Deregulation of the HOXA10 Homeobox Gene in Endometrial Carcinoma: Role in Epithelial-Mesenchymal Transition

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Abstract

Homeobox genes encode transcription factors that control cell differentiation and play essential roles in developmental patterning. Increasing evidence indicates that many homeobox genes are aberrantly expressed in cancers, and that their deregulation significantly contributes to tumor progression. The homeobox gene HOXA10 controls uterine organogenesis during embryonic development and functional endometrial differentiation in the adult. We investigated whether HOXA10 expression is deregulated in endometrial carcinomas, and how counteracting this aberrant expression modifies tumor behavior. We found that down-regulation of HOXA10 expression in endometrial carcinomas strongly correlates with increased tumor grade and is associated with methylation of the HOXA10 promoter. Enforced expression of HOXA10 in endometrial carcinoma cells inhibited invasive behavior in vitro and tumor dissemination in nude mice. The inhibitory effect of HOXA10 on invasive behavior was attributable at least in part to the ability of HOXA10 to induce expression of the epithelial cell adhesion molecule E-cadherin by down-regulating expression of Snail, a repressor of E-cadherin gene transcription. These findings reveal a novel role for HOXA10 deregulation in the progression of endometrial carcinoma by promoting epithelial-mesenchymal transition. (Cancer Res 2006; 66(2): 889-97)

Introduction

It is widely recognized that many of the molecular pathways that underlie tumor progression are aberrations of processes that control normal embryonic development. Epithelial-mesenchymal transition (EMT) is a dynamic process whereby epithelial cells lose polarity and cell-cell adhesion, acquire a migratory phenotype, and switch to a mesenchymal-like gene expression program. This process is essential for normal morphogenesis, and reactivation of the EMT program is regarded as a primary mechanism underlying carcinoma progression and invasive behavior (1, 2). Homeobox genes have increasingly been thought to also represent important hubs in the intimate relationship between tumor progression and embryogenesis. These genes encode transcription factors that control developmental patterning and specify the unique morphologic identities of adult tissues (3, 4). Several homeobox genes that are normally expressed in embryonic tissues are activated in tumors (4, 5). One example is PAX2, which is normally expressed during early urogenital development, and is expressed in renal tumors (6). PAX2 is thought to promote cell survival, and inhibiting PAX2 expression in cancer cells induces apoptosis (7). On the other hand, many homeobox genes that are normally expressed in differentiated tissues are lost or down-regulated in tumors. Nkx3.1 controls prostate morphogenesis and is normally expressed in the prostate during development and in the adult (8). Nkx3.1 is frequently deleted in prostate cancers (9), and enforced expression of Nkx3.1 in prostate cancer cells inhibits cell growth (10). Many other homeobox genes contribute to tumor progression by promoting cell growth and/or survival (4, 5). However, given the important role of homeobox genes in cell differentiation, surprisingly little is known regarding their role in EMT.

Endometrial carcinoma is the fourth most frequently diagnosed cancer in women. Grade 1 (G1) endometrioid carcinomas of the endometrium are typically well-differentiated, and are mostly detected while still confined to the uterine corpus (11). Grade 3 (G3) endometrioid carcinomas are mostly solid, whereas uterine papillary serous carcinoma (UPSC) is characterized by papillary patterns of growth. Although histologically distinct, G3 endometrioid carcinoma and UPSC share several common features including high-grade nuclear atypia and poor responsiveness to chemotherapy, radiation, and progesterin therapy (11–13). G3 endometrioid carcinoma and UPSC have greater propensity for myometrial and lymphatic/vascular space invasion than G1 tumors (11–13), but the mechanisms underlying the progression of these high-grade tumors are poorly understood.

Several studies indicate that the HOXA10 homeobox gene controls uterine organogenesis during embryonic development and functional endometrial differentiation in the adult. HOXA10 expression becomes restricted to the uterine primordium during normal Mullerian duct differentiation, and is maintained in the adult uterus (14). Female mice with a targeted disruption of HoXA10 produce normal embryos, but their uteri are unable to support embryo implantation (15, 16). Implantation normally occurs during the mid-secretory phase of the menstrual cycle, a period during which the endometrium undergoes extensive glandular differentiation and HOXA10 expression in the endometrial glands dramatically increases (14). A role for HOXA10 in endometrial differentiation is also supported by our recent findings that aberrant activation of HOXA10 in ovarian surface epithelium-derived cells gives rise to the endometrioid-like features of endometrioid ovarian carcinoma (17).

Because HOXA10 plays an essential role in endometrial development, we speculated that its expression is deregulated in endometrial carcinomas. In this study, we found that down-regulation of HOXA10 expression in endometrial carcinomas correlates with increased tumor grade and is associated with HOXA10 promoter methylation. Enforced expression of HOXA10 in endometrial carcinoma cells inhibited invasive behavior, and this inhibitory effect was attributable, at least in part, to the ability of...
HOXA10 to up-regulate E-cadherin by suppressing the expression of the Snail transcription factor. These findings reveal that silencing of HOXA10 expression in the endometrium could contribute to tumor progression by promoting EMT.

**Materials and Methods**

**Cell lines, cDNAs, and antibodies.** The endometrial carcinoma cell lines SPEC2 and KLE were obtained from Isaiah Fidler (University of Texas MD Anderson Cancer Center, Houston, TX) and American Type Culture Collection (Rockville, MD), respectively. The HOXA10 cDNA clone was provided by Corey Largman (University of California VA Medical Center, San Francisco, CA). The rabbit polyclonal antibody to HOXA10 has been previously described (17). Other antibodies used are as follows: actin, β1 integrin, cadherin-6, Snail (Santa Cruz Biotechnology, Santa Cruz, CA), E-cadherin (Zymed Laboratories, San Francisco, CA), vimentin (NeoMarkers, Fremont, CA), Ki-67 (Vector Laboratories, Burlingame, CA). Secondary antibodies were obtained from Kirkegaard & Perry Laboratories (Gaithersburg, MD).

**Tissue microarrays and immunohistochemistry.** Tissue microarrays were constructed from core biopsies of endometrial carcinoma specimens at the University of Texas MD Anderson Cancer Center using a Beecher Instruments (Silver Spring, MD) tissue arrayer. Use of specimens was approved by the Institutional Review Board. Cases included the following: normal secretory endometrium (n = 10), G1 (n = 26), G2 (n = 37), G3 (n = 58) endometrioid carcinoma, and UPSC (n = 30). H&E-stained slides from paraffin blocks of each case were analyzed by a gynecologic pathology fellow (R. Broadus) to confirm tumor histotype and grade. For each case, two separate core biopsies were taken from blocks that were representative of the tumor histotype and grade as a whole. Staining with HOXA10 antibody (1:200) was detected with streptavidin peroxidase by using a LSAB staining kit (Dako Corp., Carpinteria, CA). In previous work (17), we found that HOXA10 is expressed in normal mouse endometrial epithelium but not in fallopian tube epithelium. Slides of tissues of mouse endometrium and fallopian tube were stained simultaneously with microarray slides as internal positive and negative controls, respectively. HOXA10 staining intensity was scored independently by two observers (W. Cheng and H. Naora). An average staining score was determined for biopsies of each case. Histopathologic diagnosis of specimens was made independently of immunohistochemical analysis to eliminate bias in scoring staining. Epithelial staining intensity was graded on a 0 to 3 scale, where 0, no staining as assessed by staining with antirabbit secondary antibody alone; 1, weak; 2, moderate; and 3, intense staining.

**Cell culture and transfection.** SPEC2 and KLE cells were cultured in a 1:1 mixture of DMEM and Ham’s F12 medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 1 mM L-glutamine, sodium pyruvate, and penicillin-streptomycin. The plasmids pCMV-Tag3-HOXA10 and pIRES-EGFP-HOXA10 were generated by subcloning HOXA10 cDNA into pCMV-Tag3 (Stratagene, La Jolla, CA) and pIRES-EGFP (BD Biosciences Clontech, Palo Alto, CA) vectors, respectively. SPEC2 cells were stably transfected with pCMV-Tag3-HOXA10 and with empty pCMV-Tag vector using FuGENE6 (Roche, Indianapolis, IN). KLE cells were likewise transfected with pIRES-EGFP-HOXA10 and with pIRES-EGFP vector. Stably transfected clones, derived from single colonies, were selected by G418 (400 μg/mL; Invitrogen).

**Matrigel culture and invasion chamber assays.** Fifty thousand transfected cells, suspended in 500 μL of culture medium containing 5% Matrigel (BD Biosciences, Bedford, MA), were plated per well in 24-well plates. Solidified cultures were maintained for 2 weeks with medium changed every 3 to 4 days. For assaying cell invasion, 6 × 104 transfected cells were seeded into Matrigel invasion chambers (BD Biosciences). Twenty-four hours after seeding, invading cells on the lower surface of the filters were stained by using Giemsa solution, and counted in five different fields per assay under microscopy at ×100 magnification. Each assay was done in triplicate.

**Proliferation assays.** Cell proliferation was measured by using the 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) assay (Roche). One thousand cells were seeded per well in 96-well plates. Absorbance at 570 nm after MTT addition was measured daily over 5-day time course. The number of trypan blue–excluding cells in replicate plates were also directly counted by using a hemocytometer. Each assay was done in triplicate.

**Reverse transcription-PCR.** Reverse transcription using 1 μg of DNase I-treated total RNA was done using Superscript II reverse transcriptase (Invitrogen) in a reaction volume of 20 μL. One microliter of the reverse transcription reaction was used for amplification using Platinum Taq DNA polymerase (Invitrogen). Detection of HOXA10 and actin expression has been previously described (17). Other primers are as follows: (Snail) sense, 5’-TGGGGAATCGGCGACCC-3’; antisense, 5’-CTTAGAGAGGCTCCCGC-3’; (Slug) sense, 5’-GCCCTCCAAAAAGCCAAATCAG-3’; antisense, 5’-GTGGTGTCTACAGACGACCC-3’. Amplification was done as follows: denaturation at 94°C for 1 minute, annealing at 61°C for 1 minute and extension at 72°C for 2 minutes for 37 cycles to detect Snail expression, and denaturation at 95°C for 1 minute, annealing at 58°C for 1 minute and extension at 72°C for 1 minute for 40 cycles to detect Slug expression. Titers were done to ensure a linear range of amplification.

**Western blot and immunofluorescence staining.** Cell lysates were prepared by using M-PER buffer (Pierce, Rockford, IL). Equal amounts of protein (20 μg per lane) were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes (Amersham Pharmacia Biotech, Piscataway, NJ). Membranes were probed with antibodies to E-cadherin (1 μg/mL), β1 integrin (1:100), cadherin-6 (1:200), and actin (1:100). For cell staining, cells were fixed in 4% paraformaldehyde and permeabilized for 10 minutes in 0.1% Triton X-100. Cells were stained with antibodies to E-cadherin (2 μg/mL), vimentin (0.5 μg/mL), and Snail (1:50) for 1 hour. Staining was detected using Texas red–conjugated secondary antibodies.

**Methylation-specific PCR.** Two micrograms of genomic DNA, isolated from cultured cells and tissues, were treated with 0.2 mol/L NaOH for 10 minutes at 37°C, then with 3.75 mol/L hydroquinone and 2.6 mol/L sodium bisulfite (Sigma, St. Louis, MO) and incubated for 16 hours at 50°C. DNA was purified using the Wizard DNA Purification System (Promega, Madison, WI). Samples were desulfonated with 0.3 mol/L NaOH, precipitated with ethanol, and suspended in 20 μL of distilled water. DNA was analyzed using primer sets located within CpG islands in the HOXA10 promoter. Primers specific for unmethylated DNA were 5’-TGGAGAGTGTTTGGTG-3’ (sense) and 5’-AAATACAAATACATACC-3’ (antisense; 113 bp product). Primers specific for methylated DNA were 5’-CGGAGACGCGGTTCGTGC-3’ (sense) and 5’-AATAATACATAATGATTAA-CAATCC-3’ (antisense; 122 bp product). PCR amplification of 1 μL bisulfite-treated DNA template was done in a 10 μL reaction containing 2 μmol/L forward and reverse primers, 1.25 mmol/L deoxynucleotide triphosphates, and 0.5 units of HotStarTaq DNA polymerase (Qiagen, Valencia, CA). Amplification conditions were as follows: 15 minutes starting at 95°C, 35 cycles at 95°C for 30 seconds, 60°C (methylated) or 53°C (unmethylated) for 30 seconds, and 72°C for 30 seconds followed by a final extension at 72°C for 10 minutes. PCR products were resolved by electrophoresis on a 6% acrylamide gel.

**Propagation of cells in nude mice.** Animal studies were approved by the Institutional Animal Care and Use Committee. Five 4-week-old female nude mice per group were inoculated i.p. at one site in the lower abdomen with 3 × 106 cells of each cell line. At 3 months thereafter, mice were killed by CO2 asphyxiation. Paraffin-embedded tissues collected from mice were stained with H&E, and with antibodies to HOXA10 (1:200), Ki-67 (1:1,000), E-cadherin (2 μg/mL), and Snail (1:100).

**Statistical analysis.** Statistical significance of differences in HOXA10 staining intensity between groups of normal and malignant cases was calculated by the Mann-Whitney U test. Statistical significance of differences in cell proliferation and invasion between vector control and HOXA10-transfected cells was calculated by Student’s t test. P > 0.05 was considered not significant.

**Results**

Down-regulation of HOXA10 expression correlates with tumor grade. We examined whether HOXA10 expression is
deregulated in endometrial carcinomas by performing immunohistochemical analysis of tissue microarrays of clinical specimens. Strong HOXA10 staining was observed throughout glands of normal secretory endometrium as likewise reported by others (14). Epithelial staining was reduced in G1 endometrioid carcinomas as compared with normal secretory endometrium, reduced in G2 tumors as compared with G1 tumors, and significantly weaker in G3 tumors (Fig. 1A and B). The majority of UPSC cases exhibited little or no HOXA10 staining, as likewise found for G3 endometrioid carcinomas (Fig. 1A and B). These results indicate that down-regulation of HOXA10 expression in endometrial carcinomas correlates with increased tumor grade, and raises the possibility that this down-regulation promotes tumor growth and/or invasive properties.

**Enforced expression of HOXA10 in endometrial carcinoma cells does not affect cell proliferation.** To determine the effect of HOXA10 on the proliferation of endometrial carcinoma cells, we stably expressed HOXA10 in two model cell lines. The SPEC2 cell line was established from a UPSC tumor (18). The KLE cell line was established from a poorly differentiated endometrial carcinoma (19). These cell lines have been used as models of UPSC and poorly differentiated endometrial carcinoma by others (20, 21), and were ideal for this study because they have almost undetectable levels of endogenous HOXA10 expression (Fig. 2A). KLE and SPEC2 cell lines were each stably transfected with HOXA10 cDNA and with empty vector. Two clones from each transfection, isolated from single colonies, were used for further study (Fig. 2A). Cell proliferation was measured by MTT assays and by cell counts. No significant difference was observed in proliferation rates of vector-transfected SPEC2 cell lines (Vec-6 and Vec-8) and of HOXA10-transfected SPEC2 cell lines (A10-4, A10-42; Fig. 2B). Similarly, HOXA10 had no significant effect on the proliferation of KLE cells (Fig. 2B). Cells of the stably transfected lines were also inoculated into female nude mice. Tumors that formed in mice were stained with antibodies to

**Figure 1.** Expression of HOXA10 in endometrial tissues. A, immunohistochemical staining of HOXA10 in specimens of (i) normal secretory endometrium, (ii) G1, (iii) G2, (iv) G3 endometrioid carcinomas, and (v) UPSC; bar, 200 μm. B, summary of HOXA10 staining in clinical specimens. For each case, epithelial staining intensity was scored as either 0, no staining; 1, weak; 2, moderate; or 3, intense. Each symbol represents an individual case. Statistical significance of differences in staining intensity between the indicated groups of cases was calculated by the Mann-Whitney U test. P > 0.05 were considered not significant (n.s.).
Both vector- and SPEC2 cells were inoculated i.p. at one site in the lower abdomen. To mimic this behavior, transfected mice. UPSCs behave similarly to serous ovarian carcinomas in their invasive behavior of tumor cells propagated in female nude mice. Sections of tissues collected from sacrificed mice were stained with HOXA10 antibody; arrows, tumors. Staining of HOXA10 in normal vaginal epithelium, seen in both panels, served as a positive control; bar, 200 μm. D, SPEC2-derived tumor tissues were stained with antibody to Ki-67; bar, 20 μm.

HOXA10 (Fig. 2C) and to the proliferation marker Ki-67 (Fig. 2D). No difference was observed between the proportion of Ki-67-positive cells in tumors derived from vector control cells and in tumors derived from HOXA10-transfected cells (Fig. 2D).

**Enforced expression of HOXA10 promotes cell adhesion and inhibits invasive behavior.** We initially investigated the possibility that HOXA10 regulates the adhesive properties of endometrial carcinoma cells by examining the growth of transfected cells in semisolid Matrigel cultures. Vector control SPEC2 cells grew as small clusters of loosely aggregated cells (Fig. 3A). In contrast, HOXA10-transfected SPEC2 cells grew as large, well-formed spheroids. HOXA10-transfected KLE cells, but not vector control KLE cells, also formed tightly aggregated spheroids (Fig. 3A). These findings indicated that HOXA10 expression in endometrial carcinoma cells promotes cell-cell adhesion. HOXA10-transfected SPEC2 and KLE cells exhibited a decreased ability to invade in Matrigel invasion chamber assays as compared with vector control cells (Fig. 3B). The possibility that the decreased number of invading HOXA10-expressing cells could be due to reduced proliferation can be eliminated because HOXA10 was found not to significantly affect cell proliferation (Fig. 2B).

To confirm our findings, we investigated whether HOXA10 alters the invasive behavior of tumor cells propagated in female nude mice. UPSCs behave similarly to serous ovarian carcinomas in their propensity for i.p. spread (13). To mimic this behavior, transfected SPEC2 cells were inoculated i.p. at one site in the lower abdomen. Both vector- and HOXA10-transfected SPEC2 cells formed solid, bulky tumors at the injection site (Fig. 3C). However, vector control tumors were more locally invasive and extensively involved the ovaries (Fig. 3C). Animals inoculated with vector control cells (Vec-6 and Vec-8) developed tumors at sites distant from the injection site such as the omentum and diaphragm within 3 months (Fig. 3D). In contrast, diaphragmatic implants were not observed in any of the mice inoculated with cells of the HOXA10-transfected lines (A10-4 and A10-42) within the same period (Fig. 3D). Only one of five animals injected with A10-4 cells developed omentum implants. Expressing HOXA10 therefore seems to modify the behavior of endometrial carcinoma cells by inhibiting their ability to disseminate.

**HOXA10 up-regulates E-cadherin expression.** Down-regulation of E-cadherin expression plays a central role in the loss by epithelial cells of polarity and cell-cell adhesion, concomitant with the acquisition of migratory properties (1, 2). Because HOXA10 inhibited invasive behavior of endometrial carcinoma cells, we investigated whether HOXA10 up-regulates E-cadherin expression. Western blot analysis revealed that vector control SPEC2 and KLE cells expressed very little E-cadherin, whereas E-cadherin levels were markedly elevated when HOXA10 was expressed (Fig. 4A). HOXA10-transfected cells exhibited strong E-cadherin staining on cell surfaces and also increased intracellular staining. In contrast, very little staining was observed in vector control cells (Fig. 4B). HOXA10-transfected SPEC2 cells formed tumors in mice that highly expressed E-cadherin and exhibited more nesting than tumors that formed from vector control cells (Fig. 4C). Expression of the mesenchymal marker vimentin was strongly detected in vector control SPEC2 and KLE cells, but was abolished when HOXA10 was stably expressed in these cells (Fig. 4B). Inhibition of invasive behavior of endometrial carcinoma cells by progesterone is associated with down-regulation of β1 integrin and cadherin-6 (22). Expression of these cell adhesion molecules was not affected by HOXA10 (Fig. 4A). Enforced expression of HOXA10 in endometrial carcinoma cells therefore seems to inhibit invasive behavior.
behavior by up-regulating E-cadherin expression and promoting mesenchymal-to-epithelial transition.

**HOXA10 inhibits Snail expression.** Snail is a zinc finger protein that induces EMT and represses E-cadherin gene transcription (23, 24). Because HOXA10 induced E-cadherin expression, we investigated whether HOXA10 down-regulates expression of Snail. As shown in Fig. 5A, Snail transcripts were detected in vector control SPEC2 and KLE cells, but were almost undetectable in HOXA10-transfected cells. These findings were confirmed by staining cultured cells and tumors derived from transfected cells with antibody to Snail. Nuclear staining was detected in vector control KLE cells but not in HOXA10-transfected cells (Fig. 5B). Nuclear staining was also observed in cells of tumors that formed from vector control SPEC2 cells, but not from HOXA10-transfected SPEC2 cells (Fig. 5C). We also examined expression of Slug, a zinc finger protein that is closely related to Snail and also represses E-cadherin gene transcription (25). In contrast to Snail, levels of Slug transcripts were very similar in vector- and HOXA10-transfected cells (Fig. 5A). These findings indicate that HOXA10 induces E-cadherin expression by down-regulating expression of the Snail repressor but not of Slug.

**Down-regulation of HOXA10 expression is associated with promoter methylation.** Methylation of CpG islands in the promoter region is an important mechanism that silences expression of many genes in cancers, including various homeobox genes (26, 27). The HOXA10 promoter contains a CpG island–rich region that extends from −20 to −210 bp upstream of the transcription start site. We determined the methylation status of this promoter region in normal and malignant endometrial tissues by using methylation-specific PCR (MSP). As a control, we used the cervical cancer cell line SiHa that constitutively expresses HOXA10 (17). Only unmethylated and no methylated DNA was detected in SiHa cells (Fig. 6). In contrast, only methylated and no unmethylated DNA was detected in parental SPEC2 and KLE cells that do not express HOXA10 (Fig. 6). Only methylated and no unmethylated DNA were detected in five of eight G3 tumors (Fig. 6). Methylated DNA was more strongly detected than unmethylated DNA in the three other G2 tumors. In contrast, only partial methylation was detected in six of seven G1 tumors, and a seventh case exhibited only unmethylated DNA. Partial methylation was detected in all four specimens of normal endometrium, with unmethylated DNA being more strongly detected than methylated DNA in two of these cases (Fig. 6). The prevalence of HOXA10 promoter methylation in G3 tumors is strongly consistent with the lack of HOXA10 expression seen in the majority of G3 tumors, whereas the lower frequency of promoter methylation in G1 tumors

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**Figure 3.** HOXA10 promotes cell adhesion and inhibits invasive behavior. A, morphology of empty vector- and HOXA10-transfected SPEC2 and KLE cells in semisolid Matrigel culture; bar, 100 μm. B, 6 × 10⁴ transfected cells were seeded per well in Matrigel invasion chambers. Twenty-four hours thereafter, invading cells were counted in five different fields per assay under microscopy at ×100 magnification. The results of triplicate assays are shown. Statistical significance of differences in the number of invading cells was determined by Student’s t test. C, tumor tissues and urogenital organs excised from female mice inoculated with equivalent numbers (3 × 10⁶) of stably transfected SPEC2 cells as described in Fig. 2 and sacrificed 3 months thereafter. Black arrows, bladder; white triangles, ovaries. D, H&E-stained sections of tumors derived from vector control cells that involved the (i) omentum (top, pancreas) and (ii) diaphragm. In contrast, mice inoculated with HOXA10-transfected cells did not develop diaphragmatic implants (iii); bar, 200 μm.
and normal endometrium is consistent with the higher HOXA10 expression in these tissues (Fig. 1). These findings indicate that loss of HOXA10 expression in endometrial carcinomas, particularly high-grade tumors, is attributable at least in part to methylation of the HOXA10 promoter.

**Discussion**

A hallmark of EMT is loss of E-cadherin expression that is mediated by a battery of transcriptional repressors, the best-characterized of which is Snail (1, 2). E-cadherin plays a central role in the differentiation and histotypic organization of all types of epithelial cells, and an inverse correlation between E-cadherin expression and tumor grade has been documented in a wide variety of carcinomas (1, 2). On the other hand, the specialized differentiation patterns that are unique to each type of epithelial cell and, indeed of all cell types, are governed by specific repertoires of homeobox genes (3, 4). Our study reveals that HOXA10, a homeobox gene that controls endometrial differentiation during embryogenesis and in adulthood, regulates E-cadherin expression, and that down-regulation of HOXA10 expression could contribute to the progression of endometrial carcinoma by promoting EMT. There is evidence that other homeobox genes induce expression and/or activity of E-cadherin in a tissue-specific manner (28, 29). However, this is the first report indicating that a regulatory interaction exists between a homeobox gene and the transcriptional machinery that represses E-cadherin gene expression. This regulatory interaction seems to be highly specific, as HOXA10 was found to inhibit expression of Snail but not of Slug, SIP1 and Twist also repress E-cadherin gene transcription via E boxes that are targeted by Snail and Slug (30), and it has yet to be determined whether HOXA10 regulates the expression of these repressors. Nevertheless, our findings are consistent with increasing evidence that the various repressors of E-cadherin gene transcription are regulated by distinct signaling pathways in different cell types (1, 2, 30). One example of differential regulation is that mediated by metastatic tumor antigen 3 (MTA3) in breast epithelial cells. MTA3 is a component of the Mi-2/NuRD complex that has been found to repress transcription of Snail, but not of Slug (31). It has yet to be determined whether HOXA10 directly represses Snail gene transcription or inhibits Snail expression by an indirect mechanism.

![Figure 4](cancerres.aacrjournals.org)
The tissue-specificity of homeobox gene expression and function, and the tissue specificity of the regulatory pathways that control Snail expression prompt the question as to what receptor-mediated signal(s) could regulate HOXA10 in endometrial epithelial cells. One possible candidate is the progesterone receptor (PR). Endometrial differentiation during the secretory phase of the menstrual cycle is driven by progesterone-stimulated induction of HOXA10 expression (32). The induction by progesterone of the differentiated, secretory phenotype is primarily mediated through the PR-B isoform (22). Loss of PR-B in high-grade, poorly differentiated endometrial carcinomas is thought to be a driving force of tumor progression (33, 34). Progesterone can inhibit the invasive behavior of endometrial carcinoma cells that express PR-B (22). It is possible that loss of PR-B and progesterone-responsiveness in endometrial carcinoma cells leads to methylation of the HOXA10 promoter, and that the silencing of HOXA10 expression in turn leads to activation of Snail expression, inhibition of E-cadherin expression and increased invasive behavior.

This possible model has an interesting analogy to the study by Fujita et al. (31), which revealed that loss of functional estrogen receptors (ER) in aggressive breast cancers results in decreased expression of MT3, a component of a transcriptional complex that normally represses Snail transcription. The implication of the study of Fujita et al. is that ER status controls EMT in breast cancers. It is possible that PR-B status could likewise control EMT in endometrial carcinomas. It should be pointed out that loss of E-cadherin expression alone does not constitute EMT, and that multiple mechanisms contribute to invasive behavior. Inhibition of invasive behavior of endometrial carcinoma cells by progesterone is associated with down-regulation of several cell adhesion molecules such as β1 integrin and cadherin-6 (22). However, HOXA10 had no effect on β1 integrin and cadherin-6 levels in KLE and SPEC2 cells (Fig. 4A). This suggests that HOXA10 cannot repress all of the pathways that mediate invasive behavior and that are inhibited by progesterone. Progesterone not only inhibits the invasive behavior of endometrial carcinoma cells, but also inhibits cell proliferation (22). The notion that not all signaling pathways emanating from PR-B are regulated by HOXA10 is supported by our findings that HOXA10 expression did not significantly affect the proliferation of endometrial carcinoma cells. This observation is consistent with findings that the rate of epithelial cell proliferation in the uteri of Hoxa10-deficient mice in response to estrogen is very similar to the rate observed in wild-type mice (35).

Several parallels exist between our findings and those of Taylor and colleagues. As likewise found by these investigators (36), we detected intact HOXA10 expression in a subset of G1 and G2 endometrioid carcinomas. However, we observed that HOXA10 expression significantly decreases with tumor grade, such that G3 endometrioid carcinomas and UPSCs had lower HOXA10 expression than G1 and G2 tumors. Taylor et al. did not examine G3 endometrioid carcinomas and UPSCs. In our study, 86% of the cases that extended beyond the uterine corpus (stages II-IV) were G3 tumors and UPSCs. Sixty-one percent of the cases that extended beyond the uterine corpus (stages II-IV) were G3 tumors and UPSCs. Sixty-one percent of the cases that extended beyond the uterine corpus (stages II-IV) were G3 tumors and UPSCs. Sixty-one percent of the cases that extended beyond the uterine corpus (stages II-IV) were G3 tumors and UPSCs.
expressing HOXA10 inhibits invasive behavior. Interestingly, Taylor and colleagues also found that HOXA10 inhibits the invasive behavior of breast cancer cells (37), but it is unclear whether this inhibition occurs by the same mechanism as in endometrial carcinoma cells. The same group reported that the normal midsecretory increase of HOXA10 expression is abolished in the endometrium of women with endometriosis (38). Endometriosis is a benign condition, but exhibits many of the characteristics of malignancy. A hallmark of endometriosis is acquisition of the ability of endometrial tissue to invade other normal tissues. Epithelial cells derived from endometriotic lesions have been found to be as invasive as carcinoma cells in Matrigel assays (39), and to have reduced expression of PR-B and of E-cadherin (40, 41). Down-regulation of HOXA10 expression could therefore be responsible for the acquisition of invasive behavior of endometrial epithelial cells in endometriosis as well as in endometrial carcinoma.

HOXA11, a neighbor of HOXA10 in the HOX gene cluster, also plays an important role in regulating uterine development. As likewise observed in Hoxa10-deficient mice, female mice with a targeted disruption of Hoxa11 have implantation defects (42). HOXA10 and HOXA11 have similar patterns of expression during the menstrual cycle and both genes are up-regulated by progesterone (32, 43). Whereas Hoxa10 is expressed uniformly throughout the uterus, Hoxa11 is more highly expressed in the posterior than in the anterior uterine segment (17). The HOXA11 promoter has been found to be frequently methylated in endometrial carcinomas, and the rate of HOXA11 promoter methylation is significantly higher in recurrent than in nonrecurrent tumors (27). These observations are strikingly similar to the correlations that we found between recurrent HOXA10 promoter methylation, down-regulation of HOXA10 expression, and aggressive clinical behavior, and raise the possibility that down-regulation of HOXA11 in addition to HOXA10 could promote the progression of endometrial carcinoma.

The findings that HOXA10 expression is down-regulated in endometriosis as well as in endometrial carcinomas implies that loss of HOXA10 expression alone is insufficient to induce endometrial malignancy. This notion is supported by the fact that Hoxa10 mutant mice do not develop endometrial tumors.

Furthermore, this implication is consistent with several studies which indicate that loss of expression of a homeobox gene that is normally expressed in differentiated tissues is alone insufficient to induce malignancy. Inactivation in mice of Nkx3.1, a regulator of prostate morphogenesis, leads to the development of prostatic intraepithelial neoplasia, however, these lesions do not progress to carcinoma (8). CDX2 controls the differentiation of intestinal epithelium and is down-regulated in colorectal cancers, particularly high-grade tumors (44). Although CDX2 heterozygous mutant mice do not develop spontaneous malignant tumors, they develop adenomatous intestinal polyps and have increased sensitivity to chemically induced colon carcinoma as compared with wild-type mice (45). This suggests that loss of homeobox gene expression can collaborate with other oncogenic events to induce malignancy. Indeed, inactivation of Nkx3.1 cooperates with loss-of-function of Pten to induce prostate carcinoma (46). PTEN mutations are the most frequently detected genetic alterations in endometrioid carcinomas followed by microsatellite instability, and mutations of K-ras and β-catenin (47). In contrast, p53 mutations occur in 10% to 20% of endometrioid tumors, but occur in almost 90% of UPSCs (47). It is possible that loss of HOXA10 expression could cooperate with one or more of these genetic alterations to induce endometrial carcinoma, and that the histologic type of tumor depends on the specific combination of these molecular events.

References

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