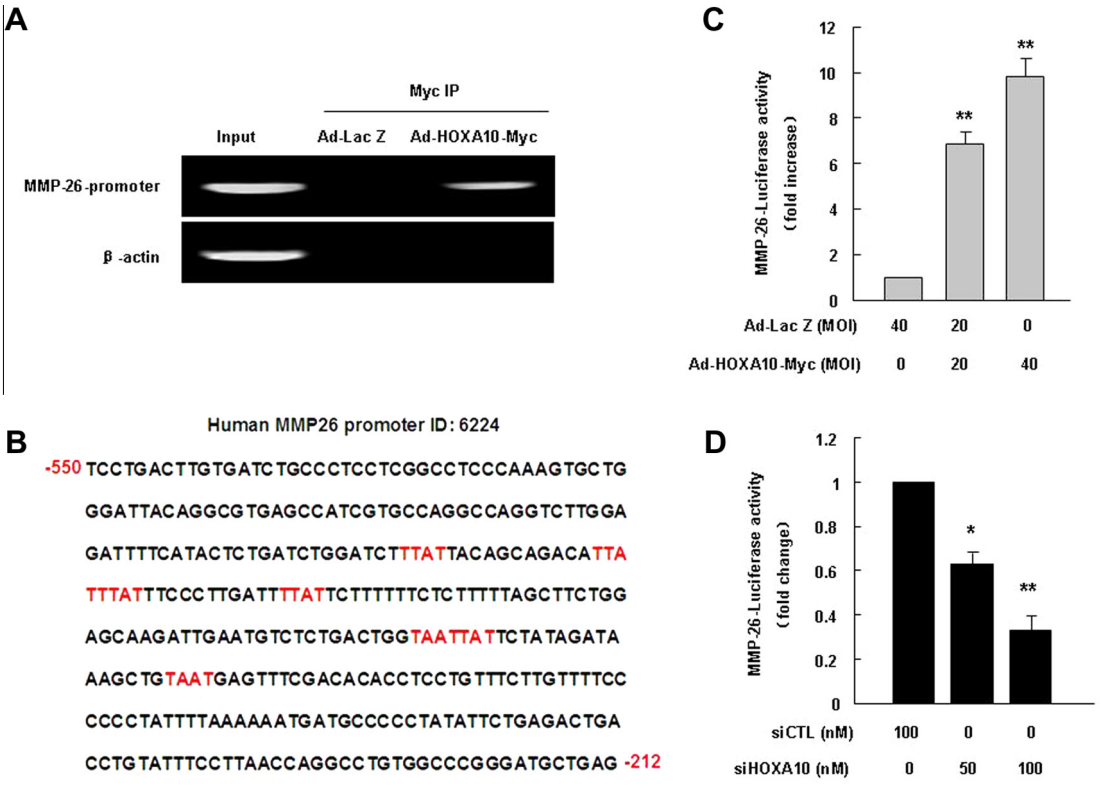

Fig. 1. Identification of MMP-26 as a HOXA10 target gene in human endometrial Ishikawa cells. (A) Chromatin immunoprecipitation assays showed that HOXA10 interacted with the MMP-26 promoter in Ishikawa cells. (B) Schematic representation of the HOXA10 consensus binding sites (TTAT and TAAT) within the MMP-26 promoter region. (C) Overexpression of HOXA10 in Ishikawa cells enhanced human MMP-26 promoter activity demonstrated by luciferase assays ($n = 3$). $^{**}P < 0.01$ compared with Ad-LacZ alone. (D) HOXA10 knockdown suppressed human MMP-26 promoter activity ($n = 3$). $^{*}P < 0.05$, $^{**}P < 0.001$ vs. siCTL alone.

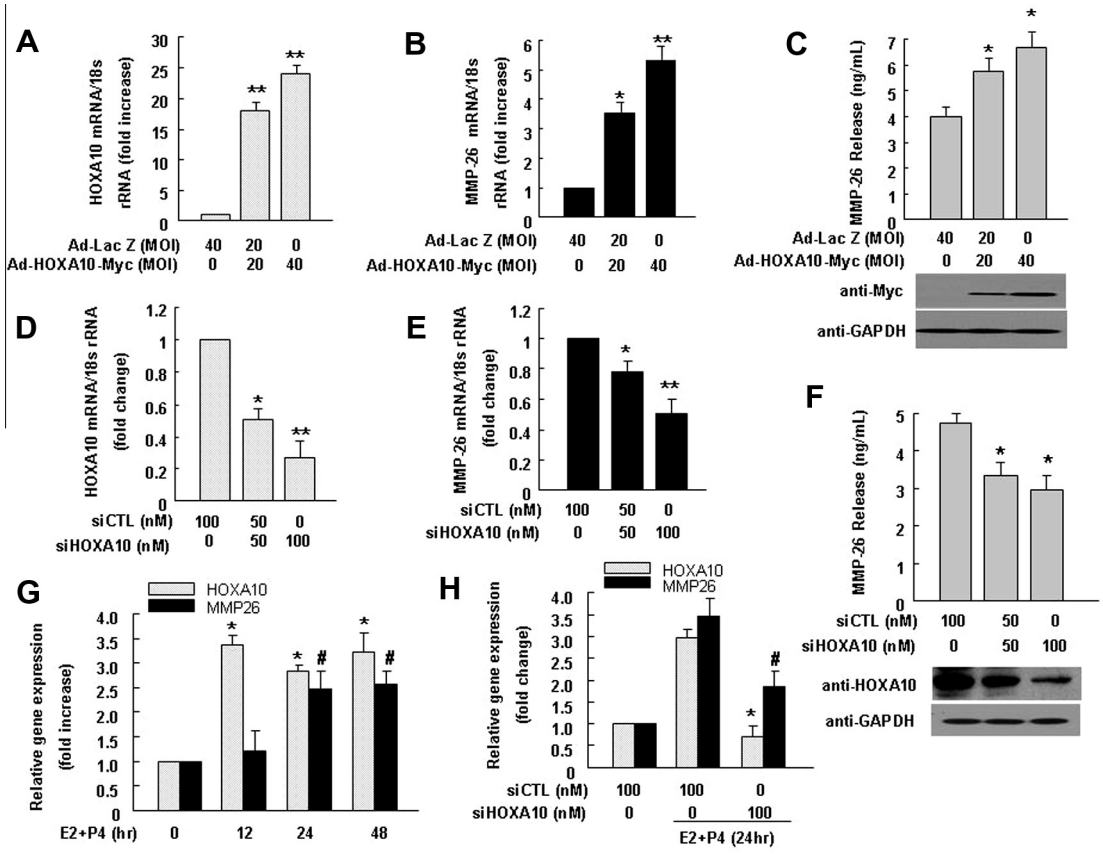

Fig. 2. Effect of HOXA10 on MMP-26 expression. Ishikawa cells were transduced with Ad-LacZ or Ad-HOXA10-Myc at the indicated MOI for 2 days. (A) HOXA10 and (B) MMP-26 mRNA expression levels were measured using qRT-PCR ($n = 3$). $^{*}P < 0.05$, $^{**}P < 0.001$ compared with Ad-LacZ. (C) Release of MMP-26 into the medium was measured by ELISA ($n = 3$), $^{*}P < 0.05$ compared with Ad-LacZ alone. Exogenous HOXA10 protein expression was determined by Western blot analysis using an anti-Myc antibody. Ishikawa cells were transfected with siHOXA10 (50 nM and 100 nM) and siCTL (50 nM and 100 nM) for 72 h. (D) HOXA10 mRNA and (E) MMP-26 mRNA expression were measured by qRT-PCR ($n = 3$). $^{*}P < 0.05$, $^{**}P < 0.001$ vs. siCTL alone. (F) MMP-26 released into the medium was measured by ELISA ($n = 3$); $^{*}P < 0.05$ compared with siCTL alone. Endogenous HOXA10 protein expression was determined by Western blot analysis using an anti-HOXA10 antibody. (G) Ishikawa cells were treated with estrogen (E2) (10 nM) and progesterone (P4) (1 μM) for different times periods as indicated. HOXA10 and MMP-26 mRNA expression levels were measured by qRT-PCR ($n = 3$). $^{*,\#}P < 0.05$ compared with Ad-LacZ. (F) Ishikawa cells were transfected with siHOXA10 (100 nM) and siCTL (100 nM) for 72 h. The cells were then treated with estrogen (E2) (10 nM) and progesterone (P4) (1 μM) for 24 h. HOXA10 and MMP-26 mRNA expression levels were measured by qRT-PCR ($n = 3$). $^{*,\#}P < 0.05$ compared with siCTL alone.

3.3. Identification of the HOXA10 functional binding sites within the human MMP-26 promoter region

HOXA10 transactivates target gene promoters through preferential binding to a consensus core of TTAT or TAAT sequences [19]. Based on the human MMP-26 promoter sequence deposited in the transcriptional regulatory element database (accession No. 6224), we identified seven conserved binding elements within the cloned MMP-26 promoter fragment. To localize the HOXA10-binding site within the MMP-26 promoter, we generated five MMP-26-Luc reporter constructs that were used in luciferase reporter assays: FL-Luc, F1-Luc, F2-Luc, F1-Luc Mu and F2-Luc Mu (Fig. 3A). We found that HOXA10 overexpression in 293T cells activated the luciferase activity of the FL-Luc, F1-Luc and F1-Luc MU constructs by greater than 20-fold. In contrast, HOXA10 overexpression induction of the MMP-26 promoter did not obviously alter the luciferase activity of the F2-Luc and F2-Luc Mu constructs (Fig. 3B). These data suggest that HOXA10 induces MMP-26 promoter activity through the transactivation of three consecutive TTAT sites located between −450 and −410 bp in the human MMP-26 promoter.

To further identify which of the consecutive TTAT units is required for HOXA10 binding, we performed ABCD assays using biotinylated double-stranded oligonucleotides. The WT, MU-1, MU-2 and MU oligonucleotides derived from the MMP-26 promoter sequence are shown in Fig. 3C. As shown in Fig. 3D, Myc-tagged HOXA10 protein strongly bound to WT and MU-2, but not to the oligonucleotide with mutations in all three TTAT units (MU). These data demonstrate that the first conserved TTAT unit (−442 to −439) is required for specific HOXA10 binding.

3.4. Activation of MMP-26 by HOXA10 promotes embryo adhesion in vitro

MMP-26 was localized exclusively to epithelial cells of both the gland and luminal surface, implying that it may be essential for implantation. We therefore fused a Flag tag to the C-terminus of MMP-26 and expressed the fusion protein in a recombinant adenovirus vector. We subsequently infected Ishikawa cells with this adenovirus to upregulate MMP-26 expression and secretion (Fig. 4A and B). Using an *in vitro* adhesion/attachment model with BeWo spheroids, we found that overexpression of Ad-HOXA10 or Ad-MMP-26 in Ishikawa cells could increase the ratio of BeWo spheroid adhesion by approximately 20% compared with the expression of Ad-LacZ (55.1 ± 3.53%, 53.3 ± 4.01% and 31.8 ± 2.24%, respectively; Fig. 4D, gray bars). An antibody-blocking assay further demonstrated that the HOXA10- and MMP-26-mediated promotion of BeWo spheroid adhesion to Ishikawa cells was more significantly inhibited by pre-treatment with an antibody specific to MMP-26 compared with control mouse IgG (For

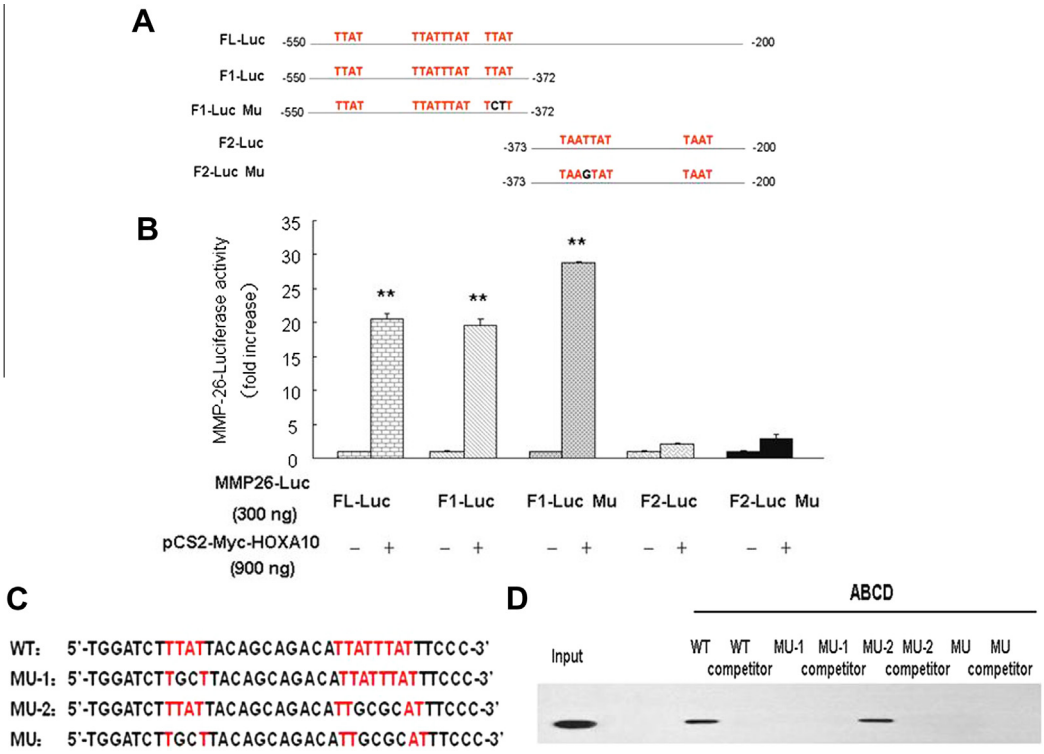

Fig. 3. Identification of functional HOXA10 binding sites within the human MMP-26 promoter region. (A) Schematic representation of human MMP-26 promoter mutants. (B) The effects of HOXA10 on the activity of human MMP-26 promoter mutants ($n = 3$). $^{**}P < 0.01$ compared with empty vector. (C) Schematic representation of the MMP-26 WT, MMP-26 MU-1, MMP-26 MU-2 and MMP-26 MU promoter core regions used in the ABCD assays. (D) The ABCD assays and Western blot analysis illustrate the binding of HOXA10 to the different MMP-26 promoter deletion mutants.

HOXA10, the attachment rates were 55.1 ± 3.53% (control IgG) vs. 42.9 ± 3.78% (MMP-26 antibody); for MMP-26, the attachment rates were 53.3 ± 4.01% (control IgG) vs. 39.7 ± 3.41% (MMP-26 antibody), $P < 0.05$; Fig. 4D, black bars). In addition, Western blot analysis (Fig. 4E) indicated that the overexpression of HOXA10 and MMP-26 could increase the phosphorylation of the focal adhesion kinase (FAK) protein at Tyr 925 in Ishikawa cells, implying that MMP-26 could active FAK signaling pathways to regulate embryo-endometrial interactions.

These results suggest that MMP-26 secreted from the glandular epithelium is essential for embryo adhesion.

4. Discussion

Successful implantation of a good quality human embryo in a receptive endometrium requires complex interactions between multiple factors [1–2]. As a known marker of endometrial receptivity, the HOXA10 transcription factor regulates the downstream target genes that are also involved in implantation [13–15]. In the present study, we used chromatin immunoprecipitation to identify MMP-26 as a novel HOXA10 target gene in human endometrial epithelial cells.

MMP-26 is a novel enzyme that displays a unique expression pattern and is different from all other known endometrial MMPs. MMP-26 mRNA is localized to epithelial cells and upregulated during the early secretory phase of the cycle immediately preceding the period of embryo implantation [20]. The regulation of MMP-26 is a complicated process. Several reports have indicated that estrogen regulates MMP-26 expression through a direct estrogen receptor-mediated pathway (ESR-I) and indirectly through the regulation of other pathways in women [21]. Recently, using an ovariectomized hormone-treated rhesus macaque model, Almeida et al. confirmed that endometrial MMP-26 expression is dependent on the presence of progesterone in the early secretory phase, while it gradually becomes refractory to progesterone stimulation in the late secretory phase [22].

HOXA10 acts downstream of estrogen and progesterone to regulate endometrial function [8,12]. We found seven conserved HOXA10 binding elements (TTAT or TAAT) within the cloned MMP-26 promoter fragment; thus, it is reasonable to presume that HOXA10 directly regulates MMP-26 expression. In this study, via ChIP/PCR, ABCD and qRT-PCR analysis, we observed that HOXA10 bound specifically to a conserved TTAT unit (−442 to −439) within the MMP-26 promoter and increased MMP-26 expression. Importantly, the knockdown of endogenous HOXA10 expression in Ishikawa cells significantly attenuated the estrogen- and progesterone-mediated induction of MMP-26 mRNA expression. However, the significance of the HOXA10 regulation of MMP-26 secretion in the human endometrium is not clear.

Matrix metalloproteinases (MMPs) and their inhibitors have been shown to play direct roles in the process of embryonic implantation [23]. As previously reported, MMP-26 is exclusively expressed in the epithelial compartment during the early secretory phase and is barely expressed in stromal cells [20]. However, we found that HOXA10 also regulated MMP-26 expression in human stromal cells and MMP-26 secretion from the mid-secretory endometrium (data not shown); thus, MMP-26 may degrade the basement membrane of endometrial cells to facilitate embryo attachment and the subsequent invasion of the underlying maternal endometrial tissue. In this study, we observed that the upregulation of MMP-26 expression and secretion could increase the attachment of BeWo spheroids to Ishikawa cells, and this adhesion effect was significantly inhibited *in vitro* by MMP-26 blocking antibodies. Additionally, the overexpression of HOXA10 and MMP-26 activated integrin-FAK signaling pathways; however, the detailed

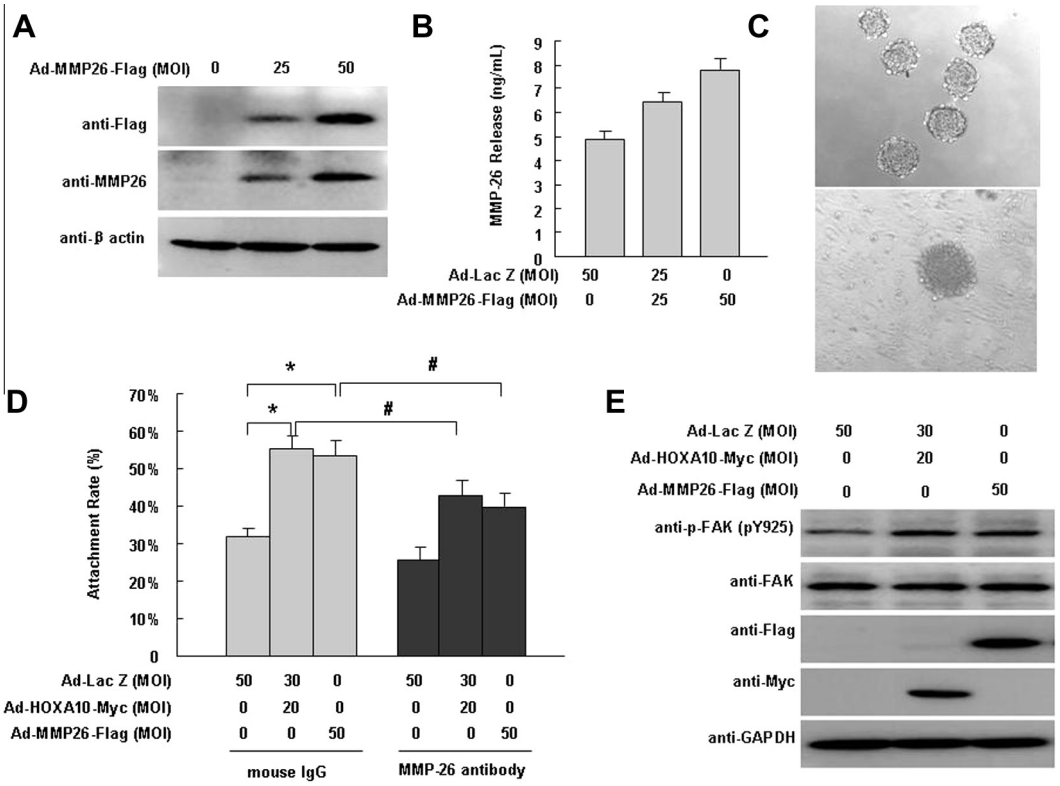

Fig. 4. HOXA10 upregulates MMP-26 expression and mediates BeWo spheroid adhesion to Ishikawa cells. (A, B) Ishikawa cells were transduced with Ad-MMP26-Flag at the indicated MOI for 2 days. MMP-26 protein expression was measured by Western blot and ELISA. (C) BeWo spheroids (150–200 μm diameter) were attached to Ishikawa cells after 1.5 h of co-culture. (D) Adhesion experiments with BeWo spheroids attached to the Ishikawa cell monolayer. The data are the average of three independent experiments, $n = 3$. ANOVA was used to compare the percentage of the attached spheroids in each treatment with that of the control. The error bars indicate ±SD of three independent experiments; $^{*,\#}P < 0.05$ versus the control group. (E) Overexpression of MMP-26 enhances FAK phosphoryation at Tyr 925. Ishikawa cells were infected with the indicated adenoviruses for 48 h, and whole-cell lysates were analyzed by Western blotting with the indicated antibodies.

molecular mechanisms of this process require further investigation.

Integrins are the most intensely studied group of cell adhesion molecules in the endometrium [23]. Integrins bind the ECM or secreted proteins containing an arginine-glycine-aspartic acid (RGD) tripeptide and are involved in trophoblast adhesion and migration. A mid-secretory phase increase in endometrial epithelial αVβ3 integrin is the result of an increase in β3 abundance after day 19, which is itself an indirect effect of steroids mediated by the transcription factor HOXA10 [13]. It has been shown that matrix metalloproteinase 2 (MMP-2) binds and becomes activated by αVβ3 integrin on the surface of cultured melanoma cells [24]. Unfortunately, we were unable to detect an interaction between MMP-26 and β3 integrin by immunoprecipitation. Further evidence supporting a correlation between integrins and MMPs was presented in a recent report, which demonstrated that αVβ3 integrin cooperates with matrix metalloproteinase 9 (MMP-9) in breast cancer cell migration [25]. Furthermore, MMP-9 is thought to be a key mediator of matrix degradation during implantation [26]. MMP-26 can directly cleave the inactive pro-MMP-9 protein into the mature active gelatinase isoform [27]. Collectively, MMP-26 secreted from the glandular epithelium regulates embryo-endometrial interactions, which may be dependent on cooperation between αVβ3 integrin and MMP-9.

In conclusion, to our knowledge, our study provides the first evidence that MMP-26 is a novel HOXA10 downstream target gene and induces the HOXA10-mediated embryo attachment process. A better understanding of how the endometrial receptivity marker gene HOXA10 regulates the implantation process will improve the pregnancy rate in IVF programs and may improve the prognosis for diseases such as endometriosis.

Competing interests

The authors declare that there is no conflict of interest related to this paper.

Acknowledgments

This work was supported by the State Key Development Program for Basic Research of China (973 Project No. 2010CB945104), the National Natural Science Foundation of China (Nos. 81170570, 30900727, 81070492, 81370683) and University Graduate Research and innovation project (Nos. CXZZ13_D587).

References

[1] H. Singh, L. Nardo, S.J. Kimber, J.D. Aplin, Early stages of implantation as revealed by an in vitro model, Reproduction 139 (2010) 905–914.
[2] S.K. Dey, H. Lim, S.K. Das, J. Reese, B.C. Paria, T. Daikoku, H. Wang, Molecular cues to implantation, Endocr. Rev. 25 (2004) 341–373.
[3] Y.E. Koot, G. Teklenburg, M.S. Salker, J.J. Brosens, N.S. Macklon, Molecular aspects of implantation failure, Biochim. Biophys. Acta 2012 (1822) 1943–1950.
[4] C. Zhang, M.J. Large, R. Duggavathi, F.J. DeMayo, J.P. Lydon, K. Schoonjans, E. Kovanci, B.D. Murphy, Liver receptor homolog-1 is essential for pregnancy, Nat. Med. 19 (2013) 1061–1066.
[5] S. Pawar, E. Starosvetsky, G.D. Orvis, R.R. Behringer, I.C. Bagchi, M.K. Bagchi, STAT3 regulates uterine epithelial remodeling and epithelial-stromal crosstalk during implantation, Mol. Endocrinol. 27 (2013) 1996–2012.
[6] X. Sun, L. Zhang, H. Xie, H. Wan, B. Magella, J.A. Whitsett, S.K. Dey, Kruppel-like factor 5 (KLF5) is critical for conferring uterine receptivity to implantation, Proc. Natl. Acad. Sci. USA 109 (2012) 1145–1150.
[7] I. Satokata, G. Benson, R. Maas, Sexually dimorphic sterility phenotypes in Hoxa10-deficient mice, Nature 374 (1995) 460–463.
[8] H. Eun Kwon, H.S. Taylor, The role of HOX genes in human implantation, Ann. N. Y. Acad. Sci. 1034 (2004) 1–18.

[9] S.K. Das, Regional development of uterine decidualization: molecular signaling by Hoxa-10, Mol. Reprod. Dev. 77 (2010) 387–396.
[10] L.H. Zhu, L.H. Sun, Y.L. Hu, Y. Jiang, H.Y. Liu, X.Y. Shen, X.Y. Jin, X. Zhen, H.X. Sun, G.J. Yan, PCAF impairs endometrial receptivity and embryo implantation by down-regulating beta3-integrin expression via HOXA10 acetylation, J. Clin. Endocrinol. Metab. 98 (2013) 4417–4428.
[11] G.S. Daftary, H.S. Taylor, Pleiotropic effects of Hoxa10 on the functional development of peri-implantation endometrium, Mol. Reprod. Dev. 67 (2004) 8–14.
[12] Y. Gui, J. Zhang, L. Yuan, B.A. Lessey, Regulation of HOXA-10 and its expression in normal and abnormal endometrium, Mol. Hum. Reprod. 5 (1999) 866–873.
[13] G.S. Daftary, P.J. Troy, C.N. Bagot, S.L. Young, H.S. Taylor, Direct regulation of beta3-integrin subunit gene expression by HOXA10 in endometrial cells, Mol. Endocrinol. 16 (2002) 571–579.
[14] P.J. Troy, G.S. Daftary, C.N. Bagot, H.S. Taylor, Transcriptional repression of peri-implantation EMX2 expression in mammalian reproduction by HOXA10, Mol. Cell. Biol. 23 (2003) 1–13.
[15] H. Sun, L. Chen, G. Yan, R. Wang, Z. Diao, Y. Hu, C. Li, HOXA10 suppresses p/CAF promoter activity via three consecutive TTAT units in human endometrial stromal cells, Biochem. Biophys. Res. Commun. 379 (2009) 16–21.
[16] D. Cermik, B. Selam, H.S. Taylor, Regulation of HOXA-10 expression by testosterone in vitro and in the endometrium of patients with polycystic ovary syndrome, J. Clin. Endocrinol. Metab. 88 (2003) 238–243.
[17] G.S. Daftary, H.S. Taylor, Hydrosalpinx fluid diminishes endometrial cell HOXA10 expression, Fertil. Steril. 78 (2002) 577–580.
[18] X. Shen, Y. Hu, Y. Jiang, H. Liu, L. Zhu, X. Jin, H. Shan, X. Zhen, L. Sun, G. Yan, H. Sun, Kruppel-like factor 12 negatively regulates human endometrial stromal cell decidualization, Biochem. Biophys. Res. Commun. 433 (2013) 11–17.
[19] G.V. Benson, T.H. Nguyen, R.L. Maas, The expression pattern of the murine Hoxa-10 gene and the sequence recognition of its homeodomain reveal specific properties of Abdominal B-like genes, Mol. Cell. Biol. 15 (1995) 1591–1601.
[20] R. Pilka, C. Whatling, H. Domanski, S. Hansson, P. Eriksson, B. Casslen, Epithelial expression of matrix metalloproteinase-26 is elevated at mid-cycle in the human endometrium, Mol. Hum. Reprod. 9 (2003) 271–277.
[21] J. Liu, B. Cao, Y.X. Li, X.Q. Wu, Y.L. Wang, GnRH I and II up-regulate MMP-26 expression through the JNK pathway in human cytotrophoblasts, Reprod. Biol. Endocrinol. 8 (2010) 5.
[22] C.C. Almeida-Francia, C.S. Keator, K. Mah, L. Holden, C. Hergert, O.D. Slayden, Localization and hormonal regulation of endometrial matrix metalloproteinase-26 in the rhesus macaque, Hum. Reprod. 27 (2012) 1723–1734.
[23] O. Guzeloglu-Kayisli, M. Basar, A. Arici, Basic aspects of implantation, Reprod. Biomed. Online 15 (2007) 728–739.
[24] P.C. Brooks, S. Stromblad, L.C. Sanders, T.L. von Schalscha, R.T. Aimes, W.G. Stetler-Stevenson, J.P. Quigley, D.A. Cheresh, Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin alpha v beta 3, Cell 85 (1996) 683–693.
[25] M. Rolli, E. Fransvea, J. Pilch, A. Saven, B. Felding-Habermann, Activated integrin alphavbeta3 cooperates with metalloproteinase MMP-9 in regulating migration of metastatic breast cancer cells, Proc. Natl. Acad. Sci. USA 100 (2003) 9482–9487.
[26] M.G. Martinez-Hernandez, L.A. Baiza-Gutman, A. Castillo-Trapala, D.R. Armant, Regulation of proteinases during mouse peri-implantation development: urokinase-type plasminogen activator expression and cross talk with matrix metalloproteinase 9, Reproduction 141 (2011) 227–239.
[27] Y.G. Zhao, A.Z. Xiao, R.G. Newcomer, H.I. Park, T. Kang, L.W. Chung, M.G. Swanson, H.E. Zhau, J. Kurhanewicz, Q.X. Sang, Activation of pro-gelatinase B by endometase/matrilysin-2 promotes invasion of human prostate cancer cells, J. Biol. Chem. 278 (2003) 15056–15064.