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Regional Development of Uterine Decidualization: Molecular Signaling by Hoxa-10

SANJOY K. DAS*

Reproductive Sciences, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio

SUMMARY

Uterine decidualization, a key event in implantation, is critically controlled by stromal cell proliferation and differentiation. Although the molecular mechanism that controls this event is not well understood, the general consensus is that the factors derived locally at the site of implantation influence aspects of decidualization. *Hoxa-10*, a developmentally regulated homeobox transcription factor, is highly expressed in decidualizing stromal cells, and targeted deletion of *Hoxa-10* in mice shows severe decidualization defects, primarily due to the reduced stromal cell responsiveness to progesterone (P₄). While the increased stromal cell proliferation is considered to be an initiator of decidualization, the establishment of a full-grown functional decidua appears to depend on the aspects of regional proliferation and differentiation. In this regard, this article provides an overview of potential signaling mechanisms mediated by *Hoxa-10* that can influence a host of genes and cell functions necessary for propagating regional decidual development.

INTRODUCTION

Embryo implantation is a complex process that requires two-way interactions between the implantation-competent blastocyst and the receptive uterus (Psychoyos, 1973; Dey, 1996), and involves complex growth regulatory mechanisms leading to molecular, cellular, physiological, and functional changes in the uterus and embryo. Any aberration of this process leads to the human infertility, which remains a major global concern both socially and economically. While the underlying cause of human infertility has been potentially challenged by successful utilization of in vitro fertilization and embryo-transfer techniques; the success rate of implantation remains disappointingly low, which is considered primarily due to our poor understanding regarding the uterine receptivity during the transfer of embryos. Therefore, there is a clear need to unravel the complexities of basic mechanisms of embryo development and implantation. Although a significant progress has been made during the last two decades (Paria et al., 2002; Dey et al., 2004; Wang and Dey, 2006), a deeper understanding is needed to optimize success.

EARLY EMBRYO–UTERINE DIALOGUE IN EARLY PREGNANCY

The establishment of a differentiated uterus for supporting embryo development and implantation is primarily dependent on the coordinated effects of ovarian estrogen and progesterone (P_4) (Psychoyos, 1973; Paria et al., 1993). In the rodent, the first conspicuous sign that the implantation process has been initiated is an increased endometrial vascular permeability at the sites of blastocyst implantation. This can be visualized as discrete blue bands along the uterus after an intravenous injection of a blue dye solution (Psychoyos, 1973; Huet and Dey, 1987; Paria et al., 1993). This increased localized vascular permeability coincides with the initial attachment reaction between the uterine luminal epithelium and blastocyst trophoctoderm (Enders and Schlafke, 1967) and is considered to be one of the earliest prerequisite events in the implantation process (Psychoyos, 1973). In the mouse, the attachment reaction occurs in the evening (2200–2300 hr) of day 4 of pregnancy, and is preceded by uterine luminal closure which in turn results in an intimate apposition of the trophoctoderm with the luminal epithelium (Enders and Schlafke, 1967; Finn and McLaren, 1967; Psychoyos, 1973; Enders, 1976; Das et al., 1994). The attachment reaction is followed by localized stromal cell decidualization and luminal epithelial apoptosis at the sites of blastocyst implantation (Parr et al., 1987). This results in subsequent adherence and penetration by trophoblast cells through the underlying basement membrane (Enders and Schlafke, 1967). Invasion of trophoblast cells continues through the stroma in a regulated manner by the remodeling of the extracellular matrix.

In mice, the molecular and cellular events leading to the uterine receptivity for implantation depend upon the priming of the uterus by P_4 and estrogen [estradiol-17 β , (E_2)] (Dey et al., 2004). A similar steroid hormonal regulation is required for the cell-type specific proliferation and differentiation in the uterus during the periimplantation period (Finn and Martin, 1967; Huet-Hudson et al., 1989). For example, preovulatory ovarian E_2 secretion causes proliferation and differentiation of the epithelium during the first 2 days of pregnancy. In contrast, rising P_4 levels from the newly formed corpora lutea result in a shift of proliferation from the epithelium to the stroma on Day 3. Stromal cell proliferation is potentiated by preimplantation ovarian E_2 on day 4 (the day of implantation). This E_2 secretion is also necessary for the increased endometrial capillary permeability at the sites of implanting blastocysts, and decidualization in the presence of the blastocyst or other decidualogenic stimuli (Finn and Martin, 1967; Yoshinaga, 1980; Huet-Hudson et al., 1989).

In order to initiate the implantation, the onset of the uterine receptive state occurs only for a limited period during pregnancy, pseudopregnancy, or when the uterus is appropriately prepared with P_4 and E_2 . For example, in mice, uterine receptivity can be divided into neutral, receptive, and non-receptive (refractory) phases (Dey et al., 2004). Normally, uteri are prereceptive on day 3 of pregnancy or pseudopregnancy under the rising ovarian P_4 levels. On Day 4, the P_4 -primed uterus becomes receptive when complemented with preimplantation ovarian E_2 secretion. Thus, E_2 is a critical determinant to specify the window of uterine receptivity for implantation (Ma et al., 2003). In fact, studies have shown that estrogen at different concentrations can initiate implantation; however, the duration of the window of uterine receptivity can be prolonged at lower concentrations of estrogen but rapidly closes at its higher levels (Ma et al., 2003). In contrast, P_4 alone is adequate for the

induction of implantation in the hamster, guinea pig, rabbit, and pig (Deanesly, 1960; Orsini and Meyer, 1962; Perry et al., 1973; George and Wilson, 1978). E₂ of embryonic origin may participate in implantation in these species (Pakrasi et al., 1985; Reese et al., 2008). In this regard, it is important to note that there is no evidence for E₂ synthesis in the mouse blastocyst (Stromstedt et al., 1996). Blastocysts implant only in the receptive uterus (Day 4), which then automatically enters into the nonreceptive state on Day 5. Similar uterine phases can be produced by ovariectomy on the morning of day 4 of pregnancy or pseudopregnancy before the preimplantation E₂ secretion. Under this condition, blastocysts in the pregnant uterus or transferred blastocysts into the pseudopregnant uterus undergo dormancy and fail to initiate the attachment reaction (delayed implantation). This condition can be maintained by continued P₄ treatment when the uterus remains in the neutral (similar to prereceptive) state, but is terminated by a single E₂ injection with blastocyst activation and attainment of uterine receptivity. Current molecular and genetic evidence indicates that several developmentally important, and E₂/P₄ regulated-signaling molecules, including homeobox transcription factors, cytokines, growth factors, lipid mediators serve as autocrine, paracrine, and juxtacrine factors, help to specify uterine receptivity (Lim et al., 2002; Paria et al., 2002; Dey et al., 2004; Wang and Dey, 2006; Kennedy et al., 2007; Lee et al., 2007).

UTERINE DECIDUALIZATION

The presence of blastocysts or artificial stimuli can trigger differentiation of stromal cells to decidual cells in the receptive uterus. The decidual cell reaction or decidualization is always preceded by increased uterine vascular permeability (Psychoyos, 1973; Kennedy, 1986). Pulse-labeling experiments with ³H-thymidine suggest that decidual cells originate from undifferentiated stromal cells (Galassi, 1968). The decidualization is first initiated at the antimesometrial site where blastocysts implant, which then spreads to the mesometrial pole, the presumptive site of placentation. Normally, this defines the implantation chamber in an antimesometrial–mesometrial direction in alignment with the anterior–posterior axis of the embryo. Generally, the decidualization process is characterized by stromal cell proliferation and differentiation into specialized type of cells (decidual cells) with polyploidy. In the mouse, the differentiating stromal cells in the antimesometrial location at the site of blastocyst form the primary decidual zone (PDZ) in the afternoon on Day 5. PDZ is avascular and epithelioid in nature (Paria et al., 1999). By Day 6, stromal cells next to the PDZ continue to proliferate and differentiate into polyploid decidual cells, forming a zone around the PDZ, termed the secondary decidual zone (SDZ). SDZ is fully developed by Day 7, while the PDZ degenerates progressively by apoptosis and by day 8 most of these cells disappear. After Day 8, the placental and embryonic growth slowly replaces the SDZ. A similar, although not identical (Paria et al., 2001; Bany and Cross, 2006), pattern of decidualization can be initiated by the application of artificial stimuli to a receptive pseudopregnant uterus, or one that has been appropriately primed by ovarian steroids (Dey, 1996). Using these model systems, cellular and molecular changes that occur during decidualization under the control of P₄ and E₂ can be examined. Numerous signaling molecules including homeo-box transcription factors, cell-cycle molecules, and growth factors have been proposed to play roles in the progression of this process (Lim et al., 2002; Paria et al., 2002; Dey et al., 2004; Wang and Dey, 2006).

Hoxa-10's ROLE IN UTERINE RECEPTIVITY, IMPLANTATION, AND DECIDUALIZATION

Hoxa-10 is a member of the homeobox or Hox multigene family of transcription factors (Satokata et al., 1995). This gene family shares a highly conserved 61-amino-acid sequence element called the homeobox that encodes for helix-turn-helix DNA-binding domain (Krumlauf, 1994). In the mammalian system, four *Hox* clusters (a–d) have been identified in different chromosomes, and they evolved through gene duplication (Krumlauf, 1994). In general, genes localized at the 3'-end of each cluster generally undergo activation first during early embryogenesis in the anterior region, while genes at the 5'-end of the clusters express late to the posterior regions of the developing embryo (McGinnis and Krumlauf, 1992; Krumlauf, 1994). *Abdominal B* (*AdbB*) is the most 5'-end gene within the *Drosophila* homeotic complex. In mammals, several *AdbB*-like genes have been identified, and they are located at the 5-ends of the Hox a, c, and d clusters (Benson et al., 1995). *Hoxa-10* is an *AdbB*-like Hox gene expressed in the developing genitourinary system in vertebrates (Dolle et al., 1991; Izpisua-Belmonte et al., 1991), and in the adult uterus during pregnancy (Benson et al., 1996). The role of Hoxa-10 in development has been defined by gene targeting experiments (Rijli et al., 1995; Satokata et al., 1995).

Null mutation of *Hoxa-10* in mice causes female infertility, which is primarily due to defective implantation and decidualization. Hoxa-10 mutant mice exhibit partial oviductal transformation of the proximal uterus, although this is shown to be unrelated to the fertility defects (Benson et al., 1996). Studies have revealed that a small percentage (~40%) of *Hoxa-10*^{-/-} mice can initiate the implantation reaction. This is consistent with the observed normal expression of several implantation related genes, such as *leukemia inhibitory factor* (*LIF*), *heparin-binding EGF* (*HB-EGF*), *amphiregulin*, and *cyclooxygenase-1* (*COX-1*) in the uterine epithelium of *Hoxa10*^{-/-} mice (Lim et al., 1999). In this regard, it is interesting to note that Hoxa10 expression is normal in *Lif* knockout uterus (Daikoku et al., 2004), which exhibits no sign of embryo–uterine interaction (Stewart et al., 1992). Overall, these studies suggest that uterine deletion of *Hoxa-10* do not completely antagonize the uterine receptivity in order to induce blastocyst attachment with the uterine luminal epithelium.

Previous studies have identified that *Hoxa-10* is primarily a P₄-regulated gene in the uterine endometrium (Ma et al., 1998; Taylor et al., 1998; Lim et al., 1999; Godbole et al., 2007). In case of mice, *Hoxa-10* is upregulated in the uterine stroma by P₄ in a protein synthesis-independent fashion, and this upregulation can be inhibited by progesterone receptor (PR) antagonist RU-486, suggesting PR's requirement for this induction (Ma et al., 1998). A similar observation in respect of hormonal regulation of *Hoxa-10* and its antagonism by P₄/PR signaling blocker was also reported in vitro with the human endometrial stromal cells (Taylor et al., 1998). Consistent with these observations, studies have also shown that *Hoxa-10* is regulated in the uterus during early pregnancy and its expression is primarily associated with cellular proliferation (Huet-Hudson et al., 1989; Satokata et al., 1995; Daikoku et al., 2004). For example, *Hoxa-10* is expressed on Days 1 and 2 of pregnancy in the luminal and glandular epithelium, expands to the stroma on Day 3, and becomes restricted to the stroma on Day 4. Following implantation by Day 5, a dramatic upregulation

of this gene occurs in stromal cells undergoing decidual transformation surrounding the implanting blastocyst. On Days 6–8, the expression progressively expands to the decidualizing stroma on both mesometrial and antimesometrial poles of the implantation site (Satokata et al., 1995; Daikoku et al., 2004). In this regard, gene knockout studies have shown that cellular proliferation within the stroma is severely compromised in the *Hoxa-10*^{-/-} mice in response to P₄ and E₂, although epithelial cell proliferation is unaffected in response to E₂. Consistent with this notion, certain uterine genes (prostaglandin receptors *EP3* and *EP4*) that are regulated by P₄ appeared to be aberrantly expressed in the uterine stroma of *Hoxa-10*^{-/-} mice (Lim et al., 1999).

In humans, *HOXA-10* expression in endometrial epithelial and stromal cells increases during the mid-secretory phase of the menstrual cycle, corresponding to the window of implantation (Taylor et al., 1998; Gui et al., 1999). In nonhuman primates (bonnet monkeys), *HOXA-10* protein expression appears to be different in the functionalis and basalis zones of the endometrium during the luteal phase (Godbole et al., 2007). Further studies revealed that this gene is primarily controlled by P₄, and the signals generated by the implanting embryo suggest that *HOXA-10* also plays an important role in early pregnancy of the nonhuman primates. Overall, these studies suggest that the endometrial expression of *Hoxa-10* plays an important role in the control of uterine receptivity, implantation, and decidualization (Satokata et al., 1995; Benson et al., 1996; Lim et al., 1999).

It has been well recognized that as a transcriptional factor, *Hoxa-10* can influence a host of uterine genes under the direction of P₄. In this regard, recent microarray analysis of P₄-responsive genes in the uteri of ovariectomized *Hoxa-10* null versus wild-type mice shows that many genes, including cell-cycle inhibitory genes p15 and p57, are upregulated in the null mice, implicating a negative impact on cell proliferation in the absence of *Hoxa-10* (Yao et al., 2003). Similarly, studies have shown that the downregulation of *Hoxa-10* is correlated with the upregulation of p57 during the progression of differentiation of human endometrial stromal cells in vitro, suggesting p57 acting as a downstream regulator of *Hoxa-10* presumably controls the exit of cell-cycle activity (Qian et al., 2005). In this regard, it is also worth mentioning that there is a class of cyclins: cyclin G1 and cyclin G2, which normally participate as inhibitory cyclins in the cell-cycle circuitry, undergo aberrant upregulation in the *Hoxa-10*^{-/-} uteri (Yue et al., 2005), suggesting that cyclin G isoforms negatively influence cell proliferation. For example, on the day of uterine receptivity (Day 4), our in situ hybridization results show that these cyclins are dramatically induced in uterine epithelial and stromal cells of *Hoxa-10* mutant mice as compared to wild-type littermates (Fig. 1). These results suggest that *Hoxa-10* is a key player for the regulation of cyclin G's in the uterus. The role of cyclin G isoforms in uterine biology is not yet known. The generation of cyclin G1 null mice has been reported (Kimura et al., 2001; Jensen et al., 2003); these mice survive to adulthood and display reduced tumor susceptibility due to increased tumor suppressive function of p53. However, the reproductive phenotype of these null mice has not yet been reported, and we are currently exploring this possibility. To our knowledge, no report of cyclin G2 deletion in mice is available.

Previously we reported that cyclin D3 expression follows the pattern of stromal cell proliferation and differentiation with the onset of decidualization (Tan et al., 2002).

Additionally, we have shown that the expression of cyclin D3 and p21, dramatically repressed in *Hoxa-10*^{-/-} uteri after application of a decidualogenic stimulus (Das et al., 1999; Rahman et al., 2006), indicating that impaired stromal cell proliferation/differentiation is a potential cause for the defective decidualization in the null mice. Recently, a similar notion is also revealed after the suppression of *Hoxa-10* during the progression of human endometrial stromal cell decidualization (Lu et al., 2008). Overall, these results suggest that the *Hoxa-10* mutation elicits defects that are relevant to the alteration of stromal cell responsiveness to P₄, leading to the implantation and decidualization defects.

Recent chromatin immunoprecipitation studies (Sun et al., 2009) provide evidence that *Hoxa-10* is a direct regulator for the control of *p/CAF* gene in human endometrial stromal cells. Studies revealed that *HOXA-10* interacts with the *p/CAF* promoter comprising three consecutive TTAT elements for its ability to suppress *p/CAF* gene expression. *P/CAF*, a histone acetyltransferase, commonly mediates gene transcriptional regulation through interaction with p300/CBP and various other regulators. In this regard, the control of *p/CAF*-mediated physical interaction and functional modification of *PTEN* is particularly interesting (Okumura et al., 2006). Furthermore, it is worth mentioning that conditional deletion of *PTEN* has been implicated in relation to the onset of endometrial cancer in mice (Daikoku et al., 2008). However, the physiologic significance of *HOXA-10/p/CAF/PTEN* signaling in uterine decidualization at the site of implantation remains unknown.

Hoxa-10 AND DEVELOPMENT OF REGIONAL DECIDUALIZATION

Cyclin D3 normally associates with *cdk4* or *cdk6* for cell proliferation. Coordinate expression of *cdk4* and cyclin D3 at the site of implantation on day 5 suggests that these regulators play roles in proliferation of decidualizing stromal cells. However, their suppression of expression in the PDZ on the afternoon of day 5 supports a view that cell proliferation activity of *cdk4/cyclin D3* ceases with the development of the PDZ. Similarly, on Day 6, the expression of *cdk4* and cyclin D3 in the mesometrial decidualizing stroma is again consistent with their role in proliferation. In contrast, down-regulation of *cdk4* in the antimesometrial SDZ on day 6 perhaps supports differentiation of stromal cells in this zone. Interestingly, on this day, a switch from *cdk4* to *cdk6* with continued expression of cyclin D3 in stromal cells within the SDZ is noted primarily due to terminal differentiation in association with decidual cell polyploidy. In this regard, we have recently noted that *Hoxa-10* deficiency causes loss of pole specific expression of *cdk4* and *cdk6* in the uterine decidual bed (Rahman et al., 2006). For example, the region-specific expression pattern for *cdk4* in the wild-type mice at the mesometrial pole and for *cdk6* at the antimesometrial pole is completely aberrant during decidualization on day 7 in *Hoxa-10* null mice (Fig. 2).

Recent microarray studies for the comparison of decidualization in *Hoxa-10*^{-/-} mice versus wild-type littermates have led us to the identification of numerous genes that are primarily involved in six functional categories, viz., growth regulation, tissue remodeling, immunomodulation, metabolism, membrane transport, and signal transduction (Rahman et al., 2006). Interestingly, several genes such as *hepatocyte growth factor (Hgf)*, *Snail2*, and *growth differentiation factor 10 (Gdf10)* are shown to be aberrantly expressed during decidualization in the *Hoxa-10* null mice as compared to that of wild-type mice. More

specifically, in the wild-type uteri, *Hgf* expression is localized to a population of cells at the mesometrial decidual bed, in contrast to the *Hoxa-10*^{-/-} uteri, with signals present in a few layers of subluminal stromal cells at the antimesometrial pole (Fig. 3). With respect to *Snail2* in wild-type mice, the expression is primarily detected at the mesometrial decidualizing stroma and in undifferentiated stroma below the muscle layer at the antimesometrial pole (Fig. 3). In contrast, the expression of *Snail2* in *Hoxa-10*^{-/-} mice is distributed primarily in sub-luminal decidualizing stromal cells (Fig. 3). In the case of *Gdf10*, expression is limited to the PDZ on Day 7 wild-type implantation sites whereas its expression is totally aberrant with apparent localization in the undifferentiated stroma in *Hoxa-10* null mice (Fig. 4). Furthermore, *Gdf10* expression at the interimplantation region (IIS) had enhanced expression in the subluminal stromal cells in *Hoxa-10*^{-/-} uteri as compared to wild-type uteri (Fig. 4). Overall, these results suggest that *Hoxa-10* is necessary to control region-specific decidual target genes, suggesting a developmental regulation by *Hoxa-10*.

The biological effects of Hgf are mediated by its high affinity binding membrane receptor, c-met protooncogene product (c-Met) (Trusolino et al., 1998). In the uterine endometrium, Hgf/c-Met signaling participates in multiple processes. In particular, human endometrial stromal cells expressing c-Met can be activated by its own Hgf during endometriosis (Yoshida et al., 2004). Studies have also provided evidence that Hgf regulates trophoblast cell proliferation and endometrial invasion during early pregnancy (Patel et al., 2000; Dokras et al., 2001; Lala and Chakraborty, 2003), uterine epithelial cell morphogenesis (Sugawara et al., 1997; Spencer and Bazer, 2002), and endothelial cell angiogenesis (Bussolino et al., 1992). Genetic deletion of Hgf or c-Met in mice shows identical phenotypes with primary defects in the development of placenta, liver, and muscle (Bladt et al., 1995; Schmidt et al., 1995; Uehara et al., 1995), resulting in death in utero. Thus, a conditional deletion approach is needed to study Hgf signaling in uterine decidualization, which is currently under progress in our laboratory. *Snail2*, a zinc finger transcriptional repressor, is a member of the Snail family, which constitutes *Snail1/Sna* and *Snail2/Slug/Snai2*. This family of proteins was first identified as regulators of cell migration during development (Nieto et al., 1994) and epithelial–mesenchymal transition (EMT) (Hemavathy et al., 2000a). Different signaling pathways including TGF- β , BMP, FGF, and Wnt are implicated in the induction of Snail family members during progression of EMT (Hemavathy et al., 2000b). The downstream signaling events mediated by Snail members regulate cell death and survival (Inukai et al., 1999; Vega et al., 2004) and suppress E-cadherin expression in epithelial cells (Batlle et al., 2000). *Snail2* null females were reported to be fertile, but no data were shown to support this conclusion (Jiang et al., 1998). Thus, it emphasizes a need for re-evaluation of *Snail2*'s role in the aspects of early implantation. In this regard, it should be recalled that mice lacking cannabinoid receptor CB1 were claimed to have normal fertility, but it has now been conclusively shown by us that these mice have major reproductive defects (Wang et al., 2004). *Gdf10*, a member of the transforming growth factor- β superfamily, is primarily involved in cellular differentiation processes (Cunningham et al., 1995). Although previous studies demonstrate that this gene is regulated in the mouse uterus during the cycle and early pregnancy, genetic deletion does not reveal any detectable fertility defects (Zhao et al., 1999).

Hoxa-10 PLAYS A ROLE FOR THE DIFFERENTIATION OF uNK CELLS

Uterine NK (uNK) cells are the most common lymphocytes found in the decidualizing uterus during the first half of pregnancy in rodents and humans (Croy et al., 1996; King, 2000; Liu and Young, 2001; Moffett-King, 2002; Dosiou and Giudice, 2005). After recruitment from the circulation, uNK cells rapidly proliferate and differentiate into a class of terminally differentiated cells, eventually acquiring cytoplasmic granules for various cytolytic mediators, including perforin, serine proteases like granzymes, phosphatases, mucin-1, and IFN- γ . Successful differentiation of uNK cells is believed to play important roles in nurturing the embryo, normal placentation, and uterine tissue remodeling. Interestingly, utilizing *Hoxa-10* knockout mice, we recently showed that the expression of several granzymes (viz., granzymes A, C, E, and G) and perforin genes is dramatically depressed in the decidual bed, as compared to that of wild-type mice (Rahman et al., 2006). These observations suggest that differentiation of uNK cells is a downstream event of *Hoxa-10* during decidualization mice. Studies have shown that *Hoxa-10* deletion in mice causes immunological disturbances primarily through the increase of T lymphocytes proliferation under the direction of P₄ in the uterine stroma (Yao et al., 2003). Previous studies have shown that the mouse uterine decidual bed at the mesometrial pole contains an increased number of viable leukocytes, as assessed by the expression of CD45 and CD90 (Croy et al., 1996; Collins et al., 2009). Furthermore, it has also been reported that CD45- and CD90-positive cells serve as precursors of uNK cells that undergo proliferation and differentiation in response to uterine decidualization signals in mice (Croy et al., 1996; Ain et al., 2004). In this regard, we observed that CD45- or CD90-positive cells are abundantly present in the mesometrial pole of the deciduoma on day 7 of pseudopregnancy in both wild-type and *Hoxa-10* null mice, suggesting these presumptive leukocytes are not a limiting factor for the aspects of uNK cell differentiation in *Hoxa-10* null mice (Rahman et al., 2006). Furthermore, it should be noted that this uNK cell differentiation defect is not primarily due to the lack of overall decidualization in *Hoxa-10* null mice, since the expression of uNK cell differentiation markers is clearly exhibited in another decidualization defective model with p21/cdkn1a double null mice (Ping et al., 2008). It should be further noted that several cell-cycle regulatory genes (viz., cyclin E, cyclin A, and cdk1) that are regulated during decidualization were not aberrant in the *Hoxa-10* null mice on day 7 of pseudopregnancy as compared to that of wild-type mice (Rahman et al., 2006), suggesting that all decidualization aspects are not compromised in *Hoxa-10* null mice. Overall, these results suggest that differentiation of uNK cells is severely compromised during decidualization with *Hoxa-10* deficiency. These results are clinically relevant since dysregulated immune function is implicated in recurrent pregnancy loss in humans (Dosiou and Giudice, 2005).

Hoxa-10 AND ENDOMETRIOSIS

Endometriosis is a common gynecological disease that remains an enigma. The pathogenesis of this disease is characterized by endometrial tissue growth outside the uterus and results in inflamed lesions, pain, and reduced fertility. Although heightened estrogenic activity and/or reduced P₄ responsiveness are considered to be involved in the etiology of this disease, neither the extent of their participation nor the underlying mechanisms are clearly

understood. Previous studies have shown that the uteri from endometriotic patients failed to show the mid-secretory rise of *Hoxa-10* expression as compared to the controls (Taylor et al., 1999), indicating that *Hoxa-10* may contribute to the etiology of infertility in patients with endometriosis. In this regard, recent studies suggest that epigenetic alteration by increased DNA methylation in the CpG clusters of *Hoxa-10* gene promoter may be responsible for this aberration of *Hoxa-10* expression (Wu et al., 2005; Kim et al., 2007). Utilizing a proteomic approach, we have recently identified the immunophilin FKBP52 as one of the *Hoxa-10*-mediated signaling molecules in the mouse uterus (Daikoku et al., 2005). Genetic deletion of *FKBP52* in mice establishes its critical role in embryo implantation, and they exhibit an increased P₄ resistance in uterine functions (Tranguch et al., 2005). Using *FKBP52*^{-/-} mice, recent studies showed that the loss of FKBP52 enhances the growth of endometriotic lesions with increased inflammation, cell proliferation, and angiogenesis (Hirota et al., 2008). Additionally, studies also showed a remarkable downregulation of FKBP52 in cases of human endometriosis, suggesting a potential role of FKBP52 in the etiology of human endometriosis. Furthermore, these studies provide evidence that P₄ resistance is favorably implicated in endometriosis and other gynecological diseases. Overall, these results suggest that *Hoxa-10* may be a potential therapeutic target to alleviate the problem with endometriosis.

Recent studies by Penna et al. (2009) have shown that Calpain5, a member of the cytoplasmic cysteine protease family involved in cellular differentiation and apoptosis (Suzuki et al., 2004), is expressed in the human endometrial stroma during the menstrual cycle and in the decidua. Based on transfection experiments in human endometrial stromal cells for the expression or suppression of HOXA 10, it has been revealed that Calpain5 acts as a downstream target of HOXA 10. Furthermore, studies have shown that the expression of Calpain5, like that of HOXA 10, is aberrantly downregulated in human endometriotic tissues (Gui et al., 1999; Taylor et al., 1999; Kim et al., 2007; Penna et al., 2009), suggesting that a HOXA 10–Calpain5 connection may be either directly or indirectly involved in the progression of endometriosis.

MATERNAL NUTRITION AND *Hoxa-10*

The role of maternal nutrition to establish pregnancy and maintenance of embryonic development has been well documented, yet very few reports show how specific nutrients can affect early implantation and decidualization. In this regard, the role of vitamin D in the reproductive function and pregnancy establishment in rodents is interesting (Halloran and DeLuca, 1980; Yoshizawa et al., 1997; Daftary and Taylor, 2006). While studies have implicated that vitamin D is not absolutely necessary for reproduction, female rats fed a vitamin D-deficient diet show diminished reproduction success with reduced litter size (Halloran and DeLuca, 1980; Daftary and Taylor, 2006). Additionally, female knockout mice for vitamin D receptors are unable to reproduce and exhibit defects of uterine development and decidualization (Yoshizawa et al., 1997). Similarly, the null mice for 25-hydroxyvitamin D-1 α -hydrolase, an enzyme necessary for the synthesis of vitamin D from its precursor 25-hydroxyvitamin D, show uterine hypoplasia and infertility primarily due to combined uterine and ovarian defects (Panda et al., 2001). Moreover, studies have shown that intraluminal injection of vitamin D in the rat uterus on Day 5 of pseudopregnancy

enhances uterine decidual response (Kwiecinski et al., 1989; Halhali et al., 1991). Overall, these data suggest that vitamin D has an essential role in fertility, necessary for differentiation of decidual cells.

Recent studies have implicated a direct role of vitamin D in the regulation of HOXA-10 in human endometrial stromal cells (Du et al., 2005). Studies have shown that the exposure of vitamin D to the human endometrial stromal cells causes enhancement of gene expression for *HOXA-10*. Transfection experiments using the HOXA-10 upstream regulatory sequence containing two putative vitamin D response elements (VDRE) into luciferase reporter constructs have revealed an activation of the reporter gene by vitamin D treatment, suggesting vitamin D can regulate HOXA-10 gene expression through direct interaction of vitamin D/ receptor complex at the promoter site. Furthermore, electrophoretic mobility shift assays clearly demonstrate that VDR binds to the HOXA-10 VDRE in the presence of 1,25-(OH)₂D₃. Overall, these studies suggest that vitamin D upregulates HOXA-10 expression via VDR/VDRE interaction in the HOXA-10 regulatory region. In summary, the influence of vitamin D on fertility likely occurs through regulation of uterine cell differentiation by the direct transcriptional activation of HOXA-10.

WORKING MODEL

Based on above discussions, a working model for regional developmental mechanism of decidualization by Hoxa-10 is presented in Figure 5. Hoxa-10 is expressed under the direction of ovarian P₄ in uterine stromal cells either prior to or during decidualization. Hoxa-10 mediates implantation-induced signaling pathways that include the regulation of stromal cell proliferation/differentiation and uNK cell differentiation, either in parallel or in a dependent fashion, to ultimately control a full complement of regional decidual development.

CONCLUDING STATEMENT

The process of decidualization is common to both mice and humans, although stromal cells in the human uterus undergo decidualization during the receptive phase (secretory phase) of each menstrual cycle in the absence of an embryo. Since Hoxa-10 is expressed in the receptive uterus and undergoes expressional alteration during decidualization or with endometrial pathogenesis (such as endometriosis), unraveling the downstream signaling pathway mediated by Hoxa-10 is clearly an interesting area of research that requires in-depth future investigation. It is not possible to use humans to study embryo–uterine interactions in early pregnancy. Mouse models serve to provide mechanistic approaches in defining a molecular basis of implantation/decidualization. Physiological functions of specific factors can be examined more mechanistically by manipulation of gene expression. Thus, the research using mice as an animal model will provide valuable information to better understand the intricacies of human pregnancy. Despite significant progress in IVF technology, the pregnancy success rate remains low, primarily due to higher incidence of unexplained pregnancy loss. Failure of pre-implantation embryo development, implantation, and placentation are major determinants for pregnancy loss. Basic research to better understand these events will help alleviate problems of infertility.

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Abbreviations

E₂	estradiol-17(beta)
EMT	epithelial-mesenchymal transition
Hox	homeobox
P₄	progesterone
PDZ	primary decidualization zone
SDZ	secondary decidualization zone
uNK	uterine natural killer lymphocytes

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Figure 1.

In situ hybridization for the expression of cyclin G1 and cyclin G2 genes in the uteri of wild-type (WT) and *Hoxa-10*^{-/-} mice on Day 4 of pseudopregnancy. Representative dark-field photomicrographs of longitudinal uterine sections are shown at 40×. le, luminal epithelium; s, stroma; myo, myometrium.

Figure 2.

In situ hybridization of *cdk4* and *cdk6* during experimentally induced decidualization on d7 of pseudopregnancy in *Hoxa-10*^{-/-} mice as compared to wild-type (WT) littermates. M, mesometrial pole; AM, antimesometrial pole. Bars: 100 μ m. *Note:* The region-specific expression of *cdk4* or *cdk6* in the corresponding mesometrial or antimesometrial location, as indicated by arrows in the wild-type mice, is lost in *Hoxa-10*^{-/-} mice.

Figure 3.
In situ hybridization of *Hgf* and *Snail2* during experimentally induced decidualization on day 7 (d7) of pseudopregnancy in *Hoxa-10* mutants as compared to wild-type (WT) littermates. M, mesometrial pole; AM, antimesometrial pole. Bars: 100 μ m.

Figure 4.

In situ hybridization of *Gdf10* at the embryo implantation sites (IS) and interimplantation regions (IIS) on Day 7 (d7) of pregnancy in *Hoxa-10* mutants as compared to wild-type (WT) littermates. M, mesometrial pole; AM, antimesometrial pole; E, embryo; pdz, primary decidual zone; le, luminal epithelium; s, stroma. Bars: 100 μ m.

Figure 5.

A scheme depicting potential roles of Hoxa-10 in mediating regional development of decidualization.