REVIEW

Progesterone governs endometrial proliferation-differentiation switching and blastocyst implantation

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Abstract. Blastocyst implantation contains the following three processes: apposition, attachment, and invasion of the blastocyst. Ovarian hormone progesterone (P_4) regulates these processes exquisitely. P_4 -induced molecular communications between the endometrial epithelium and stroma as well as endometrial proliferation-differentiation switching (PDS) until blastocyst attachment are fundamental steps in blastocyst implantation. Based on the knowledge obtained from the previous studies of mouse models by my group and others, this article outlines how P_4 directs the uterus to complete blastocyst implantation.

Key words: Blastocyst implantation, Uterine receptivity, Progesterone, Endometrial proliferation-differentiation switching

Introduction

Infertility and recurrent miscarriage are one of global health problems. Despite enormous efforts by medical experts, their underlying mechanisms remain unclear. Blastocyst Implantation is a process of the first embryo-maternal encounter in pregnancy. It contains the following three processes: apposition, attachment, and invasion of the blastocyst. A blastocyst attaches to the receptive uterine luminal epithelium to start pregnancy. Then, the blastocyst invades into the endometrial stroma under the epithelium, and the stroma becomes decidua, which supports growth and survival of blastocyst [1-7]. Because it is estimated that implantation failure causes 75% of unsuccessful conceptions [8], it should be an obligatory mission to elucidate the mechanism and solutions of implantation failure.

Successful blastocyst implantation is the result of suitable molecular crosstalk between the uterus and the blastocyst during these processes. During these processes, the ovarian steroid hormone progesterone (P_4), that is called a “hormone of pregnancy”, plays an essential role by thoroughly managing uterine functions [1, 9-12]. P_4 is produced by ovarian corpus luteum after ovulation, and

later, the main source of P_4 secretion changes from ovary to trophoblast during first trimester of pregnancy. In the clinical situation, luteal support by P_4 improves the implantation rate [13-15], indicating significant role of P_4 in human embryo implantation.

Studies using mouse models are frequently conducted in the research field of embryo implantation [3, 6]. Particularly, some genetically-engineered mice provide us valuable piece of information to elucidate molecular functions of P_4 in blastocyst implantation [9-12]. This article presents the literature about the role of P_4 in mouse blastocyst implantation by my group and others in order to support better comprehension of molecular and cellular basis for human blastocyst implantation. The current article is separated into three parts; (1) biological function of P_4 during mouse blastocyst implantation, (2) molecular mechanisms of P_4 action in mouse blastocyst implantation, (3) key molecular mechanisms in mouse blastocyst implantation beyond P_4 action.

Biological Function of P_4 during Mouse Blastocyst Implantation

Uterine receptivity to the blastocyst in mice

An implantation-competent blastocyst is one of two indispensable components for embryo implantation, because a major reason of implantation failure is a poor-quality embryo [16]. The other essential element is uterine receptivity defined as a capacity to accommodate the competent blastocyst in the uterus [3, 6]. The receptive mouse uterus displays stromal proliferation and epithelial

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Fig. 1 Blastocyst attachment and invasion as important steps of mouse blastocyst implantation

differentiation as an indicator of proper arrangement for blastocyst implantation [17], which is governed by ovarian hormones. P_4 -dependent morphological alteration termed “pre-decidualization” occurs during this step [5, 6]. Increased production of ovarian steroid hormones estradiol- 17β (E_2) and P_4 provides the uterus with a capacity of blastocyst attachment after pre-decidualization. Consequently, blastocyst attachment takes place in the uterus. Then, stromal cells surrounding the embryo begin to differentiate concomitantly with polyploid formation. This stromal differentiation is termed “decidualization” [5, 6]. If the blastocyst attachment fails, any competent blastocysts are never able to stick to the uterus (Fig. 1). The uterus permits blastocysts to attach to itself within the limited period. This limited period is termed “implantation window” [5, 6]. Luminal epithelium around the blastocyst exfoliates, and trophoblast begins to invade into the stroma, which is termed “blastocyst invasion” (Fig. 1) [5, 18]. In this way, uterine receptivity is involved in embryo implantation.

P_4 directs mouse blastocyst implantation

Mouse models are employed most commonly among the animal experimental models [3, 6]. Vaginal plug is noticed in the morning on the next day of ovulation and mating, and this day is defined as day 1 of pregnancy. The uterus appears swollen under the influence of E_2 surge on day 1, and luminal epithelial cells proliferate outstandingly. Circulating P_4 levels rises on day 3 due to its production from newly-formed corpus luteum. Elevated P_4 takes over E_2 and becomes a dominant ovarian hormone by day 4 morning. Notably, P_4 provides uterine

receptivity, and changes uterine cell proliferation status dynamically. The stromal cells begins to proliferate, and in contrast, the luminal epithelial cells declines to proliferate and begins to differentiate [17], which I here name “endometrial proliferation-differentiation switching (PDS)”. These changes in time- and cell-specific proliferation and differentiation is accompanied by the expression of several genes critical for uterine receptivity. Concomitant ovarian secretion of E_2 under continuous effects of P_4 on day 4 morning provides initiating signals to the uterus for the communications between the blastocyst and the uterus on day 4 evening. Dormant blastocyst is activated by E_2 -derived uterine factors, and the uterus turns to be receptive. Under the influence of ovarian hormones, the implantation-competent blastocyst as well as the receptive uterus is prepared through the molecular communications between the blastocyst and uterus [3, 6]. The blastocyst trophoctoderm adheres to the luminal epithelium on day 4 midnight, and stromal cells surrounding the blastocyst initiate differentiation, change their stromal morphology into epithelioid type containing polyploidy, and form a new endometrial layer around the blastocyst as a process of decidualization. Stromal vascular permeability is markedly increased at the attachment sites surrounding the blastocyst. An intravenous injection of Chicago Blue Dye solution can visualize the attachment site with increased vascular permeability. Trophoblast cells invade into the stroma on day 5 evening, and blastocyst implantation is completed [3, 6, 18].

Molecular Mechanisms of P₄ Action in Mouse Blastocyst Implantation

P₄-PR signaling in mouse blastocyst implantation

P₄ acts through progesterone receptor (PR), which regulates the expression of P₄ responsive genes transcriptionally and the critical pathways for pregnancy event including ovulation and implantation. Genetic alteration of PR and its related molecules in female mice has exposed the roles of P₄ in pregnancy. PR knockout female mice showed infertility due to ovulation failure [9], suggesting that P₄-PR signaling is essential for ovulation. Although PR knockout mice are very powerful and useful to analyze the molecular pathway in ovulation, but not to dissect the role of P₄ on blastocyst implantation.

Stability of PR complex impacts on PR function. Functionally-mature PR complex contains a receptor monomer, a 90-kDa heat shock protein (Hsp90) dimer, the cochaperone p23, and one of four cochaperones which include tetratricopeptide repeat (TPR) repeat that binds to Hsp90 [19]. The immunophilin cochaperone 52-kDa FK506 binding protein (FKBP52) is one of TPR-containing chaperones, binding both Hsp90 and PR, stabilizing the structure of PR complex, thus reinforcing P₄-PR signaling [11, 19]. Deficiency of FKBP52 deteriorates uterine P₄-PR signaling, but does not pose complete demise, because minimal binding of P₄ to PR is retained [11, 19]. P₄ supplementation rescues uterine PR signaling on the CD1 background, which is a noteworthy aspect of FKBP52 knockout mice unlike PR knockout mice [12]. Furthermore, FKBP52 knockout mice show normal ovulation with normal P₄ secretion [12]. Based on the findings of FKBP52 knockout mice, P₄-PR signaling is essential in the process of blastocyst implantation.

Endometrial PDS is governed by P₄-PR signaling

As mentioned above, endometrial stroma begins to proliferate, and luminal epithelium attenuates proliferation and begins to differentiate [17], which is named “endometrial PDS”. In general, cell differentiation is compatible with poor cell proliferation, and distinct switching between proliferation and differentiation occurs in many cell types [20-23]. Our study clearly demonstrated that an injection of PR antagonist RU486 hampers endometrial PDS and blastocyst implantation in wild-type (WT) mice [17]. FKBP52 knockout uterus has uterine P₄ resistance with persistent epithelial cell proliferation without much stromal cell proliferation on day 4, which is recovered by P₄ supplementation [17]. These findings indicate that P₄-PR signaling directs the uterus and encourages endometrial PDS in the peri-implantation period (Fig. 2). In addition, PDS in the receptive uterus

occurs not only in mice but in humans [17]. As far as I know from the literature, all types of genetically-modified mice lacking endometrial PDS show implantation failure [12, 17-19, 24-28].

PR has two isoforms, PR-A and PR-B, and PR-A is primarily responsible for uterine function during pregnancy, contributing to endometrial PDS [10, 29]. However, it is expected that PR-B does not have critical function in pregnancy, because systemic ablation of PR-B does not lead to any problems in pregnancy outcome [10, 29]. These findings suggest that P₄-PR-A signaling serves endometrial PDS and uterine receptivity.

Balance between E₂ and P₄ signaling fine-tunes endometrial PDS and uterine receptivity

Both lack and excess of E₂ levels can prevent blastocyst implantation [30]. Implantation failure due to the superiority in E₂ effects is also observed in knockout mouse models other than FKBP52 knockout mice. Uterine specific deletion of nuclear receptor co-activator 2 (NCOA2) encoding steroid receptor co-activator 2 (SRC2) causes implantation failure through the disorder of the optimization of PR function by NCOA2 in mice [31]. Another mouse study demonstrated that nuclear receptor co-activator 6 (NCOA6) does not act as a coactivator but promotes the ubiquitination and degradation of ER α , attenuating E₂-ER α signaling in the peri-implantation period [26]. Uterine NCOA6 knockout mice causes the accumulation of ER α and enhances E₂ sensitivity, leading to implantation failure [26]. Imbalanced hormonal signaling as well as implantation failure are rescued by the injection of estrogen receptor antagonist ICI-182780 [26]. Protein tyrosine phosphatase Src homology 2 domain containing protein tyrosine phosphatase-2 (SHP2), a classic cytoplasmic protein, is primarily located in the nuclei of uterine cells during implantation, and nuclear SHP2 enhances SRC kinase-mediated ER α tyrosine phosphorylation, facilitates ER α binding to PR promoter, and promotes the ER α transcription activity in the peri-implantation mouse uterus [27]. A recent mouse study revealed that B lymphoma Mo-MLV insertion region 1 homolog (BMI1), a component of the polycomb repressive complex-1 (PRC1), regulates PR ubiquitination in a polycomb complex-independent manner, and uterine deletion of BMI1 causes implantation failure due to the disruption of uterine P₄ responsiveness [28]. In women with history of miscarriage, low expression levels of endometrial BMI1 is related to poor PR responsiveness [28]. These findings suggest that BMI1 controls endometrial PR function by post-transcriptional regulation of PR, leads to successful implantation in both mice and humans.

Mice with uterine deficiency of signal transducer and

Fig. 2 P₄-PR signaling pathway and endometrial proliferation-differentiation switching (PDS) regulated by the molecular crosstalk between luminal epithelium and stroma. P₄, Progesterone; PR, progesterone receptor; FKBP52, 52-kDa FK506 binding protein; ERα, estrogen receptor α; miR-200a, microRNA-200a; FGF, fibroblast growth factors; FGFR, fibroblast growth factor receptor; HAND2, heart and neural crest derivatives-expressed protein 2; IHH, Indian hedgehog; PTCH1, Patched-1; NCOA6, nuclear receptor co-activator 6; BMI1, B lymphoma Mo-MLV insertion region 1 homolog; SHP2, Src homology 2 domain containing protein tyrosine phosphatase-2.

activator of transcription 3 (STAT3), known as a downstream molecule of leukemia inhibitory factor (LIF) during blastocyst implantation [32], demonstrated implantation failure with relatively higher uterine influence of E₂-ERα than P₄-PR signaling [33], but it is not fully elucidated how STAT3 influences E₂/P₄ signaling in the peri-implantation uterus.

P₄-induced endometrial PDS is modified by HAND2 and IHH in mice

A basic helix-loop-helix transcription factor, heart and neural crest derivatives-expressed protein 2 (HAND2), is expressed in the endometrial stroma under the influence of P₄-PR signaling and hinders endometrial PDS through suppression of fibroblast growth factor, despite no con-

tribution to stromal proliferation [25]. HAND2 acts as a blocker of epithelial E₂ signaling during blastocyst implantation. Uterine HAND2 knockout mice shows defective blastocyst attachment [25], suggesting that stromal HAND2 causes blastocyst attachment by way of P₄-induced differentiation of luminal epithelium.

Indian hedgehog (IHH), a downstream factor of PR, is strongly expressed in the luminal epithelium of mouse uterus immediately before blastocyst attachment [34, 35]. IHH acts through its receptor Patched-1 (PTCH1). PTCH1 is localized in the stroma, and induces stromal proliferation [34-36]. One of two major downstream targets in IHH signaling is a transcriptional factor GLI [34], and the other is a nuclear receptor chicken ovalbumin upstream promoter-transcriptional factor (COUP-TFII)

Fig. 3 Key molecular pathways and processes in the steps of blastocyst implantation.

P₄, Progesterone; PR, progesterone receptor; PDS, proliferation-differentiation switching; FOXA2, forkhead box protein A2; LIF, leukemia inhibitory factor; STAT3, signal transducer and activator of transcription 3; HIF2α, hypoxia inducible factor 2α.

[37]. GLI contributes to stromal proliferation [34] and COUP-TFII keeps the balance between E₂/P₄ signaling [37]. Taken together, the interactions between the endometrial epithelium and stroma under the hormonal control is involved in endometrial PDS (Fig. 2).

Epigenetic regulation of P₄-PR signaling by microRNA is involved in endometrial PDS

Our previous study showed that endometrial PDS occurs in a spatial manner, between the uterus and cervix [17]. Blastocysts do not implant in the cervix but in the uterus in normal pregnancy. The mouse uterus displays endometrial PDS, and in contrast, the cervix does not exhibit any changes of proliferation and differentiation both in the epithelium and stroma. The human uterus also presents dynamic endometrial PDS between the proliferative phase and the secretory phase, unlike the human cervix with no significant changes in the proliferation status [17]. Based on these findings, we hypothesized that there is the different machinery for the reduced P₄-PR signaling in the cervix unlike the uterus. Indeed, P₄-PR signaling weakens in the cervix due to microRNA (miR)-200a in two different pathways. One is the reduction of PR levels in the cervix by posttranscriptional regulation by miR-200a [17]. The other is local metabolism of P₄ in the cervix, in which miR-200a upregulates 20α-hydroxysteroid dehydrogenase, a P₄-metabolizing enzyme, through down-regulation of STAT5 [17, 38]. Moreover, the expression levels of miR-200a are low in the receptive uterus compared to the pre-receptive one (unpublished observation), indicating that miR-200a makes uterine P₄-PR signaling appropriate for successful implantation.

Molecular Mechanisms of Blastocyst Implantation beyond P₄ Action

Involvement of LIF and FOXA2 in mouse blastocyst attachment

Uterine P₄-PR signaling is critical not only in the process of uterine receptivity acquisition but even after blastocyst attachment throughout pregnancy (Fig. 3). Recent studies performed by us and other groups clarified key regulators of blastocyst attachment and invasion other than P₄-PR signaling. Forkhead box protein A2 (FOXA2) regulates blastocyst attachment [39], and hypoxia inducible factor 2α (HIF2α) controls blastocyst invasion [18]. As mentioned above, E₂ is an initiator for blastocyst attachment. Leukemia inhibitory factor (LIF), an IL-6 family cytokine, is secreted from the endometrium in response to E₂ secreted by ovaries and plays a crucial role in blastocyst attachment. In a delayed implantation mouse model with ovariectomy on day 4 of pregnancy and later hormone supplementation, LIF can induce blastocyst attachment on behalf of E₂ [40]. However, it remains unclear how LIF allow blastocyst attachment. FOXA2 is necessary for development of endometrial glands in mouse newborn pups [39]. FOX transcription factors are involved in cell proliferation, differentiation and longevity of many tissues and organs. FOXA2 is expressed in the glandular epithelium of mouse [39]. In mice, deletion of FOXA2 in the entire uteri shows complete loss of endometrial gland [39]. In addition, deletion of FOXA2 in uterine epithelium shows reduction of LIF expression and failure of blastocyst attachment which LIF supplementation can recover [39], suggesting critical roles of E₂-FOXA2-LIF pathway in blastocyst attachment.

Involvement of HIF2 α in mouse blastocyst invasion

Uterine molecules associated with blastocyst invasion have rarely been investigated. Oxygen concentration of endometrial surface of the early human pregnancy is known to be lower than that of inner endometrium [41], suggesting the uterine cavity and endometrial surface are hypoxic state during blastocyst implantation. Hypoxia inducible factor (HIF) is a major transcriptional factor inducible by low oxygen tension [42] and HIF2 α is expressed in the mouse uterus during blastocyst implantation [43]. Our recent study revealed that mice with deletion of HIF2 α in the entire uterus are infertile due to implantation failure [18]. Treatment with P₄ and LIF rescues decidual growth arrest and aberrant position of implantation sites in uterine HIF2 α knockout mice, respectively, but does not restore pregnancy failure. Uterine HIF2 α knockout mice show persistence of the intact luminal epithelium [18], which blocks direct contact between stroma and blastocyst, inactivation of PI3K-AKT pathway (embryonic survival signal), and failed blastocyst invasion [18]. Mice with stromal deletion of HIF2 α are infertile due to impaired blastocyst invasion, and those with epithelial deletion of HIF2 α show normal fertility, suggesting the critical role of stromal HIF2 α in blastocyst invasion. Taken together, our study revealed that stromal HIF2 α allows trophoblast invasion through detachment of the luminal epithelium and activation of an embryonic survival signal. Thus, HIF2 α is one of few uterine molecules regulating blastocyst invasion (Fig. 3).

Summary

To improve fertility rate, there remain many problems to solve. One of these issues is recurrent implantation failure regardless of good-quality embryo transfer [4,

44]. Several molecules which are employed within very limited time are involved in the opening of implantation window. Basic research is required to dissect the mechanism of implantation failure and to establish its effective treatment. One possible mechanism of implantation failure is “P₄ resistance” [12, 19]. P₄ supplementation for fertility treatment is very common in humans, and its effectiveness on patients with luteal insufficiency is established [13]. However, P₄ treatment does not always help infertile patients suffering from implantation failure. Thus, the current fertility therapy can not cure patients with severe P₄ resistance. FKBP52 knockout mouse is a well-designed experimental model which reflects the influence of P₄ resistance on implantation failure, and therefore, this mouse model may assist our comprehension of human infertility. In addition, it is notable that our group discovered uterine HIF2 α knockout mice as a new mouse model for the investigation of blastocyst invasion. I believe that better understanding of P₄ functions must be of great help in developing novel approaches of infertility and contraception.

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Disclosure

The authors declare that they have no conflict of interest.

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