Biochemical and Biophysical Research Communications 379 (2009) 16–21

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

HOXA10 suppresses p/CAF promoter activity via three consecutive TTAT units in human endometrial stromal cells

Haixiang Sun [a,b,1], Linjun Chen [b,1], Guijun Yan [b], Ruina Wang [a], Zhenyu Diao [b], Yali Hu [b], Chaojun Li [a,c,*]

[a] College of Life Sciences, Nanjing Normal University, 1 Wenyuan Road, Nanjing 210046, PR China
[b] Reproductive Medicine Center, The Affiliated Drum Tower Hospital of Nanjing University, Medical School, Nanjing 210008, PR China
[c] Jiangsu Key Laboratory for Molecular and Medical Biotechnology, Nanjing Normal University, Nanjing 210046, PR China

ARTICLE INFO

Article history:
Received 17 November 2008
Available online 11 December 2008

Keywords:
HOXA10
p/CAF
Endometrium

ABSTRACT

Implantation is the first maternal–embryo crosstalk that only occurs during a finite period called the 'implantation window'. HOXA10, a homeobox transcription factor, plays an important regulatory role during this period. However, the target genes of HOXA10 involved in implantation and decidualization have not been identified. Using a chromatin immunoprecipitation screen, we identified the p300/CBP-associated factor (p/CAF) as a direct HOXA10 target gene *in vivo*. Adenovirus-mediated overexpression and siRNA-specific knockdown of HOXA10 altered p/CAF promoter activity via interaction with the three consecutive TTAT element units in human endometrial cells. These results indicate that p/CAF is a novel HOXA10 target gene, and HOXA10 promotes human endometrial development, at least in part, through the regulation of p/CAF gene.

Embryo implantation is one of the earliest events in human, and all mammalian, reproduction and determines whether pregnancy will proceed successfully. This process begins with close interaction between the embryo and the uterus. Numerous studies have suggested that the process of embryo implantation is likely to be mediated via a number of signaling and adhesion molecules. Hox genes, an evolutionarily conserved family of transcription factors, have been reported to play critical roles in controlling these physiological processes [1–3].

Like other homeobox genes, HOXA10 is expressed in both the embryonic and the adult reproductive tracts and is found predominantly in the uterus. Previous studies have found that HOXA10 expression is dependent on menstrual cycle stage [4], and that levels of HOXA10 expression are diminished in the endometria of women with endometriosis, polycystic ovary syndrome [5], and idiopathic infertility [6]. These findings indicate that HOXA10 may play an important role in development of the uterus and may be essential for endometrial development and fertility. In fact, blockade of HOXA10 expression via HOXA10 antisense oligonucleotide administration into the uterine lumen results in decreased rate of implantation [7]. In adult HOXA10-deficient female mice, implantation was found to be severely compromised, and defective decidualization led to recurrent pregnancy loss and infertility [2,8]. These results further support the critical role of HOXA10 in the regulation of endometrial stromal cell physiological function.

Recent evidence has demonstrated that HOXA10 reduces the expression of insulin-like growth factor-binding protein (IGFBP1) in decidualizing stromal cells and controls the decidualization process by preventing premature differentiation and apoptosis [9,10]. However, the precise molecular mechanisms involved in HOXA10 regulation of human endometrial stromal cell physiological function remain undefined.

To identify HOXA10 target genes in the endometrium, we performed chromatin immunoprecipitation assays and identified the p300/CBP-associated factor (p/CAF) as a novel HOXA10 target gene in human endometrial stromal cells (hESC). We found that HOXA10 directly binds to three consecutive nucleotide sequences of TTAT and suppresses p/CAF promoter activity. Our results suggest that p/CAF may be a novel gene involved in fertility.

Materials and methods

Human endometrial stromal cell culture. Human endometrial stromal cells were isolated from normal endometrial tissues of normal cycling women by endometrial biopsy at the time of diagnostic laparoscopy for fallopian tube obstruction. Histological examination of the endometrium was normal. This study was approved by Drum Tower Hospital Research and Ethics Committee, and patient consent was obtained before biopsy.

HESCs were isolated as previously described [11]. Briefly, endometrial tissues were minced and enzymatically digested with 0.1%

* Corresponding author. Address: College of Life Sciences, Nanjing Normal University, 1 Wenyuan Road, Nanjing 210046, PR China. Fax: +86 25 858 91870.
E-mail address: lichaojun@njnu.edu.cn (C. Li).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2009 Published by Elsevier Inc.
doi:10.1016/j.bbrc.2008.11.144

collagenase I (Invitrogen, Carlsbad, CA) in Dulbecco's modified Eagle's medium/F12 (DMEM/F12, Invitrogen, Carlsbad, CA), for 1 h at 37 °C. Stromal cells were separated from intact glands by filtration of the digested tissue through 40 μM gauze. After centrifugation at 400*g* for 5 min, the pellet was resuspended in DMEM/F12 and 10% heat-inactivated fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA). The endometrial stromal cells were then maintained in DMEM/F12, supplemented with 10% FBS and 50 IU/ml–50 μg/ml penicillin–streptomycin at 37 °C in a humidified environment with 5% CO_2. The purity of the cultured stromal cells was better than 95%, as determined by immunohistochemical staining with polyclonal antibodies against vimentin and cytokeratin (Santa Cruz Biotechnology, CA). The cells were used between passages 2 and 10 in this study.

Construction of adenovirus. Adenovirus harboring HOXA10 was made using AdMax (Microbix). Briefly, pBHGlox E1 and 3Cre, including the E1 adenoviral genome, were cotransfected with the pDC shuttle vector containing the gene of interest into Ad293 cells using Fugene 6 (Roche). Adenovirus bearing LacZ was obtained from Clontech. The viruses were propagated in Ad293 cells and purified using $CsCl_2$ banding followed by dialysis against 10 mmol/l Tris-buffered saline with 10% glycerol. Titration was performed on Ad293 cells using Adeno-X Rapid Titer kit (Clontech) according to the manufacturer's instructions.

Chromatin immunoprecipitation (ChIP) assay. ChIP was performed based on the protocol provided in the kit with some modifications (ChIP assay kit by Upstate Biotechnology). Briefly, hESCs (70–80% confluence) were infected with Ad-LacZ and Ad-HOXA10 (50 MOI) for 72 h, washed with PBS, and cross-linked with 1% formaldehyde for 15 min at room temperature. Cross-linking was stopped with the addition of glycine (0.125 M final concentration) for 10 min. Cells were washed twice with cold PBS and then harvested in lysis buffer A (20 mM Tris–HCl, pH 8.0, 85 mM KCl, 1 mM EDTA, 0.5 mM EGTA, 0.5% Nonidet P40, 1 mM PMSF, 1 μg/ml aprotinin, and 1 μg/ml pepstatin A), and pelleted by centrifugation. Cell pellets were then lysed in nuclear lysis buffer B (50 mM Tris–HCl, pH 8.0, 10 mM EDTA, 1% SDS, with protease inhibitors mentioned above). Cell samples were sonicated in ice to yield genomic DNA fragments that were approximately 500–1000 bp in size. Precleared sonicates were then incubated with HOXA10 antibody (2 μg, Santa Cruz) and rotated overnight. Protein A/G beads (Upstate Biotechnology) were added for 3 h, and the collected beads were washed extensively. Immunocomplexes were eluted by incubation at 65 °C for 30 min and then at room temperature for 15 min with fresh elution buffer (1% SDS; 0.1 M $NaHCO_3$). Cross-links were reversed by incubation for 5 h at 65 °C with a final concentration of 0.3 M NaCl. The eluates were incubated with proteinase K, and the DNA was purified using a phenol:chloroform extraction with an ethanol precipitation. Finally, the purified DNA fragments were cloned into a pUC19 vector and sequenced to confirm their identity.

Luciferase reporter assay. About 1.0 kb of the p/CAF promoter sequence (−941/−26) and its deletion mutants were amplified by PCR and cloned in pGL3-basic vector (Promega) with KpnI/NheI sites. Preconfluent (75–80%) endometrial stromal cells and Ishilkawa cells, in six-well plates, were transduced with Ad-LacZ and Ad-HOXA10 at the MOI indicated for 48 h. Then the cells were transfected with 600 ng cDNA luciferase reporter constructs using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). Forty-eight hours after transfection, cell lysates were assayed for luciferase activities using the dual Luciferase Assay System (Promega). We used *Renilla* luciferase as a control for transfection efficiency. Values for firefly luciferase were normalized to *Renilla* luciferase under the control of the thymidine kinase promoter in the pRLTK vector. Transfections were performed in duplicate and experiments were repeated five times.

Western blot. Proteins were prepared and separated on SDS–PAGE as described [12]. Immunoblotting was performed with the primary antibodies to HOXA10 (1:1000 dilution; Santa Cruz), p/CAF (1:200 dilution; Santa Cruz), myc-HRP (1:5000 dilution; Invitrogen), and α-tubulin (1:5000 dilution; Sigma). Immunodetection was accomplished using a goat anti-rabbit secondary antibody or rabbit anti-mouse secondary antibody and the enhanced chemiluminescence kit (Amersham Corporation).

Gene silencing with small interference RNA. Two pairs of siRNA oligonucleotides for human HOXA10 (sense strand, 5′-UAA GGU ACA UAU UGA ACA GAA ACU C-3′ (HOXA10 siRNA-A) 5′-GAG UUU CUG UUC AAU AUG UAC AUU A-3′ (HOXA10 siRNA-B)) and a pair of Control siRNA oligonucleotides were purchased from Invitrogen (Carlsbad, CA).

HESCs and Ishilkawa cells grown to 50% confluence were transfected with target-specific siRNA (20 nM) and control siRNA (20 nM) with Lipofectamine 2000 in serum-free Opti-MEM medium according to the manufacturer's recommendation. Six hours post-transfection, fresh complete DMEM/F12 medium was added, and the cells were cultured for an additional 72 h for protein analyses of HOXA10.

Avidin–biotin conjugate DNA precipitation assay. The following double-stranded oligonucleotides were used, all of which were biotinylated at the 3-end of the sense strand: p/CAF wild: 5′-TCG ATA TTT ATT CTT TTG AAT TAT GCT TAT ATC TTT T-3′, p/CAF reverse: 5′-TGC ATA TTT ATG CTT ATT TTG AAT TAT ATC TTT T-3′ and p/CAF mutation: 5′-TCG ATA TCG ATT CTT TTG AAC GAT GCC GAT ATC TTT T-3′. An avidin–biotin conjugate DNA precipitation (ABCD) assay was performed by incubating 500 μg cell extracts derived from hESCs with 500 pmol of each double-stranded DNA extract immobilized on streptavidin agarose in binding buffer (10 mM Tris, pH 8.0, 150 mM NaCl, 0.5% Triton X-100, 0.5 mM DTT, 0.5 mM EDTA, 10% glycerol, 20 mg/ml poly[dI–dC], and protease inhibitors). After 3 h of incubation at 4 °C, the beads were washed four times with the same buffer and proteins were resolved by SDS–PAGE, followed by electrotransfer onto polyvinylidine fluoride membrane and probed with the anti-HOXA10 antibody. Chemiluminescence was accomplished using a goat anti-rabbit secondary antibody and the enhanced chemiluminescence kit.

Statistics. All values are expressed as means ± SE. Differences between mean values were analyzed using a two-tailed Student *t*-test. Significant differences were noted when $P < 0.05$.

Results

HOXA10 binds to the p/CAF promoter in human endometrial stromal cells

Previous studies indicated that the transcription factor HOXA10 plays an important role in endometrial physiological functions [2,13,14]. However, the transcriptional targets of HOXA10 remain poorly understood. In this study, we successfully established stromal cell monolayer cultures from endometrial biopsies. More than 95% of the cells in the cultures were stromal cells, which are characterized by their spindle shape, positive vimentin staining, and negative cytokeratin immunohistochemical reaction (Fig. 1A). To identify the novel, direct HOXA10 target genes, the soluble chromatins from cultured hESCs were immunoprecipitated with specific antibodies against HOXA10, and the purified DNA fragments from the HOXA10–chromatin complexes were cloned and sequenced. We obtained a total of 86 clones and sequenced 20 clones derived from hESCs. Among the sequenced clones, four appeared to be from promoter regions, which correspond to bases −1085 to −478 of the promoter for the p/CAF gene.

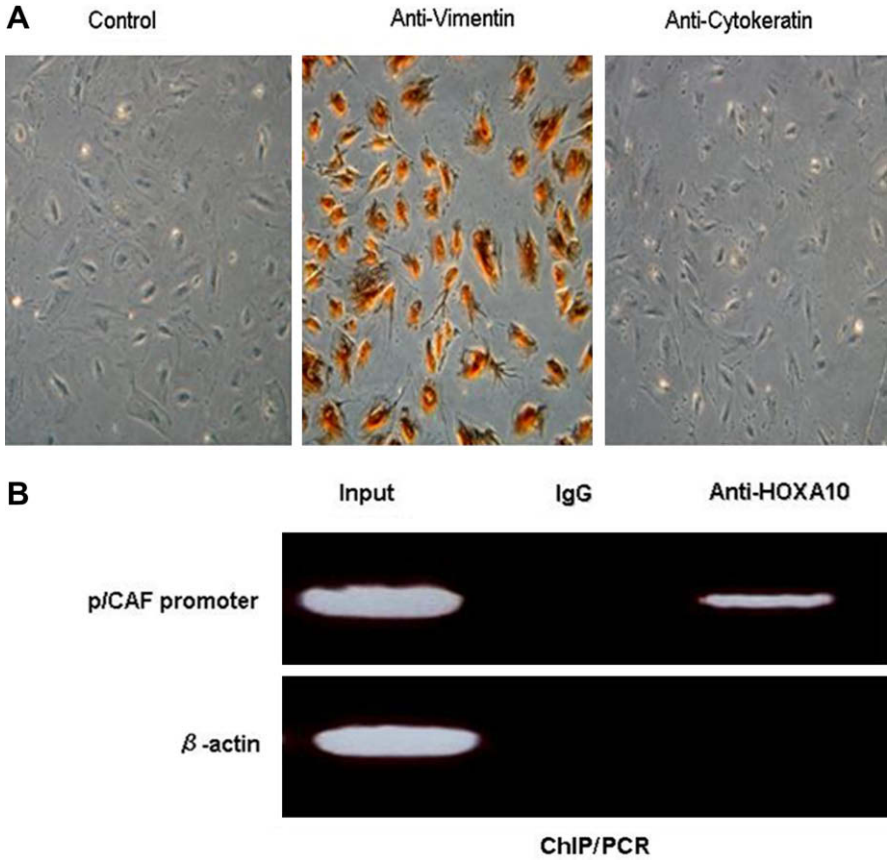

Fig. 1. Identification of HOXA10 target gene in human endometrial stromal cells. (A) Cultured human endometrial stromal cells were stained with anti-cytokeratin and anti-vimentin antibodies. (B) Chromatin immunoprecipitation assays showed interaction of HOXA10 with the p/CAF promoter in hESCs. Soluble chromatin from hESCs was immunoprecipitated with an antibody against HOXA10 or normal rabbit IgG as a negative control. Precipitated DNA fragments were then analyzed by PCR amplification using primers against the human p/CAF promoter region or the β-actin coding region.

The conventional ChIP-PCR analysis was further used to investigate whether the p/CAF promoter is a direct target for HOXA10 *in vivo*. After immunoprecipitation with a specific antibody against HOXA10 and normal rabbit IgG, the immunoprecipitated DNAs were analyzed by PCR with the specific primer pairs derived from the cloned p/CAF promoter fragment. As shown in Fig. 1B, the p/CAF promoter was effectively recovered from immunoprecipitates of HOXA10, but not from control normal rabbit IgG. No PCR product was obtained from HOXA10 or normal rabbit IgG immunoprecipitates using control β-actin primers. These data demonstrate that endogenous HOXA10 is associated with the chromatinized p/CAF promoter in hESCs.

Overexpression of HOXA10 suppresses human p/CAF promoter activity and down-regulates p/CAF expression

To explore the functional role of HOXA10 binding to the p/CAF promoter, p/CAF promoter activity and its expression were analyzed in intact cells. After transfection with myc-tagged HOXA10 adenovirus, the p/CAF promoter-luciferase (Luc) reporter construct was transiently transfected into both hESCs and Ishikawa cells. We found that overexpression of HOXA10 decreased luciferase activity by 70–85% in both hESCs and Ishikawa cells compared to control LacZ adenovirus (Fig. 2A and B). Moreover, overexpression of HOXA10 markedly inhibited endogenous p/CAF expression in a dose-dependent manner, and maximal inhibition was approximately 90% in hESCs (Fig. 2C). These data demonstrated that HOXA10 binds to p/CAF promoter, inhibits p/CAF promoter activity and thus down-regulates p/CAF expression.

HOXA10 knockdown activates p/CAF promoter activity

To further investigate the functional role of endogenous HOXA10 in the regulation of p/CAF promoter activity, we performed loss-of-function studies using the RNA interference technique. Transfection of HOXA10-specific siRNA significantly inhibited HOXA10 expression by approximately 80% in hESCs (Fig. 3A). Furthermore, knockdown of HOXA10 increased basal p/CAF promoter activity by greater than twofold in hESCs (Fig. 3B) and approximately twofold in Ishikawa cells (Fig. 3C) compared with control siRNA.

Three consecutive TTAT elements in p/CAF promoter are required for HOXA10 functional binding

The transcription factor HOXA10 consensus binding site has been defined by random binding site selection [15]. This site contains a TTAT core sequence shared by a subset of *HOX* genes and flanked by nucleotides that together make up the 12-bp element 5′-AA(A/T)TTTATTAC-3′. We visually scanned the cloned p/CAF promoter fragment sequence and found five potential sites located at base pair −1085 to −468 relative to the start of p/CAF transcription. Interestingly, the three TTAT sites near base pair −690 are consecutive with no intervening base pairs between sequences. These three repeats resemble the three consecutive TTAT sites in the p21 promoter [16], suggesting that they are potentially transcription factor-binding sites. To test this hypothesis, a series of promoter-Luc reporter plasmids corresponding to p/CAF promoter deletion mutants were generated and used in luciferase reporter

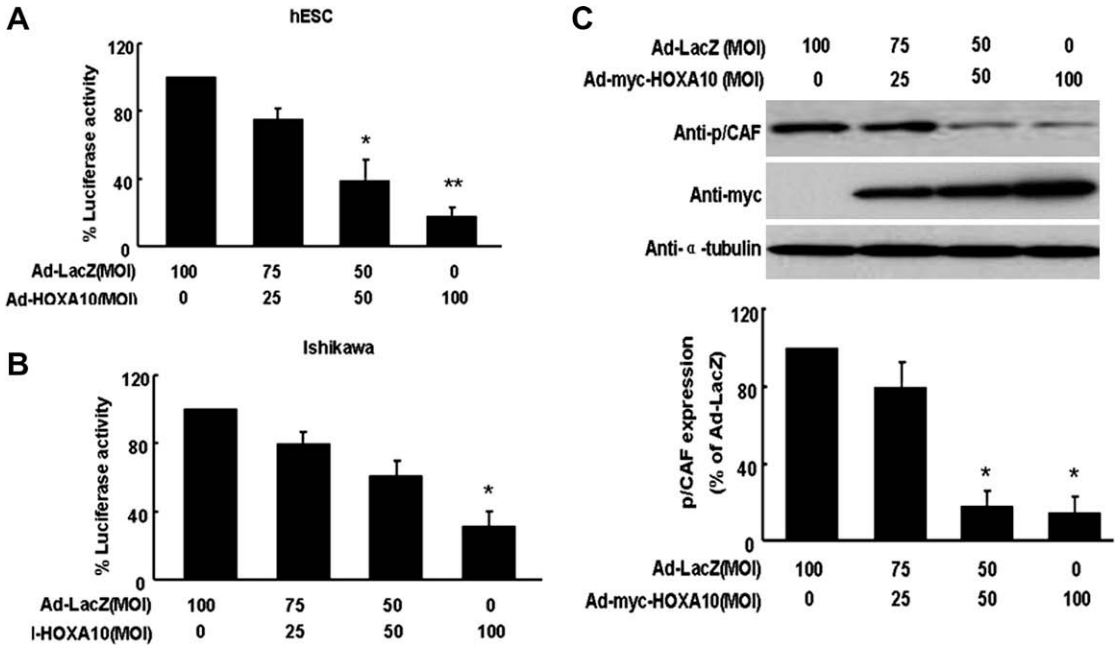

Fig. 2. Overexpression of HOXA10 reduced human p/CAF promoter activity and down-regulated p/CAF expression. hESCs (A) and Ishilkawa cells (B) in six-well plates were transfected with Ad-LacZ and Ad-HOXA10 at the MOI indicated for 48 h. Cells were then transfected with 600 ng p/CAF promoter-Luc reporter plasmid. Forty-eight hours later, cell lysates were collected and assayed for luciferase activity ($n = 5$). (C) hESCs in a 100-mm plate were transfected with myc-tagged HOXA10 adenovirus at the indicated MOI. Forty-eight hours after transduction, cell lysates were collected for Western blot analysis. $^{*}P < 0.05$; $^{**}P < 0.001$ vs Ad-LacZ alone.

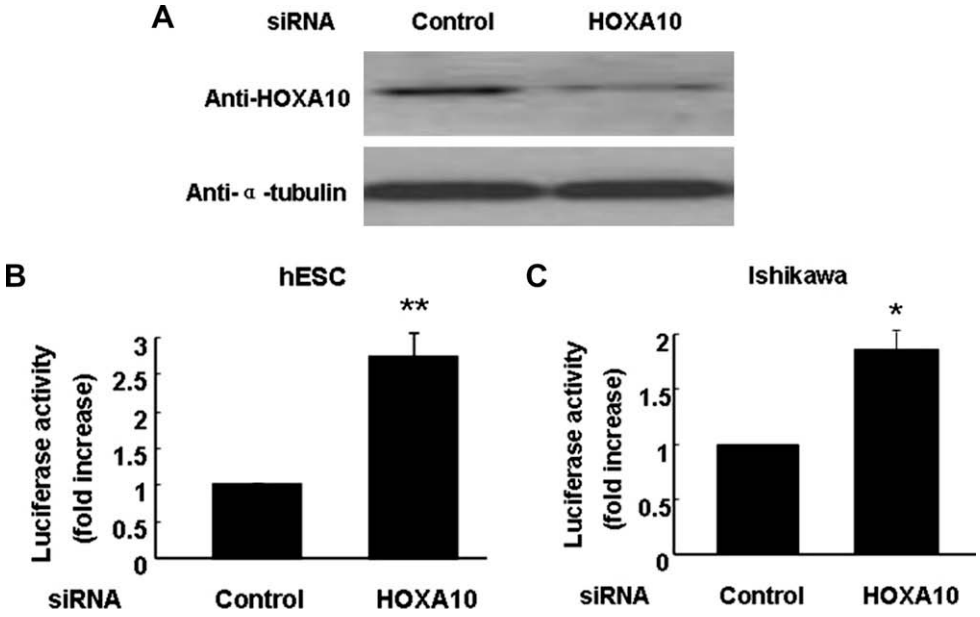

Fig. 3. HOXA10 knockdown activated p/CAF promoter activity. (A) Western blot showed the expression of HOXA10 in hESCs transfected with HOXA10-specific siRNA and control siRNA. (B,C) Knockdown of HOXA10 activated p/CAF promoter activity both in hESCs (B) and Ishilkawa cells (C). hESCs and Ishilkawa cells were transfected with HOXA10-specific siRNA and control siRNA, then cells were transfected with 600 ng p/CAF promoter-Luc reporter plasmid. Forty-eight hours after transfection, cell lysates were collected and assayed for luciferase activity ($n = 5$). $^{*}P < 0.05$; $^{**}P < 0.001$ vs control siRNA alone.

assays (Fig. 4A). For the constructs *P1LUC* and *P2LUC*, which each contain three consecutive TTAT sites, HOXA10 binding was retained. However, deletion of just one of the three consecutive TTAT sites resulted in a loss of binding (Fig. 4A). These results indicate that the three consecutive TTAT elements located between base pairs −710 to −674 relative to the p/CAF promoter are required for HOXA10-stimulated reporter activity.

To determine whether the three consecutive TTAT units are required for HOXA10 binding, we divided the three core TTAT elements, and generated 3 p/CAF-Luc reporter constructs: namely, p/CAF WT, p/CAF REV, and p/CAF MUT (Fig. 4B). Because the p/CAF promoter was identified as a novel *in vivo* target for HOXA10 (Fig. 1B), we next studied whether HOXA10 could interact with these three consecutive TTAT elements *in vitro*. We performed ABCD assays using biotin conjugated, double-stranded oligonucleotides containing the sequences shown in Fig. 4B. The whole cell lysates extracted from intact hESCs were mixed with each oligonucleotide that was immobilized on streptavidin agarose. We found

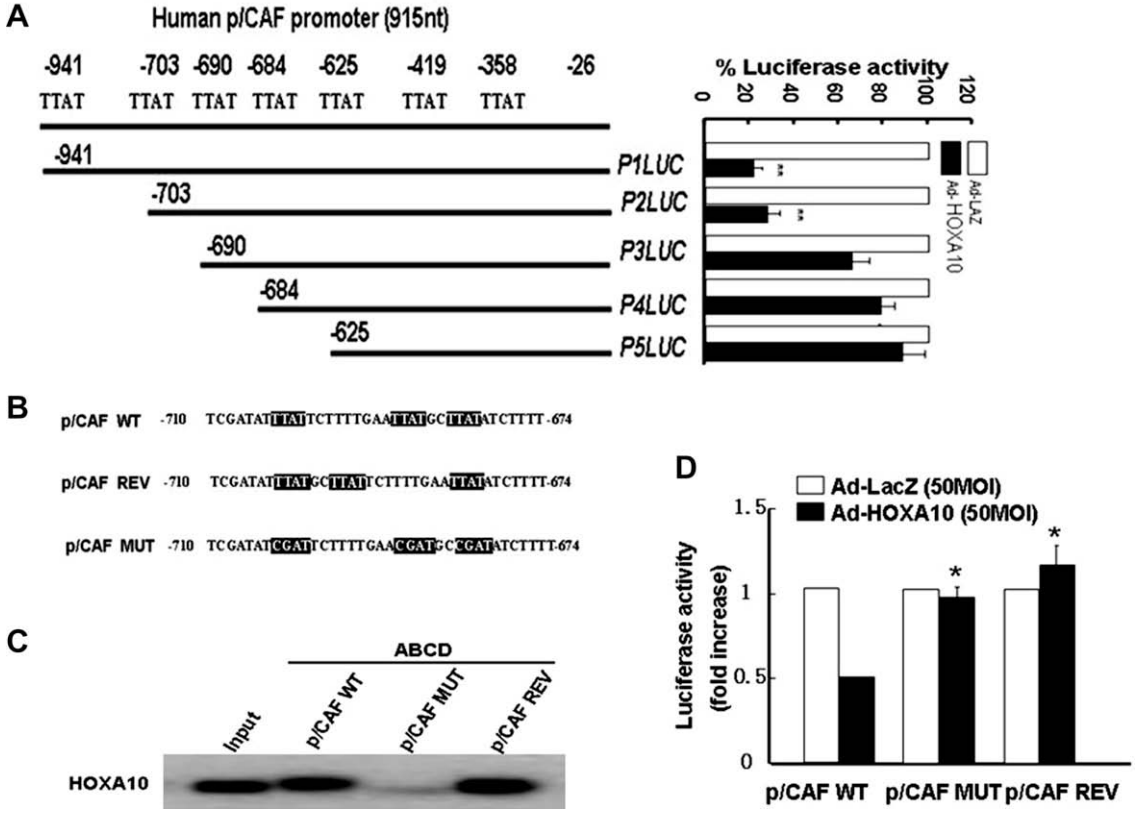

Fig. 4. Identification of the HOXA10 functional binding sites within the human p/CAF promoter region. (A) The deletion mutants of p/CAF promoter altered the inhibitory effects of HOXA10 on human p/CAF promoter activity. (B) Schematic representation of the p/CAF WT, p/CAF REV, and p/CAF MUT promoter core regions used in an ABCD assay and in later luciferase reporter assays. (C) The ABCD assays were performed using biotinylated double-stranded p/CAF WT, p/CAF REV, and p/CAF MUT with whole cell extract from hESCs. After washing the beads, proteins were detected by Western blot analysis. The input lane represents 3% of the total volume of whole cell extracts used for the ABCD binding assay. (D) Effects of HOXA10 on various p/CAF promoter-Luc reporter construct activities. hESCs were transfected with Ad-LacZ and Ad-HOXA10 at 50 MOI for 48 h, then cells were transfected with 600 ng of different p/CAF promoter-Luc reporter plasmids. Forty-eight hours after transfection, cells lysates were collected and assayed for luciferase activities ($n = 5$). $^{*}P < 0.05$; $^{**}P < 0.001$ vs control.

that endogenous HOXA10 strongly binds to p/CAF WT and p/CAF REV, but not p/CAF MUT (Fig. 4C).

To examine the role of the three consecutive TTAT elements in the functional suppression of HOXA10 on p/CAF, we studied luciferase activities of the three p/CAF-Luc reporter constructs after HOXA10 overexpression. We found that HOXA10 overexpression decreased luciferase activity of the p/CAF WT construct by approximately 50% but did not alter luciferase activity of the p/CAF MUT construct. Unexpectedly, although our above results showed that HOXA10 could bind to the p/CAF REV construct, in our luciferase activity assays, we found that overexpression of HOXA10 did not change luciferase activity of the p/CAF REV construct relative to control experiments (Fig. 4D). These data demonstrate that all three consecutive TTAT element units (−710 to −674), found near the p/CAF promoter (−1085 to −468), are required for HOXA10-specific binding and HOXA10 functional suppression of p/CAF promoter activity.

Discussion

HOXA10 is expressed in the endometrial glands and stromal cells throughout the menstrual cycle, with highest expression at the time of implantation. The requirement for maternal HOXA10 in the peri-implantation uterus has previously been demonstrated. Recent evidence further suggested that HOXA10 may be essential for controlling the decidualization process to prevent premature differentiation and apoptosis [16]. HOXA10 contains a 61-amino acid helix–turn–helix DNA-binding motif, which regulates a number of genes, such as cyclin-dependent kinase inhibitor 1A [17], genes of the Wnt pathway [18], ITGB3 [19], IGFBP1 [9], EMX2 [19], and FK506 binding protein 4 [20]. Although the target genes of HOXA10 have been characterized in human and primate endometrium, the potential molecular targets of HOXA10 that have key functions in implantation of the human embryo remain unknown. In the present study, using chromatin immunoprecipitation, we identified p/CAF as a novel target gene of HOXA10 in hESCs. The interaction of HOXA10 with the p/CAF promoter was further confirmed by ChIP/PCR and ABCD assays. Moreover, adenovirus-mediated overexpression of HOXA10 decreased p/CAF promoter activity, and siRNA-specific knockdown of HOXA10 increased p/CAF promoter activity both in hESCs and Ishilkawa cells. Furthermore, we found that all three consecutive TTAT elements are required for HOXA10 functional suppression of p/CAF promoter activity. These results suggest that p/CAF is a novel downstream molecular target of HOXA10, which may play an important role in the regulation of endometrial receptivity.

P/CAF has been described as a coactivator that mediates the transcription of many genes. Similar to many other transcriptional coactivators, this factor possesses intrinsic histone acetylase activity [21]. The role of p/CAF in regulation of transcription has been investigated in several studies, and its requirement for histone acetyltransferases (HATs) and coactivator has been described in the context of nuclear receptor and growth factor-mediated activation. Previous studies have shown that p/CAF interacts physically

and functionally with PTEN [22]. It is well known that PTEN is necessary for embryonic development [23], embryo implantation [24,25], cell migration, proliferation, and apoptosis [26,27]. During the process of implantation, there are many cells at the implantation site undergoing apoptosis [25], and this phenomenon has been suggested to play an important role in the regulation of endometrial decidualization and trophoblast invasion. Thus, it would be interesting to further examine the p/CAF–PTEN signaling pathway in the regulation of HOXA10 functions with respect to implantation and decidualization.

In summary, this study provided the first evidence to our knowledge that p/CAF is a novel HOXA10 target gene and is down-regulated by HOXA10 through the three consecutive TTAT element units within the p/CAF promoter region. However, further studies are required to determine the physiological role of p/CAF in embryo implantation. It is possible that p/CAF may be a novel therapeutic target to overcome implantation failure and associated infertility.

References

[1] I. Satokata, G. Benson, R. Maas, Sexually dimorphic sterility phenotypes in Hoxa10-deficient mice, Nature 374 (1995) 460–463.
[2] G.V. Benson, H. Lim, B.C. Paria, I. Satokata, S.K. Dey, R.L. Maas, Mechanisms of reduced fertility in Hoxa-10 mutant mice: uterine homeosis and loss of maternal Hoxa-10 expression, Development 122 (1996) 2687–2696.
[3] C.N. Bagot, H.J. Kliman, H.S. Tyalor, Maternal Hoxa10 is required for pinopod formation in the development of mouse uterine receptivity to embryo implantation, Dev. Dyn. 222 (2001) 538–544.
[4] H.S. Taylor, A. Arici, D. Olive, P. Igarashi, HOXA10 is expressed in response to sex steroids at the time of implantation in the human endometrium, J. Clin. Invest. 101 (1998) 1379–1384.
[5] D. Cermik, B. Selam, H.S. Tyalor, 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.
[6] 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.
[7] C.N. Bagot, P.J. Troy, H.S. Tyalor, Alteration of maternal Hoxa10 expression by in vivo gene transfection affects implantation, Gene Ther. 7 (2000) 1378–1384.
[8] H. Lim, L. Ma, W.G. Ma, R.L. Maas, S.K. Dey, Hoxa-10 regulates uterine stromal cell responsiveness to progesterone during implantation and decidualization in the mouse, Mol. Endocrinol. 13 (1999) 1005–1017.
[9] J.J. Kim, H.S. Taylor, G.E. Akbas, I. Foucher, A. Trembleau, R.C. Jaffe, A.T. Fazleabas, T.G. Unterman, Regulation of insulin-like growth factor binding protein-1 promoter activity by FKHR and HOXA10 in primate endometrial cells, Biol. Reprod. 68 (2003) 24–30.
[10] J.J. Kim, H.S. Taylor, Z. Lu, O. Ladhani, J.M. Hastings, K.S. Jackson, Y. Wu, S.W. Guo, A.T. Fazleabas, Altered expression of HOXA10 in endometriosis: potential role in decidualization, Mol. Hum. Reprod. 13 (2007) 323–332.
[11] S. Fernandez-Shaw, S.C. Shorter, C.E. Naish, D.H. Barlow, P.M. Starkey, Isolation and purification of human endometrial stromal and glandular cells using immunomagnetic microspheres, Hum. Reprod. 7 (1992) 156–161.
[12] G.J. Yan, S. Chen, B. You, J.X. Sun, Activation of sphingosine kinase-1 mediates induction of endothelial cell proliferation and angiogenesis by epoxyeicosatrienoic acids, Cardiovasc. Res. 78 (2008) 308–314.
[13] R. Krumlauf, Transforming the Hox code, Curr. Biol. 2 (1992) 641–643.
[14] K. Block, A. Kardana, P. Igarashri, H.S. Taylor, In utero diethylstilbestrol (DES) exposure alters Hox gene expression in the developing mullerian system, FASEB J. 14 (2000) 1101–1108.
[15] 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.
[16] Z. Lu, J. Hardt, J.J. Kim, Global analysis of genes regulated by HOXA10 in decidualization reveals a role in cell proliferation, Mol. Hum. Reprod. 14 (2008) 357–366.
[17] V.C. Bromleigh, L.P. Freedman, P21 is a transcriptional target of HOXA10 in differentiating myelomonocytic cells, Genes Dev. 14 (2000) 2581–2586.
[18] 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.
[19] 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.
[20] T. Daikoku, S. Tranguch, D.B. Friedman, S.K. Das, D.F. Smith, S.K. Dey, Proteomic analysis identifies immunophilin FK506 binding protein 4 (FKBP52) as a downstream target of Hoxa10 in the periimplantation mouse uterus, Mol. Endocrinol. 19 (2005) 683–697.
[21] X.J. Yang, V.V. Ogryzko, J. Nishikawa, H.H. Bruce, N. Yoshihiro, A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A, Nature 382 (1996) 319–324.
[22] K. Okumura, M. Mendoza, R.M. Bachoo, R.A. DePinho, W.K. Cavenee, F.B. Furnari, PCAF modulates PTEN activity, J. Biol. Chem. 281 (2006) 26562–26568.
[23] B. Stiles, M. Groszer, S. Wang, J. Jiao, H. Wu, PTENless means more, Dev. Biol. 273 (2004) 175–184.
[24] G.L. Mutter, M.C. Lin, J.T. Fitzgerald, J.B. Kum, C. Eng, Changes in endometrial PTEN expression throughout the human menstrual cycle, J. Clin. Endocrinol. Metab. 85 (2000) 2334–2338.
[25] G. Correia-da-Silva, S.C. Bell, J.H. Pringle, A. Teixeira, Patterns of uterine cellular proliferation and apoptosis in the implantation site of the rat during pregnancy, Placenta 25 (2004) 538–547.
[26] O. Guzeloglu-Kayisli, U.A. Kayisli, A.L. Rafat, W. Zhang, L. Guven, A. Aydin, Regulation of PTEN (phosphatase and tensin homolog deleted on chromosome 10) expression by estradiol and progesterone in human endometrium, J. Clin. Endocrinol. Metab. 88 (2003) 5017–5026.
[27] Y. Zhu, P. Hoell, B. Ahlemeyer, J. Krieglstein, PTEN: a crucial mediator of mitochondria-dependent apoptosis, Apoptosis 11 (2006) 197–207.