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Molecular Studies on Pregnancy with Mouse Models

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Abstract

Pregnancy is a complex process that involves crosstalk among multiple cell types in both the endometrial and myometrial compartments at the maternal side to support the fetus. Genetic engineered mouse models have served as a major platform to dissect the convolute genetic interactions in a physiological context. Combining with various applications of next generation sequencing and genome editing, functional assays by mouse models have expanded the spectrum to include both coding and noncoding genome. The present review will highlight recent findings that are primarily based on studies of mouse models with emphasis on pathways for endometrial receptivity and myometrial contraction. Emerging novel technologies that may advance the research in these two aspects will also be discussed.

Keywords

Early Pregnancy; Parturition; Window of Receptivity; Mouse Model; Endometrium; Myometrium

Introduction

The uterus undergoes extensive structural remodeling and functional adaptation to support a successful pregnancy. The endometrium initially forms a receptive environment to allow embryo implantation, followed by transformation to support fetal growth. The myometrium expands both in number and size of the primary constituent smooth muscle cells to accommodate development of the conceptus, maintain uterine structural integrity, and provide contractile force at the laboring phase. After parturition, the involution process repairs and regenerates the uterus preparing for the next pregnancy. Both uterine compartments are under tight control by the ovarian steroids progesterone and estrogen. Understanding the molecular processes regulated by these hormones is critical to understanding the biology of the endometrium and myometrium during pregnancy [1, 2].

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Although cell lines have been an indispensable tool in the dissection of the mechanism of steroid hormone signaling in reproductive tissue, these studies have been limited. Cell lines rarely recapitulate the *in vivo* situation and since steroid hormone signaling changes with site of action during the progression of pregnancy, *in vivo* approaches are critical. In this case, genetically engineered mice are indispensable tools for functional assays, especially on subjects that involves communication among multiple cell types, despite the precautions that must be taken when applying knowledge learned from rodent models to human pregnancy [3, 4]. Genetically engineered animals allow the compartment specific ablation and activation of genes in the reproductive tract to determine the role of steroid hormones on specific compartments of the reproductive tract in a physiological context. In this review, we will discuss recent findings on functional assessment of genetic elements in the uterus via mouse models, focusing on biological processes under the control of progesterone at embryo implantation in the endometrium and smooth muscle contraction in the myometrium.

Progesterone Signaling Network in Pregnancy: Lessons from Understanding the Endometrial Receptivity

Critical to the establishment of pregnancy is the preparation of the endometrium for embryo implantation. Embryo implantation occurs during the period of uterine receptivity, which involves an array of biological processes under the control of the ovarian hormones [5]. This preparation for uterine receptivity requires communications between endometrial epithelial and stromal cells via progesterone and estrogen signaling that transforms the epithelia to the receptive state for subsequent embryo implantation and primes the stroma for decidualization. The ovarian hormones estrogen and progesterone act through their cognate receptors, which are members of the nuclear receptor transcription factor super family. Receptors for estrogen include ESR1 and ESR2. In the uterus ESR1 is the major mediator of estrogen action [1]. Progesterone action is mediated by the progesterone receptor, PGR, with two major isoforms, PGRA and PGRB. PGRA is the dominant form in the mouse uterus while both PGRA and PGRB play a role in human uterine functions [6]. In order to define the differential role of the nuclear receptors, genetically engineered mouse models have been indispensable.

PGR expression in the endometrium is regulated in a temporal and cell specific fashion. Prior to the preimplantation period PGR is expressed in the uterine epithelium. During the preimplantation period PGR expression is observed in the endometrial stroma. At the receptive window PGR loses expression from the epithelium but expresses predominantly in the stroma [7]. Ablation of PGR in the uterine epithelium further demonstrates that epithelial PGR is critical for uterine receptivity [7, 8]. Combining gene ablation models and transcriptomics has shown that PGR partners with GATA2 and SOX17 to promote *Ihh* gene expression in the uterine epithelium [9, 10]. The IHH ligand then signals through stromal *Ptch1* and *Ptch2*, *Nr2f2* and *Pgr* to suppresses *Hand2* dependent stromal FGF signaling, leading to a reduction of epithelial proliferation [2]. In human endometrium, patients treated with PGR modulator CDB-2914 display increased expression of IHH and its downstream targets, indicating an evolutionarily conserved mechanism to regulate IHH by PGR [11].

In the endometrial stroma, *Ptfn11*, *Esr1*, *Pgr*, *Nr2f2* and *Hand2* together regulate the communication with epithelia. Stromal ESR1 mediates estrogen-induced proliferation of epithelial cells and PGR expression before embryo implantation [12, 13]. Phosphorylation of ESR1 by the Shp2-Src kinase axis enhances *Pgr* expression via increasing ESR1 occupancy at the *Pgr* promoter [13]. The stromal PGR then joins *Nr2f2* and *Hand2* genes to mediate the epithelium-derived IHH signaling in suppression of epithelial estrogen activities in preparation of embryo implantation [13–16]. Loss of ESR1 in a subpopulation of stromal cells leads to subfertility in part due to a reduction of embryo implantation rate as well as embryo reabsorption presumably owing to defective stromal decidualization [12]. Uterine ablation of either *Ptfn11*, *Nr2f2* or *Hand2* results in endometrial progesterone resistance, complete failure of embryo implantation, and infertility [13, 15, 17], highlighting the pivotal role of this stromal signaling network in pregnancy.

The mechanism of PGR in the uterus has been further delineated by cistromic assays and CRISPR/Cas9 mediated gene editing. ChIP-Seq reveals the control of mouse *Ihh* gene in uterine epithelia through interaction among regulatory region 68 (*Rr68*, aka *Ihh19*), PGR, GATA2 and SOX17 [9, 10]. *Rr68* is a noncoding region of the mouse genome located approximately 19 kb upstream of the *Ihh* transcription start site and appears to house a uterine-specific enhancer for *Ihh*. Removal of *Rr68* diminishes *Ihh* expression specifically in the uterine epithelium without impact on other *Ihh* expressing tissues. Physiologically, *Rr68* knockout mice exhibit subfertility with impaired progesterone signaling and failure on hormone induced decidualization. Among the four known transcription factors that occupy *Rr68*, PGR is the hormone responsive transcription regulator; GATA2 and FOXA2 are pioneer factors for gene regulation; and SOX17 may modulate DNA structure in addition to its transcription factor role. Uterine ablation of either PGR, GATA2 or SOX17 attenuates *Ihh* gene expression. These findings collectively indicate that *Rr68* serves as a uterine-specific enhancer to recruit major uterine transcription factors for regulation of *Ihh* that controls uterine receptivity. Further investigations are needed to identify other components in the transcription machinery that interact with *Rr68*, to understand the mechanisms that control interaction between *Rr68* and the *Ihh* promoter, and to discover *Rr68*'s potential interaction with other hormone controlled cis-acting elements and downstream targets in addition to *Ihh* [18, 19].

ETS family Transcription factor EGR1 also regulates epithelia-stroma crosstalk [20]. *Egr1* ablation results in reduced expression of PGR, *Ihh* and other progesterone responsive markers, increased estrogen signaling, and aberrant epithelial proliferation. Physiologically, *Egr1* deficiency leads to decidualization defects and implantation failure. Given the phenotype similarity among *Egr1*, *Pgr*, *Gata2*, *Sox17* and *Ihh* mutant mice [9, 10, 21, 22], *Egr1* likely resides in the same pathway with these genes. This hypothesis finds further support by the epithelial origin of the heightened estrogen response of *Egr1* deficiency and over-representation of the EGR1 consensus binding motif in uterine PGR, GATA2 and SOX17 occupying regions [7, 9, 10]. Together these observations suggest *Egr1* as a novel member of this regulatory network for progesterone signaling that controls epithelia-stroma interaction. Whether EGR1 regulation of *Ihh* is in direct transcriptional control or involved through other members in the network remains unclear. Also, determining the EGR1 genome occupancy pattern and its downstream target genes would help to identify common

and distinct pathways as well as cis-acting elements among EGR1, PGR, GATA2, SOX17 and FOXA2.

Molecular Regulation of the Myometrium for Parturition

Parturition disorders such as preterm birth and dystocia often lead to subsequent complications to both mother and child. Mouse models for preterm birth research has been comprehensively reviewed [4]. Recent results also provide new functional indications on several pathways for the parturition process, including gonadotropic ERK signaling regulation of placenta architecture and gestation time [23], suppression of uterine inflammation and preterm birth by the lactate-*Hcar1* axis [24], promotion of uterine inflammatory gene expression by the TLR4-NF κ B-p38 signaling pathway [25], regulation of myometrial contractility by the sodium leak channel NALCN [26] and the cation channel TRPC3 [27], contribution of preterm birth by the *Faah*-mTOR-p38 signaling dependent premature decidual senescence [28], and *Ptgs1* on cervical ripening that controls timing of parturition [29, 30].

Progesterone has been clinically applied to prevent premature parturition [31]. The role of progesterone in the myometrium is to regulate the quiescence of the myometrium during pregnancy. Changes in progesterone signaling result in increased myometrial contractility and parturition. Recent studies shed light on how PGR isoforms regulate the functional switch between quiescence and labor states in myometrium during pregnancy.

Modulation of Progesterone Signaling in the Myometrium—In most species progesterone signaling decrease by way of reduced hormone levels. However, in the human, serum progesterone levels maintain at term despite of reduced progesterone signaling [32]. Several possibilities may be accounted for decreased progesterone signaling. An increase in progesterone metabolism may cause a decrease in ligand availability for receptor signaling. In the nonlaboring myometrium, progesterone signaling acts through the ZEB1/2-STAT5b pathway to suppress *miR-200a*, leading to a reduction of 20 α -HSD levels and preventing progesterone metabolism [33, 34]. At term, results from mouse models show that ovarian progesterone production is suppressed by fetal lung dependent elevation of prostaglandin. Meanwhile, 20 α HSD levels increase under the control of the prostaglandin-Nur77 axis and the MAMLD1 transcription regulator, leading to increased ovarian progesterone catabolism (reviewed in [35]). In the term laboring human myometrium, local 20 α -HSD expression increases concomitant with a reduction of local progesterone levels in the nuclei, while the cytoplasmic progesterone levels remain comparable with that at the term nonlaboring stage [36]. The mechanism that regulates the subcellular levels of progesterone in human awaits further investigation.

A shift in the progesterone receptor interaction with steroid receptor coregulators also alters the transcriptional activity of the receptor. *In vitro* studies on human cells suggest that, before laboring, progesterone binding PGRB partners with JUN/JUN homodimers to recruit transcription repressors for repression of contractile gene GJA1 expression [36], while liganded PGRB suppresses GJA1 trafficking to cell membrane for gap junction formation [37]. At the laboring stage when nuclear progesterone levels are low, unliganded nuclear

PGRA interacts with FOSL2/JUND to promoter GJA1 transcription [36]. Meanwhile, liganded cytosolic PGRA facilitates the trafficking of full length GJA1 to the plasma membrane [37]. PGR isoforms function differentially at both genomic and non-genomic levels to modulate the myometrial transcriptome profiles and mTOR-mediated subcellular localization of contractile proteins [37, 38]. Taking together, PGR partners with various co-regulators through two major isoforms to control production and distribution of contractile elements for coordinated muscle contraction.

The third mechanism is a change in the PGRA:PGRB isoforms ratio resulting in a switch from a pro-pregnancy anti-inflammatory signaling to a pro-parturition inflammatory pathway. In human, the myometrium PGRA/PGRB ratio increases amid pregnancy progression, owing to the change of PGRA levels. PGRA transcription is promoted by KLF9 and decreased by epigenetic enzymes KDM5A and HDAC1. Control of PGRA protein stability by progesterone and proinflammatory stimulation has also been reported. Moreover, post-translational modification of PGRA at serine345 with phosphorylation allows PGRA to repress PGRB dependent anti-inflammatory actions, supporting the notion that PGRA increases suppression of PGRB activities during the advance of gestation. The observations, together with aforementioned studies, indicate that progesterone signaling is regulated by a versatile network with multiple layers of control mechanisms (reviewed in [2]).

While these findings generally support the presence of differential capacity between PGRA and PGRB isoforms, the *in vivo* genomic impact and the physiological functions of these two isoforms remain unclear. These questions could be addressed by mouse models that allow myometrial-specific expression of either PGRA or PGRB isoform in smooth muscle or myometrial Cre recombinase expressing background [7, 39–41]. The impact of these two isoforms on uterine contractility could be assessed in the physiological context by measuring intrauterine pressure through telemetry [42]. Furthermore, the stage-specific genomic effects of these two isoforms can be examined by transcriptomic and cistromic assays on tissues from the myometrial-specific PGRA and PGRB expressing mice at various phases of pregnancy. Integrative analysis of this data would shed light on the convergent and divergent pathways of the PGR isoforms *in vivo*.

The emerging role of actin cytoskeleton rearrangement in parturition—A recent study links the rearrangement of actin cytoskeleton to parturition disorders [43]. Studies from multiple smooth muscle systems reveal that cytoskeletal remodeling realigns interactions between contractile units and extracellular matrix to promote force transmission and muscle contraction in response to contractile and mechanical stimulation [44]. SETD3 methylates the actin and facilitates kinetics of actin polymerization with no impact on depolymerization [43]. *Setd3* deficient mice exhibit a dystocia phenotype and fail to respond to oxytocin or prostaglandin induced parturition [43]. The role of myometrial SETD3 in muscle contractility is further investigated in a model of SETD3 depleted primary human myometrial cells, which also shows hampered contractions in response to treatment of ecobolic agents and suggests the myometrial origin of the mouse dystocia phenotype [43].

Notably, actin cytoskeleton reorganization also regulates gene expression through control of nuclear localization and protein turnover of transcription factors and coregulators, as well as

by joining in the chromatin remodeling and RNA polymerase complexes in the nucleus [45]. An interesting example is the YY1 transcription factor whose nuclear localization is controlled by actin cytoskeleton remodeling in vascular smooth muscle cells [46]. Recent evidence shows that YY1 also regulates DNA looping for promoter-enhancer interaction in the genome [47]. In the uterus, the consensus YY1 binding motif is over-represented in the PGR-A occupying sites [7]. These observations implicate a potential interaction among the pregnancy hormone, the DNA conformation regulator and actin cytoskeletal dynamics in the myometrium, which awaits future investigation.

Concluding Remarks and Future Prospects

Genetically engineered mouse models provide pathophysiological outcomes and downstream gene network information of genes of interest. Integrative analysis of omics data from mutant mice that share similar phenotypes helps to decipher the genetic regulatory codes for the target biological processes. On the horizon, using mouse uterine organoids derived from mutant mouse models would facilitate studies of mechanisms underlie the physiological changes, and provide comparative analysis to support human relevance of the research [48, 49]. Further examining the omics information at the single cell level not only can study the impact of gene mutation on uterine cell type composition and identify rare/unknown cell types, but also permit CRISPR-based high throughput functional assessment of downstream genes in given mouse models [50, 51]. The combination of NextGen Sequencing, single cell technology, organoid culture, genetically engineered mouse models and metadata data analysis will propel the reproductive research in an unprecedented pace. These approaches have and are being used to dissect out endometrial biology. Employing these approaches to investigate the role of PGR in the myometrium will lead to the identification of cofactors that work with PGR to regulate myometrial gene expression at different stages of pregnancy and may aid in the development of strategies to treat diseases of pregnancy such as preterm birth.

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Abbreviations

Abbreviation	Name
20α-HSD	20-alpha-hydroxysteroid dehydrogenase
Cas9	CRISPR associated protein 9
CDB-2914	17 α -acetoxy-11 β -[4-N,N-dimethylaminophenyl]-19-norpregna-4,9-diene-3,20-dione
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
EGR1	Early Growth Response 1
ERK	Extracellular Signal-Regulated Kinase

<i>Esr1</i>	Estrogen Receptor 1
<i>Esr2</i>	Estrong Receptor 2
ETS	Erythroblast Transformation-specific Proto-Oncogene 1
<i>Faah</i>	Fatty Acid Amide Hydrolase
FGF	Fibroblast Growth Factor
FOSL2	FOS Like 2, AP-1 Transcription Factor Subunit
FOXA2	Forkhead Box A2
GATA2	GATA Binding Protein 2
GJA1	Gap Junction Protein Alpha 1
<i>Hand2</i>	Heart and Neural Crest Derivatives Expressed 2
<i>Hcar 1</i>	hydroxycarboxylic acid receptor 1
HDAC1	Histone Deacetylase 1
<i>Ihh</i>	Indian Hedgehog
JUN	Jun Proto-Oncogene, AP-1 Transcription Factor Subunit
JUND	JunD Proto-Oncogene
KDM5A	Lysine Demethylase 5A
KLF9	Kruppel Like Factor 9
MAMLD1	Mastermind Like Domain Containing 1
<i>miR-200a</i>	MicroRNA 200a
mTOR	Mechanistic Target of Rapamycin Kinase
NALCN	Sodium Leak Channel, Non-selective
NFκB	Nuclear Factor-kappaB
<i>Nr2f2</i>	Nuclear Receptor Subfamily 2 Group F Member 2
Nur77	Orphan Nuclear Receptor Subfamily 4 group A member 1
p38	p38 mitogen-activated protein kinase
PGR	Progesterone Receptor
PGRA	Progesterone Receptor isoform A
PGRB	Progesterone Receptor isoform B
<i>Ptch1</i>	Patched 1

<i>Ptch2</i>	Patched 2
<i>Ptgs1</i>	Prostaglandin-Endoperoxide Synthase 1
<i>Ptpn11 (Shp2)</i>	protein tyrosine phosphatase non-receptor type 11
<i>Rr68 (Ihh19)</i>	Regulatory region 68
SETD3	SET Domain Containing 3
SOX17	SRY-Box 17 (Sex Determining Region Y-Box17)
<i>Src</i> kinase	SRC Proto-Oncogene, Non-receptor tyrosine kinase
STAT5b	Signal Transducer and Activator of Transcription 5B
TLR4	Toll Like Receptor 4
TRPC3	Transient Receptor Potential Cation Channel Subfamily C Member 3
YY1	YY1 transcription factor
ZEB1	Zinc Finger E-Box Binding Homeobox 1
ZEB2	Zinc Finger E-Box Binding Homeobox 2

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