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ENDOMETRIAL DECIDUALIZATION: OF MICE AND MEN

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Abstract

In murine and human pregnancies, embryos implant by attaching to the luminal epithelium and invading into the stroma of the endometrium. Under the influence of the steroid hormones estrogen (E) and progesterone (P), the stromal cells surrounding the implanting embryo undergo a remarkable transformation event. This process, known as decidualization, is an essential prerequisite for implantation. It comprises morphogenetic, biochemical and vascular changes driven by the estrogen and progesterone receptors. The development of mutant mouse models lacking these receptors has firmly established the necessity of steroid signaling for decidualization. Genomic profiling of mouse and human endometrium has uncovered a complex, yet highly conserved network of steroid-regulated genes that supports decidualization. In order to advance our understanding of the mechanisms regulating implantation and better address the clinical challenges of infertility and endometrial diseases such as endometriosis, it is important to integrate the information gained from the mouse and human models.

Keywords

Decidualization; Implantation; Progesterone Receptor; Endometriosis

Introduction

In mammals, the onset of pregnancy is heralded by the implantation of an embryo into the uterus, initiating a complex and reciprocal relationship between the mother and the fetus. This maternal-fetal dialogue involves an intimate interaction between the specialized trophectodermal cells of the embryo and the receptive uterine lining of the mother. The steroid hormones estrogen (E) and progesterone (P) play a pivotal role in directing early uterine events during pregnancy. These hormones orchestrate the changes in the uterine

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epithelium that makes it competent to attach to the blastocyst and initiate the process of implantation[1,2]. Subsequently, E and P regulate a series of complex interactions at the interface between the developing embryo and the cells in the stromal compartment, leading to the formation of a differentiated maternal tissue, known as the "*decidua*," which supports embryo growth and maintains early pregnancy. Although the details of these events vary in different species, the central roles played by E and P in controlling various phases of early pregnancy are common to many mammals[3]. Ultimately, the timely and ordered regulation of the cellular and genetic changes in the endometrial tissue surrounding the implanting embryo is critical for the establishment of early pregnancy. In humans, infertility is one of the most common disturbances of reproductive health, with 10–15% of couples finding it difficult or impossible to conceive. Despite significant advances in assisted reproductive technologies (ART), many couples experience infertility as a result of failed implantation of the fertilized embryos into the uterus and subsequent loss of these embryos. The implantation rates in ART remain low, even with high-quality embryos, pointing to the importance of uterine deficiency during implantation as a major cause of pregnancy failure and infertility[4]. Therefore, it is imperative to gain a clear understanding of the cellular and genetic mechanisms underlying embryo implantation in order to better address clinical approaches to infertility.

In this review, we will focus on studies conducted in the mouse, which has served as an important animal model to investigate the regulation of uterine functions by E and P. We will discuss relevant studies conducted in endometrial cultures obtained from human biopsies. We will summarize the biological sequence of events taking place during the process of decidualization following embryo implantation, describe selected steroid hormone-controlled signaling pathways involved in this transformation and discuss how improper decidualization may lead to endometriosis, a clinical condition of endometrial dysfunction.

Part 1. The physiology and cell biology of decidualization

The mouse blastocyst reaches the uterus four days after fertilization[5,6]. The attachment of the blastocyst to the uterine epithelium initiates the process of decidualization that involves differentiation of the underlying fibroblastic stromal cells into morphologically distinct cells, termed decidual cells[7–9]. These transformed cells have unique biosynthetic and secretory properties. There are also substantial changes in the extracellular matrix. This cellular differentiation, induced by P following brief priming with E, is a prerequisite for successful implantation. Decidua is a transient tissue, which begins to develop- at the time of blastocyst attachment on day 4.5 of pregnancy. During the next 3 days of gestation, decidual cells surrounding the site of embryo attachment proliferate and differentiate extensively, eventually becoming larger, often with bi-nucleated or polyploid status[10]. Stromal cell polyploidy during differentiation eventually results in apoptosis and the latter process is thought to limit the life span of decidual cells, allowing placental expansion and development [8,9]. By the end of the invasive period (day 10.5), the decidua is totally regressed. A variety of functions have been attributed to the decidua, such as providing a source of growth factors and cytokines that support embryo development, serving an immunoregulatory role during pregnancyand regulating trophoblast invasion[1,7]. An additional role for decidual cells is to support maternal blood vessel formation in order to perfuse and nourish the developing embryo. Despite these critical functions, steroid-induced signaling molecules that participate in the formation and function of the decidual tissue remain poorly understood.

In the human, endometrial preparation for pregnancy is initiated by tissue remodeling during the secretory phase of each menstrual cycle following ovulation. In resemblance to mice, the

hallmark of early endometrial remodeling in humans is the process of decidualization. This differentiation event first begins in late secretory phase endometrial stromal cells surrounding the spiral arteries of the uterus and involves their transformation into larger, rounded decidual cells [11,12]. As a result of the differentiation, decidual cells acquire unique biochemical and cellular properties that enable them to support embryo implantation. Therefore, the decidualization of human endometrium is a requirement for successful pregnancy. In the event of embryo implantation, stromal transformation spreads beyond the perivascular regions and is prolonged, so as to complete the formation of the maternal decidual compartment.

Similar to the mouse, the participation of the steroid hormones E and P is critical for the regulation of the cyclical events in human endometrium[12–14]. Extensive proliferation of both epithelial and stromal cells occurs in response to rising E levels during the first half of the menstrual cycle, or proliferative phase. During the second half of the cycle, termed secretory phase, P action dominates in the endometrium where it induces differentiation of stromal cells. Although the developmental changes that define various stages of embryo implantation and decidualization have been described in both mice and humans, the identities of the hormone-regulated pathways that control the decidualization process remain largely unknown. In the next section, we will discuss the roles of several key steroid-hormone regulated molecules that have been identified through the efforts of many different groups and are thought to participate in the establishment of early pregnancy by controlling uterine decidualization.

Part 2. Steroid-regulated pathways controlling decidualization

2.1. Estrogen and progesterone receptors

Despite the abbreviated reproductive cycle in mice relative to that of humans, this species has served as an important animal model to study the regulation of uterine functions by E and P. The cellular actions of these hormones are mediated through intracellular estrogen receptor (ER) and progesterone receptor (PR) proteins, which are hormone-inducible transcription factors[15]. Hormone-occupied ER or PR is recruited to specific DNA response elements in the promoters of target genes. These genomic actions trigger the expression of specific gene networks in different cell types within the uterus and the products of these genes in turn mediate the hormonal effects during the reproductive cycle and pregnancy. The development of mutant mouse models lacking ER and PR has established the requirement of these hormones and their downstream signaling pathways for successful establishment and maintenance of pregnancy[16,17].

The PR knockout (PRKO) mice display a non-receptive uterus that is refractory to an artificial deciduogenic stimulus, such as the intrauterine injection of oil[17]. Thus, the PRKO mouse uterus cannot support embryo implantation. When these mutant mice are placed under chronic E stimulation, increased hyperplasia and an inflammatory response is observed, due to unopposed action of E in the uterus[2]. On the other hand, ERα-null mice have impaired uterine growth and fail to prepare for blastocyst attachment[16]. Due to the pleiotropic and multisystem effects of global ERα and PR gene ablation, the interpretation of the mutant phenotypes is complicated. Thus, we used the Cre-Lox strategy in our recent studies and have uncovered a novel role for ERα in mediating stromal cell decidualization (Mary Laws, Indrani Bagchi, and Milan Bagchi, unpublished results). Conditional targeting of ERα deletion is achieved through expression of Cre recombinase under the control of the endogenous PR promoter, avoiding the embryonic and developmental effects of global ERα ablation and allowing significant recombination activity only in the PR expressing tissues of the mature animal[18]. The ablation of ERα in PR-positive uterine cells resulted in a failure of

the mutant animals to exhibit a decidual response to an artificial stimulus, indicating an important role for ERα in decidualization.

In the human endometrium, the involvement of the ligand-bound PR is paramount to decidualization [11,19]. The expression of both isoforms of this receptor, PR-A and PR-B in human endometrial stromal cells (hESCs) has been documented. PR is thought to control decidualization through the transcriptional regulation of a variety of decidual proteins, the identities of which are presently limited. To further add to the complexity, the PR- and cAMP-dependent pathways are both vital for maintenance of the decidual phenotype of hESCs, and appear to converge on common downstream molecular targets[19]. Ultimately, the loss of appropriate PR-mediated regulation of decidualization could result in overall infertility and a variety of endometrial disorders[11]. In summary, mediation of both murine and human endometrial decidualization requires ERα and PR proteins to effect uterine stromal differentiation. The current effort in the field is to identify and characterize the molecular pathways underlying the actions of E and P and their respective receptors.

2.2. Steroid receptor chaperones

The appropriate functioning of nuclear receptors such as PR depends on their interaction with molecular chaperone proteins such as heat shock proteins and immunophilins. Two such immunophilins are FKBP4 (FKBP52) and FKBP5 (FKBP51) among which FKBP4 exhibits closely overlapping expression with PR in the uterine stroma during decidualization[20,21]. The female FKBP4-null mice are reproductively impaired despite normal development to adulthood and unaffected ovarian functions. The FKBP4-null mice cannot display a decidual response and fail to support embryo implantation. In agreement with a close functional association of FKBP4 with PR, there is an overall suppression of PR target gene expression in the FKBP4-null mice[20].

2.3. Downstream mediators of E and P actions during decidualization

A number of studies employing gene expression profiling have identified steroid-regulated pathways that control epithelial and stromal functions during implantation in mice[22,23]. Here, we describe the physiological relevance of several ER- and PR-target genes in embryo implantation and decidualization.

2.3.1. CCAAT/Enhancer Binding Protein-β (C/EBPβ)—This transcription factor belongs to a family of basic leucine zipper (bZIP) proteins, which regulate numerous biological processes, including cell proliferation, differentiation, metabolic homeostasis, acute phase inflammation and apoptosis[24]. Among the C/EBPs, C/EBPβ has been identified as a critical mediator of the biological actions of E and P in mouse uterus[25]. During normal murine pregnancy, a robust induction of C/EBPβ occurs predominantly in the stromal compartment during the decidualization phase. Similarly, C/EBPβ expression is up regulated upon experimentally induced decidualization. The decidual stage-specific expression of C/EBPβ likely arises from a complex interplay of E and P within the uterine compartments. Although a transient rise in E in the preimplantation period induces C/EBPβ in the stromal compartment of pregnant uterus, PR becomes a critical regulator of this gene as these cells become progressively differentiated. This view is strongly supported by the observation that RU486, an antagonist of PR, efficiently suppressed stromal C/EBPβ expression when administered on day 6 of pregnancy[25].

The essential role for C/EBPβ in decidualization was uncovered when mice carrying a deletion in this gene were examined. Peter Johnson and coworkers first reported that female mice lacking C/EBPβ were infertile, due to complications in ovulation[26]. However, upon further examination, functional abnormalities in the uterus also were evident in mutant

females [25]. Wild-type (WT) embryos transferred to pseudopregnant uteri of C/EBPβ-null mice failed to implant. Uterine defects noted in the mutant mice included a reduced epithelial cell proliferation in response to E, and more importantly, an impaired stromal response to a deciduogenic stimulus. There was a striking lack of expression of alkaline phosphatase (ALP), a classical early marker of stromal cell differentiation, in C/EBPβ-null stroma. These phenotypic defects were observed in the presence of exogenously administered E and P, indicating that they were independent of ovarian malfunction, and intrinsic to the uterus.

Recent studies revealed an intense expression of C/EBPβ in glandular epithelium and differentiating stroma of human endometrium during the mid-secretory phase of the cycle[27]. The biological significance of C/EBPβ function during decidualization was analyzed in primary cultures of human endometrial stromal (hESC) cells (Wei Wang, Robert Taylor, Indrani Bagchi, and Milan Bagchi, unpublished results). Numerous reports have shown that hESCs isolated from biopsies collected in the proliferative phase of the menstrual cycle can be induced to undergo decidualization *in vitro* by treatment with a "decidualization cocktail" containing P, E, and a cAMP analog[28,29]. Well-characterized biochemical markers, including prolactin (PRL) and insulin-like growth factor binding protein-1 (IGFBP-1), are induced during this process[30,31]. We observed an early enhancement of C/EBPβ expression during in vitro decidualization, indicating the possibility that this transcription factor is a critical driver of endometrial stromal differentiation. Indeed, when the transcriptional activity of C/EBPβ was attenuated using a dominant-negative mutant, the differentiation process was strongly inhibited, providing evidence that C/EBPβ is a critical regulator of human stromal decidualization. This result is consistent with previous reports documenting C/EBPβ regulation of the PRL promoter in differentiating hESCs[32].

2.3.2. Homeobox A10 (Hoxa-10)—Hoxa-10 is a transcription factor belonging to the *Hox* family of genes, which function as regulators of early development in *Drosophila* but their roles in mammalian biology are increasingly evident. In the mouse uterus, Hoxa-10 appears in the subepithelial stroma on day 3.5 and persists through day 4.5. The Hoxa-10-null females were found to be compromised in their ability to support pregnancy[33,34]. A failure of the Hoxa-10-null stromal cells to proliferate results in an impaired decidual reaction and renders these uteri non-receptive to embryo implantation. Of particular importance is the dramatic down-regulation of the uterine prostaglandin synthesizing enzyme, cyclooxygenase-2 (COX-2) in Hoxa-10-null uteri. It has been proposed that the down-regulation of the COX-2 enzyme, in addition to the prostaglandin receptor subtypes EP_3 and EP_4, contributes to the decidualization defect and failed implantation in Hoxa-10-null mice[34] Of further interest is the emerging role of Hoxa-10 in human endometrial decidualization. This factor and its close relative, Hoxa-11, have both been confirmed as targets of E and P regulation in decidualizing human endometrial stroma[35,36]. Similar to the mouse, Hoxa-10 and Hoxa-11 expression in the endometrium has been correlated to the window of implantation in the human.

2.3.3. Bone Morphogenetic Protein-2 (BMP2) and Wingless 4 (Wnt4)—BMPs are the largest family of morphogens belonging to the TGF-β superfamily of growth modulators. They were initially identified by their ability to induce ectopic formation of cartilage and bone, and were subsequently shown to influence a broad spectrum of cellular functions including proliferation, differentiation, apoptosis, migration, and adhesion in a large variety of cell types during embryonic development[37]. Recently, the decidual stage-specific expression of BMP2 and its receptor in the uterine stroma during early pregnancy was uncovered, providing a potential link between BMP2 signaling and the steroid-dependent changes underlying stromal differentiation during decidualization[38].

BMP2 is expressed in the stromal cells surrounding the implanted embryo[39]. Studies by S.K. Dey and coworkers demonstrated that when beads coated with heparin-binding epidermal growth factor were placed into pseudopregnant uteri, they induced decidualization concomitantly with BMP2 expression[40]. Administration of the antiprogestin, RU486 downregulated BMP2 expression in uterine stromal cells, indicating that the expression of BMP2 is downstream of pathways mediated via PR[41]. The functional role of BMP2 during embryo implantation was demonstrated using transgenic mice carrying a conditional deletion of this gene in mouse uterus [39]. BMP2-null mice are infertile due to the absence of a decidual response. Although the embryos attach to the uterine epithelium, the stromal cells fail to undergo decidualization. The decidual phenotype could be partially rescued through the addition of recombinant BMP2 into the uterine lumen, ruling out the possibility that a developmental defect contributes to the implantation failure.

In parallel to the creation of the BMP2-null mice, the mouse and human primary stromal cultures were utilized to provide novel insights into the role of BMP2 and its downstream signaling pathways in uterine decidualization[41]. The addition of recombinant BMP2 to the undifferentiated mESC significantly increased Smad signaling which in turn led to an increase in ALP activity and markedly accelerated the stromal differentiation program. Furthermore, when a siRNA targeted to BMP2 mRNA was transfected into undifferentiated mESCs, it efficiently suppressed BMP2 expression and also inhibited stromal differentiation as indicated by the drastically reduced expression of the well-established decidual markers. Similarly, in hESCs, treatment with exogenous BMP2 accelerated the decidual program *in vitro*, whereas attenuation of BMP2 mRNA with siRNA prevented hESC differentiation[41]. These studies indicated that BMP2-mediated canonical Smad signaling in the uterus plays a critical role in stromal cell differentiation during early pregnancy.

Microarray analyses conducted with BMP2-null mESCs revealed the involvement of Wnt4 in BMP2 signaling[41]. Like BMPs, the Wnt family of signaling proteins exert pleiotropic effects including mitogenic stimulation, cell fate specification, and differentiation[42]. An overlapping expression of BMP2 and Wnt4 was noted *in vivo* in the uterine stromal compartment during early pregnancy[40]. Most importantly, a marked induction of Wnt4 in mESC cultures was observed when Bmp2 was added to induce differentiation[41]. In contrast, the expression of other Wnts were not altered significantly in response to BMP2, suggesting that Wnt4 is a specific downstream target of BMP2 signaling in uterine stromal cells during decidualization. A recent report described the expression of several members of the Wnt family and their inhibitors in different cell types of human endometrium during the menstrual cycle[43]. While the functional significance of their expression in human endometrium is not known, the Wnt-β-catenin pathway is a potential regulator of angiogenesis in human tissues via VEGF gene expression.

2.3.4. Indian Hedghog (Ihh) and COUP-TFII—Studies by DeMayo and coworkers revealed a novel role for another morphogen, Indian Hedgehog (Ihh), in mediating epithelial and stromal cross-talk that triggers decidualization. The regulation of this factor by P and PR appears to be confined to the luminal epithelium of the receptive uterus during the window of implantation[44,45]. The expression of Patched-1 (Ptch1), the receptor for Ihh, exhibited a similar temporal pattern in the stromal compartment, supporting an epithelial-stromal communication. Conditional ablation of Ihh expression in the uterus yielded reproductive defects that are consistent with a role for epithelial Ihh in mediating PR-controlled decidualization of the subjacent stroma[46]. Recent studies using tissue recombinants indicated that the expression of Ihh in the epithelium is controlled by stromal PR[47], indicating complex interactions between epithelial and stromal compartments that requires further exploration.

Prominent among the potential target genes mediating Ihh function in the uterine stromal cells is the orphan nuclear receptor family member COUP-TFII. Insights into the reproductive functions of COUP-TFII was gained by Cre recombinase-mediated conditional excision of this gene in mouse uterus [48]. The loss of COUP-TFII in the uteri rendered these mice infertile primarily due to a loss of embryo attachment in the luminal epithelium. Furthermore, a failure of the artificially induced decidual reaction revealed a stromal impairment. Interestingly, administration of recombinant BMP2 *in vivo* led to a partial rescue of the decidualization phenotype in COUP-TFII-null uteri, indicating that BMP2 is a downstream mediator of COUP-TFII function[48]. Collectively, these findings broadly defined a dynamic pathway in which PR regulates epithelial Ihh expression and secretion into the stromal compartment. Ihh then acts via the Ptch1 receptor to induce a decidual response, which is mediated by COUP-TFII and BMP2. The COUP-TFII function in human decidualization is yet to be explored.

2.3.5. Cell-to-cell communication in the uterus: Role of Connexin 43—In mice, stromal decidualization is accompanied by a significant remodeling of the vasculature surrounding the zone of embryo implantation. The resulting angiogenic network is extensive and serves to support the proper growth and development of the implanted embryos[3]. In the human, the endometrium undergoes cyclical phases of vascular remodeling with each successive menstrual cycle. The intimate association between the growing vascular network and the decidual tissue is suggestive of a regulatory role for stromal factors in uterine angiogenesis. Recent studies revealed that an E-regulated gap junction component, Connexin 43 (Cx43), influences communication between decidual cells and blood vessels during decidualization[49]. Connexins are proteins that form transmembrane channels through which intercellular communication takes place[50]. The expression of Cx43 is regulated by E in the uterine stromal cells of pregnant mice. In order to analyze its function in decidualization, a conditional Cx43 knockout mouse was generated [49]. These mice exhibited impaired stromal differentiation in response to a deciduogenic stimulus, and more importantly, failed to display the appropriate vascular expansion needed to maintain pregnancy. A lack of proliferation of endothelial cells, which line the uterine blood vessels, and the dysregulation of several key angiogenic factors, such as vascular endothelial growth factor (VEGF) and angiopoietins 2 and 4, were evident in the absence of the Cx43 gap junctions. In hESC, siRNA-mediated knockdown of Cx43 impaired decidualization and gap junction communication in a similar fashion to mice. Collectively, these findings supported a plausible model in which Cx43 gap junctions control the paracrine secretions of decidualizing stromal cells. It is likely that certain of these paracrine factors influences the proliferation and migration of endothelial cells, thereby critically regulating blood vessel formation in the uterus[49].

Part 3. Other critical regulators of decidualization

3.1. Forkhead Box O1 (FoxO1)

The forkhead/winged-helix family contains various members that have been implicated in cell differentiation and proliferation [51]. Within this family, Forkhead Box O1 (FoxO1) was shown to be cAMP-inducible in hESCs[52]. FoxO1 displays persistent nuclear presence during decidualization, suggestive of important transcriptional activities on its part in hESCs. Further evidence has revealed that FoxO1 participates in the regulation of PRL expression at the promoter level, underlining the intimate association between this factor and endometrial decidualization[52]. It was shown that FoxO1 and C/EBPβ occupy the PRL promoter through a cooperative interaction, lending support to the notion that both these factors are critical for activating and maintaining PRL expression during the course of decidualization[11,19,52]. SiRNA-mediated silencing of FoxO1 inhibited a number of

decidualization-specific genes in hESCs, pointing again to a critical functional role for this transcription factor in human decidua[53].

3. 2. Cytokine signaling by Interleukin 11 and Interleukin 11 Receptor α (IL-11Rα)

Interleukins are secretory cytokines, which are responsible for a variety of cellular functions. Although they are not direct targets of regulation by steroid hormones E and P, their actions are nevertheless important for endometrial functions in both mice and humans. Specifically, the expression interleukin 11 (IL-11) rises transiently in the decidua upon embryo implantation, while that of its receptor, interleukin 11 receptor alpha (IL-11Rα), is localized to this tissue. Consistent with this expression pattern, mice carrying a deletion of the gene encoding IL-11Rα displayed an impaired decidual response [54,55]. A recent study showed that inhibition of IL-11 function with an antagonist disrupted the proliferation and differentiation of stromal cells in pregnant mice and of hESC *in vitro* [56]. In human endometrial stromal cells, IL-11 and its receptor are believed to control mitotic expansion of hESCs obtained from early to mid-secretory stage endometrium[57].

Part 4: Uterine dysfunctions: consequences of improper decidualization

Among the clinical diseases of the uterus, endometriosis is a major public health concern for women of reproductive age[58,59]. It is a reproductive tract disorder in which endometrial tissue is transported by retrograde menstruation and implants ectopically at abnormal sites outside the uterine cavity. Endometriosis is associated with pelvic pain and infertility. Initial attachment of the menstrual tissue, containing both glandular epithelial and stromal elements, at extrauterine sites, such as the pelvic peritoneum and ovarian surface, establishes small early lesions. These lesions proliferate, and invade the underlying tissue, leading to inflammation, and more progressive disease. The origin of the disease and its association with infertility are not well understood. However, ample evidence now exists in support of biochemical abnormalities present in the endometrium (eutopic) of women with endometriosis[60–63]. It is thought that this inherently abnormal endometrial tissue predisposes to lesions at ectopic sites. Alterations in the molecular pathways in the eutopic endometrium during implantation are likely to contribute to the infertility in these subjects. Indeed, a number of recent studies revealed dysregulated gene expression in eutopic endometrium of subjects with endometriosis within the window of implantation and indicated impaired differentiation of endometriotic stromal cells in response to P[64,65]. Some of the potential mechanisms by which the molecular pathways underlying decidualization are altered in endometriosis are summarized below.

4.1. Progesterone resistance in endometriosis

Defects in steroid hormone signaling in eutopic and ectopic endometriotic tissues have been described. Reduced expression of the PR-B isoform and P target genes, e.g., 17β-hydroxysteroid dehydrogenase-2 and glycodelin, in endometriotic tissue was also reported[61,62,66]. Similarly, other gene products that are important for endometrial stromal decidualization such as Hoxa-10 and Hoxa-11 are downregulated in endometriotic tissue[65]. Based on these findings, a hypothesis was forwarded that endometriosis is associated with altered regulation of P-responsive genes, presumably due to an aberrant PR signaling that creates a condition of P resistance in the endometriotic implants.

4.2. Aberrant Estrogen synthesis & metabolism

There is also a growing body of evidence indicating that endometriosis is also an E-dependent disease[67]. The expression of aromatase, an enzyme that catalyzes the conversion of testosterone to E, is markedly enhanced in the endometriotic tissue[68]. Recent studies described increased expression of the steroidogenic factor-1 (SF-1), an orphan nuclear

receptor that stimulates transcription of aromatase gene, in endometriotic lesions[69,70]. Additionally, the promoter region of SF-1 was found to undergo epigenetic changes, including selective DNA demethylation, in endometriotic stromal cells, allowing this factor to be overexpressed and leading to enhanced aromatase expression. It was further suggested that the increased local production of E sustains the growth of endometriotic lesions. Reduced P sensitivity coupled with the increased presence of local E implies that several steroid hormone-regulated molecular pathways might be in a state of imbalance in endometriosis.

Conclusions

Infertility in women due to impaired uterine functions is a critical health concern that requires further exploration. The involvement of steroid hormones E and P acting through their cognate receptors is critical to the precise and timely regulation of the endometrial events required for pregnancy. Beginning with embryo attachment, stromal decidualization and eventually vascularization of the endometrium require the appropriate participation of a host of genes that are regulated by E and P (Figure 1, Table 1). This review has focused on the theme of decidualization as a biological event that is both unique and essential to pregnancy in mice and humans. The use of powerful mouse genetics and manipulation of hESC culture systems, combined with informative gene expression profiling strategies in both mouse and human samples, has made it feasible to pinpoint specific molecular cues to decidualization. These and future studies will serve to illuminate the mechanisms by which E and P regulate uterine function and will serve as a framework in which to explore new therapeutic approaches to human endometrial diseases, particularly those associated with aberrant steroid hormone signaling such as excessive E action or reduced P sensitivity.

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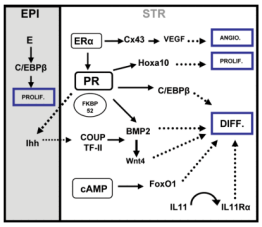

Figure 1. The molecular pathways controlling decidualization

An emerging blueprint of the molecular pathways underlying the mouse and human endometrial functions, leading to decidualization, is shown. EPI and STR represent uterine epithelial and stromal compartments, respectively. The bold arrows represent the hypothetical linear relationships between the indicated factors. The dashed arrows point to functional links for which the mechanisms are still unknown. The schematic describes only those factors that are discussed in this article. BMP2. Bone morphogenetic protein 2; cAMP, cyclic adenosine monophosphate; C/EBPβ, CCAAT enhancer binding protein-β; COUP-TFII, chicken ovalbumin upstream promoter-transcription factor II; Cx43, connexin 43; E, estrogen; ER, estrogen receptor; FoxO1, Forkhead box O1; Hoxa-10, Homeobox A-10; Ihh, Indian hedgehog; IL, interleukin; IL11Rα, interleukin receptor α, PR, progesterone receptor; VEGF, vascular endothelial growth factor; Wnt4, Wingless 4; PROLIF, proliferation; DIFF, differentiation; ANGIO, angiogenesis.

Table 1

Genes Critical for Endometrial Decidualization and Implantation.

Gene	Molecule Encoded (*Biological Category)*	Model(s)	Decidual Phenotype	Ref. #
PR (Pgr)	Progesterone Receptor *(Nuclear Receptor / Transcription factor)*	Mouse KO* † hESC	Infertility; Defective decidualization	17
Erα (Esr1)	Estrogen Receptor-α *(Nuclear receptor / Transcription factor)*	Mouse KO* ‡ Mouse KO	Infertility; Impaired uterine receptivity; Defective decidualization	16
C/EBPβ	CCAAT/Enhancer Binding Protein Beta *(Transcription factor)*	Mouse KO hESC	Infertility; Impaired uterine receptivity; Defective decidualization	25, 26 27, 32
Hoxa10	Homeobox A10 *(Transcription factor)*	Mouse KO hESC	Reduced fertility; Defective stromal proliferation	33, 34 65
FoxO1	Forkhead/winged Helix protein *Transcription factor*	hESC	Defective decidualization	52, 53
Bmp2	Bone Morphogenetic Protein-2 *(Morphogen)*	‡ Mouse KO hESC	Infertility; Defective decidualization	39 41
Wnt4	Wingless-type MMTV integration site 4 *(Morphogen)*	§ mESC	Infertility; Defective decidualization	41
Ihh	Indian hedgehog *(Morphogen)*	‡ Mouse KO	Infertility; Impaired uterine receptivity	46
COUP-TFII (Nr2f2)	Chicken Ovalbumin Upstream-Promoter Transcription Factor II *Transcription factor*	‡ Mouse KO	Infertility; Impaired uterine receptivity; Defective decidualization	48
IL-11Rα	Interleukin-11 Receptor α *Cytokine signaling*	Mouse KO	Infertility; Defective decidualization	54–56
Cx43	Connexin 43 *Gap Junctional communication*	‡ Mouse KO hESC	Reduced fertility; Impaired embryo growth; Compromised angiogenesis; Defective decidualization	49
FKBP52	*FK506 Binding Protein PR-A chaperone / Immunophilin*	Mouse KO	Infertility; Defective decidualization	20, 21

*Total knockout

‡Conditional knockout using Cre-Lox approach

KO, knockout; hESC, human endometrial stromal cells.

†Human endometrial stromal cells primary culture

§Mouse endometrial stromal cells primary culture