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The progesterone receptor regulates implantation, decidualization, and glandular development via a complex paracrine signaling network

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Abstract

Many women are affected by infertility and reproductive-associated disease such as endometriosis or endometrial cancer. Successful pregnancy is dependent on a healthy uterus that is fit to receive and support a fertilized embryo. The uterus is an endocrine organ, responsive to the presence of the ovarian steroid hormones, estrogen and progesterone, which activate transcription of target genes through the binding of their cognate receptors, the estrogen receptor and the progesterone receptor. Progesterone signaling has been demonstrated to be critical for the initiation and continuance of pregnancy. Through the induction of *Ihh*, *Wnt*, and *Bmp* pathways within the epithelial and stromal compartments of the uterus, embryo attachment and implantation occur followed by decidualization of the surrounding stroma. Furthermore, these pathways have been shown to be involved in uterine glandular development. This review highlights the integral role of uterine progesterone-mediated paracrine signaling in gland development and pregnancy.

Keywords

Progesterone; Progesterone receptor; Implantation; Paracrine signaling; Mouse models

1. Introduction

Dysfunction involving the female reproductive tract is a significant health concern in the world today. These health issues center on a woman's control of her ability to reproduce as well as her physical health. In the United States, 12% of reproductive-aged women are considered infertile and many women are affected by reproductive-associated disease such as endometriosis or endometrial cancer (Chandra et al., 2005). Due to the significant role that the progesterone receptor (PR) plays in pregnancy, increased knowledge of progesterone signaling can promote the development of more effective contraceptives and also therapeutic drugs that can enhance fertility. Identifying the role that the PR plays in the progression of uterine cancer and endometriosis will assist in the development of successful therapies for treating these devastating diseases. Over the last decade, genetic manipulation of the mouse has been pivotal in the dissection of the interactions of regulatory pathways within the mouse uterus. This review will highlight the intricate progesterone signaling network occurring in the murine uterine microenvironment. Through the elucidation of the complicated paracrine signaling network occurring downstream of PR, we can begin to

translate these findings to the human endometrium and identify new target therapies for the treatment of reproductive-associated disease.

The uterus is a complex reproductive organ composed of two major anatomical compartments, the endometrium and myometrium. The endometrium is a glandular compartment consisting of the central luminal epithelium and the surrounding stroma containing the uterine glands. The endometrium is surrounded by the two outer muscle layers of the myometrium (Bloom and Don, 1975). The uterus functions to receive the fertilized embryo from the oviduct and provide the requisite environment for the implantation of the embryo and subsequent placentation. In the murine uterus, implantation occurs five days after a fertile mating, during a period known as the “window of receptivity” in which the blastocyst has a limited time frame to implant into the uterine lumen (reviewed in Wang and Dey (2006)). The embryo then invades the uterine wall which causes the stromal fibroblasts to undergo decidualization. The decidual process involves extensive proliferation, differentiation, and vascularization of the surrounding stroma in order to support the implanting blastocyst (reviewed in Ramathal et al. (2010)). For this process to occur, the stromal and epithelial cells communicate by paracrine signaling. This communication occurring between the uterine compartments is regulated by ovarian steroid hormones.

The ovarian steroid hormones, estrogen and progesterone, control uterine physiology by regulating gene transcription through binding to their cognate receptors, the estrogen receptors (ER) and the progesterone receptor (PR). There are two ERs, ER α and ER β , and two isoforms of PR, PRA and PRB. With respect to the ERs, most of the investigations of mouse uterine biology have revolved around the ER α gene since ER β was identified at a later time. Although ER β is expressed moderately within the uterus (Kuiper et al., 1997), the uterine phenotype of the ER β knockout mouse is minor compared to that of the ER α knockout mouse (Krege et al., 1998; Lubahn et al., 1993). Here we will focus on ER α and group PRA and PRB as one PR unless the function of the individual isoforms is specified. The expression of these receptors in the uterus is dynamic. The level of expression of these receptors varies, not only with cell type, but also with the progression of pregnancy and steroid hormone stimulation (Tan et al., 1999; Tibbetts et al., 1998). In the mouse on day 1 of pregnancy, ER α is expressed in the luminal epithelial compartment (Tan et al., 1999), and in response to the ovulatory estrogen surge, promotes epithelial proliferation within the luminal epithelium (reviewed in Carson et al. (2000)). As pregnancy proceeds and serum progesterone levels rise due to the presence of the corpora lutea following ovulation, epithelial proliferation is inhibited. This inhibition also results from increasing levels of PR expression in the luminal epithelium on days 2–4 of pregnancy (Tan et al., 1999). On day 3.5 of implantation, the production of leukemia inhibitory factor (LIF) is induced by estrogen within the endometrial glands (Bhatt et al., 1991). This rise in LIF occurs just before the window of receptivity in order to initiate embryo implantation (Stewart et al., 1992). PR expression is then extinguished in the luminal epithelium during the window of receptivity on day 5 (Tan et al., 1999). Stromal proliferation then increases dramatically and consistently as decidualization occurs. Therefore, this differential expression pattern of the steroid hormone receptors and the complementary surge in hormone levels plays an integral role in early pregnancy.

In order to identify the specific effect ovarian steroid hormones have on the expression of ER α and PR, expression analysis was performed on ovariectomized mice treated with exogenous hormones (Tibbetts et al., 1998). In the absence of steroid administration, the expression of ER α was observed at high levels in the glandular epithelium and stroma, while PR expression was highest in both the luminal and glandular epithelium. Upon administration of estrogen to ovariectomized mice, ER α was found to be upregulated in the

luminal epithelium and myometrium, but was inhibited within the stromal compartment of the endometrium. PR expression was present within the stroma, myometrium, and glandular epithelium upon estrogen administration, however, PR expression within the luminal epithelium was lost. Therefore, estrogen drives PR expression from the luminal epithelium to the stroma and myometrium, while it promotes the expression of ER α within the luminal epithelial compartment. ER α was observed to be dependent on progesterone stimulation as it was found to be upregulated in the glandular epithelium and downregulated in the myometrium upon treatment with estrogen and progesterone. Progesterone decreased PR expression altogether. Therefore, estrogen and progesterone can both promote or inhibit the differential expression patterns of the steroid receptors within the uterine compartments.

In the examination of the expression patterns of ER α and PR in the regulation of cell proliferation and gene expression in the mouse uterus, it would be easy to hypothesize that the regulation of epithelial cell proliferation in the epithelial compartment by estrogen and progesterone is due to the direct interaction of the receptor in that compartment. That is, estrogen stimulates epithelial cellular proliferation and progesterone inhibits this proliferation through the binding of the ER α and PR in the epithelium. However, the simple direct action of estrogen and progesterone through their respective receptors within the epithelial compartment appears not to be the case. This is especially true when observing the action of estrogen regulation of cellular proliferation, which was first elucidated through the use of xenograft experiments and then more recently investigated using compartmental specific ablation of the receptors. Through the recombining of epithelial and stromal tissue from wild type mice and ER α ablated mice, it was demonstrated that estrogen regulation of epithelial cell proliferation is governed by ER α acting in the stromal cells and regulating a paracrine signal to the epithelium (Cooke et al., 1997). By utilizing the same technique, it was determined that stromal ER α is also essential for the downregulation of PR in the epithelium upon treatment with estrogen (Kurita et al., 2000b). Although these experiments were conducted in xenotransplantation models, recent studies using epithelial specific ablation of ER α in the mouse uterus confirmed the significant role of stromal ER α in the regulation of estrogen-induced epithelial cell proliferation (Winuthayanon et al., 2010). Tissue recombinations with epithelia and stroma from PR knockout (PRKO) and wild type mice have shown that PR expressed in the stromal cells is critical for the ability of progesterone to inhibit estrogen induced proliferation and for the expression of the progesterone regulated target gene indian hedgehog (Kurita et al., 2000a; Simon et al., 2009). However, the role of PR in each compartment has yet to be verified *in vivo*. Nonetheless, these tissue reconstitution experiments provide testament to the intricate coordination of paracrine signaling taking place in the regulation of uterine function. Furthermore, these experiments illustrate the benefit of utilizing gene ablation in mice to elucidate the cascade of signaling initiated by steroid hormone action within the uterus.

2. The progesterone receptor plays a critical role in the development and maintenance of pregnancy

2.1. Progesterone receptor action

The PR is a member of the nuclear receptor superfamily of transcription factors capable of activating and repressing transcription of their target genes (Evans, 1988). The PR molecule is comprised of domains which allow for ligand binding, DNA binding, and the activation of transcription. The latter domain allows the PR to interact with coregulators in the regulation of gene transcription. PR has two isoforms, PRA and PRB, which are transcribed from the same gene, yet utilize different start codons (Conneely et al., 1987). PRA is the smaller isoform, while PRB contains an extra 164 amino acids at the amino terminus (Conneely et al., 1989). This extra sequence forms an additional activation domain ascribing unique

functions to PRB (Sartorius et al., 1994). Homodimerization or heterodimerization of the two isoforms can occur which generates increased specificity and regulation upon binding to progesterone response elements (PREs) of target genes (reviewed in Tsai and O'Malley (1994)). In the absence of progesterone ligand, the PR is located in the cytoplasm bound by chaperone proteins. Upon binding of progesterone, the PR dimerizes, enters the nucleus, and binds DNA to regulate the expression of target genes. When bound to the promoter element, the PR interacts with coregulators which coordinate remodeling of the chromatin and the initiation of gene transcription (reviewed in Xu and Li (2003)). It has also been identified that PR can be activated independent of ligand binding and successfully travel to the nucleus for the activation of target genes (reviewed in Li and O'Malley (2003)). Finally, the PR was shown to activate the Src/Ras/MAPK signaling pathway by interacting directly with Src kinase in the cytoplasm via an SH3 interaction domain (Boonyaratanakornkit et al., 2008). This third mechanism of PR activation is ligand-dependent and was confirmed to be extra-nuclear. Therefore, PR is regulated by many factors and can itself regulate cellular function at multiple levels. Indeed, microarray analysis of PR in the mouse uterus confirms that PR activates and represses a multitude of gene pathways (Cheon et al., 2002; Jeong et al., 2005). Gene expression analysis combined with the ability to ablate PR in the mouse has proven to be beneficial in understanding the mechanism of PR signaling as well as allow for the identification of paracrine signaling pathways that regulate uterine function.

2.2. Progesterone receptor knockout mouse models demonstrate the necessity of the progesterone receptor in pregnancy

Progesterone signaling has been recognized to play a significant role in pregnancy illustrated by the PRKO mouse generated by Lydon et al. (1995). The PRKO mouse, in which both PRA and PRB isoforms are ablated, is infertile due to numerous defects in the hypogonadal-pituitary axis, ovary, and uterus. In particular, the PRKO mouse displays defects in follicular rupture in the ovary, embryo implantation, and decidualization. Since PR plays a critical role in the inhibition of estrogen-induced epithelial proliferation, the uterine epithelium is found to be unfit to receive the fertilized embryo in PRKO mice (Lydon et al., 1995). Upon administration of estrogen and progesterone, these mice exhibited extensive uterine hyperplasia, inflammation, and proliferation confirming that progesterone signaling is essential for the inhibition of estrogen-induced proliferation. Furthermore, the recruitment of leukocytes and macrophages to the uterus was demonstrated to be inhibited by progesterone signaling (Tibbetts et al., 1999). Therefore, the PR plays a critical anti-proliferative and anti-inflammatory role in the uterine epithelium that is necessary for proper embryo implantation and subsequent decidualization. Contrary to the antiproliferative role within the uterus, the PR was demonstrated to be necessary for mammary gland development during pregnancy as the PRKO mice display a lack of lobular-alveolar structures and decreased ductal side branching upon treatment with hormones (Lydon et al., 1995). Therefore, PR is required for pregnancy and global reproductive function within the murine system.

The PRKO mouse defined the role of PRA and PRB in the mouse uterus. However, since the PR isoforms display differential expression in the compartments of the mouse uterus during pregnancy, further studies were conducted to define the role of the PR isoforms (Mote et al., 2006). In order to further define the function of PR in the murine uterus, knockout mouse models specific for the PR isoforms were generated (reviewed in Conneely et al. (2002)). The female PRA isoform knockout (PRAKO) mouse is infertile and displays defects in uterine implantation and decidualization. In addition to uterine defects, these mice also display a failure to ovulate. Specifically, with respect to embryo attachment, PRA was demonstrated to be significant in the inhibition of MUC1 expression in the mouse uterine epithelium (Brayman et al., 2006). Inhibition of MUC1, an epithelial cell-surface glycoprotein, is critical for the allowance of embryo implantation (Surveyor et al., 1995).

Interestingly, administration of progesterone to the PRAKO mouse resulted in a progesterone-dependent increase in epithelial cell proliferation, indicating that the PRB isoform of this receptor mediates proliferation while PRA is involved in the repression of epithelial proliferation (Mulac-Jericevic et al., 2000). This can apply to human uterine disease, specifically endometriosis where PRB was identified as the predominant isoform (Misao et al., 1999). Therefore, this demonstrates that PRA is responsible for many uterine-related functions. Furthermore, the PRAKO mouse displays no overt phenotype in the mammary gland, suggesting that the PRA isoform is critical for the regulation of reproductive tract function but is not involved in mammary gland branching or ductal development (Mulac-Jericevic et al., 2000). In contrast, the PRB isoform knockout (PRBKO) mice are fertile but display significant defects in branching morphogenesis and alveologogenesis in the mammary gland (Mulac-Jericevic et al., 2003). Thus, in the mouse, the PRA isoform is responsible for most uterine functions while PRB is critical for mammary gland development and growth.

3. The progesterone receptor initiates paracrine signaling within the uterine microenvironment during the pre-implantation period

3.1. Hedgehog-COUP-TFII signaling within the Uterus

3.1.1. Indian Hedgehog—The PR knockout mouse models have demonstrated that early pregnancy is dependent on functional PR signaling within the uterine compartments. The differential expression of PR within the luminal epithelium at days 2 and 3 of pregnancy facilitate the subsequent downstream expression of PR targets within the epithelium and stroma. Indian hedgehog (*Ihh*) was identified as an acute PR target gene (Takamoto et al., 2002), and is a known member of the hedgehog (Hh) signaling pathway that has demonstrated to be critical for development (reviewed in (Varjosalo and Taipale, 2008)). The Hh pathway consists of three mammalian hedgehog growth factor ligands: sonic hedgehog, (*Shh*) desert hedgehog (*Dhh*), and *Ihh*. Specifically, through the use of knockout mouse models, *Ihh* has demonstrated to be necessary for proper skeletal, vascular, and gastrointestinal tract development in the growing mouse embryo (Dyer et al., 2001; Ramalho-Santos et al., 2000; St-Jacques et al., 1999). *Ihh* homozygote knockout mice exhibited perinatal lethality due to defects in skeletal development (St-Jacques et al., 1999). Due to its significance in development and since it is a confirmed PR target, the elucidation of *Ihh* function within the uterus is necessary.

Ihh signaling operates in an epithelial to mesenchymal manner within the uterus. *Ihh* transduces its signal via binding the transmembrane receptor patched-1 (PTCH1) (reviewed in Varjosalo and Taipale (2008)). PTCH1 functions to inhibit the activity of another transmembrane receptor smoothened (SMO). When a Hh ligand binds to PTCH1, PTCH1 ceases to repress SMO, resulting in an active SMO signal. This signal then activates the transcription of genes in the glioma-associated oncogene homolog (Gli) family of transcription factors and the orphan nuclear receptor Chicken Ovalbumin Upstream Transcription Factor II (COUP-TFII) (Krishnan et al., 1997). These transcription factors have shown to be responsible for activating downstream target genes of the Hh ligands by binding to Hh response elements within upstream promoters.

3.1.2. COUP-TFII—COUP-TFII (nuclear receptor subfamily 2, group F, member 2 (*Nr2f2*)) has been identified to be a critical regulator in cell differentiation and tissue development as well as angiogenesis and metabolism (reviewed in Lin et al. (2011)). Ablation of COUP-TFII results in embryonic lethality due to defects in vascular development (Pereira et al., 1999). Mice heterozygous for the ablation of COUP-TFII exhibit reproduction defects including the inability of the uterus to undergo the changes

required to support embryo implantation (Takamoto et al., 2005). Therefore, loss of only one allele of COUP-TFII can impair reproduction. Additionally, given the fact that COUP-TFII has demonstrated to be activated downstream of Hh signaling in the developing central nervous system (Krishnan et al., 1997), strong evidence exists to propose a role of a Hh–COUP-TFII axis in the regulation of reproduction. *Ihh* is expressed in the uterine epithelium on days 3 and 4 of pregnancy while COUP-TFII is expressed in the stromal cells following the induction of *Ihh* (Takamoto et al., 2002). The expression of these genes is just prior to the window of receptivity giving further support for a role of this signaling axis in preparing the uterus for embryo implantation. Due to the severe phenotype of *Ihh* knock out mice and COUP-TFII knock out mice, conditional knock out mice were individually generated for each gene in order to further ascertain the effect that the PR-induced *Ihh*-COUP-TFII signaling axis has on the regulation of pregnancy.

3.1.3. The *Ihh*–COUP-TFII axis—Through the use of effective conditional knock out mouse models prompting the individual ablations of *Ihh* and COUP-TFII within the uterus, this axis was shown to be critical for both uterine implantation and decidualization. The *PR^{cre}* mouse model which ablates floxed genes in PR containing cells (i.e. those of the pituitary gland, preovulatory granulosa cells of the ovary, uterus, and mammary gland (Soyal et al., 2005)), was implemented in these models. In the separate *PR^{cre/+}Ihh^{f/f}* and *PR^{cre/+}COUP-TFII^{f/f}* mouse models, both models displayed a similar phenotype in which embryos were unable to attach to the uterine lumen (Kurihara et al., 2007; Lee et al., 2006). Also, these mice failed to undergo decidualization upon administration of a manual scratch to mimic embryo implantation. Therefore, *Ihh* likely plays a role in transducing an epithelial to stromal signal that initiates embryo implantation and subsequent decidualization. However, the surprising finding was that the *Ihh*–COUP-TFII axis affected embryo attachment. This provided evidence that this axis plays a role in signaling from the stroma to the epithelium in order to promote embryo attachment. In summary, the *Ihh*–COUP-TFII signaling axis acts concurrently between both uterine compartments to carry out successful PR function in early pregnancy.

As was demonstrated previously, the active *Ihh*–COUP-TFII signaling axis is critical for the proper development and preparation of the uterus for the implanting embryo. Together with this axis, the steroid hormone receptors are also highly regulated in order to ensure the proper implantation of the embryo at the window of receptivity. Since estrogen signaling promotes epithelial proliferation and MUC1 expression, its downregulation is necessary for implantation to occur. Interestingly, in the *PR^{cre/+}Ihh^{f/f}* and *PR^{cre/+}COUP-TFII^{f/f}* mouse models, estrogen signaling was found upregulated within the uterine epithelium of both models suggesting that the inhibition of ER α by the PR occurs via *Ihh* and COUP-TFII (Franco et al., 2010b; Kurihara et al., 2007). In fact, when *PR^{cre/+}COUP-TFII^{f/f}* mice were treated with an ER α inhibitor, embryo attachment and decidualization were rescued (Lee et al., 2010). Therefore, COUP-TFII plays a critical role in mediating the signal from epithelial *Ihh* to other effector genes in the stroma to control embryo implantation and decidualization (Franco et al., 2010a; Lee et al., 2007). Also, COUP-TFII likely provides the stromal to epithelial signal necessary for the inhibition of epithelial proliferation directly or it could relay the signal to a target molecule. Identification of the signaling pathway from stroma to epithelium would aid in the understanding of how the stroma contributes to embryo implantation. In actuality, a recent study has demonstrated that stromally located *Hand2* plays a critical role in the inhibition of epithelial proliferation (Li et al., 2011).

3.1.4. *Hand2*—*Hand2* is a basic helix-loop-helix (bHLH) transcription factor and known downstream target of PR. Upon conditional ablation of *Hand2* using the *PR^{cre/+}* mouse (*PR^{cre/+}Hand2^{f/f}*), embryo attachment was inhibited while in contrast, mating behavior, ovulation, and neuroendocrine signaling remained intact (Li et al., 2011). In *Hand2* null

mice administered with estrogen and progesterone, instead of being inhibited by progesterone, epithelial proliferation remained persistent within the luminal epithelium. This suggests that *Hand2* is a critical mediator between active progesterone signaling and the inhibition of estrogen-induced proliferation within the epithelium. This mechanism was further elucidated and revealed that fibroblast growth factors or FGFs were upregulated in *PR^{cre/+}Hand2^{fl/f}* mice. FGFs bind to their receptors, FGFRs, to activate the ERK pathway which has been shown to be critical for inducing the activation of ER α (Kato et al., 1995). Not only were FGFs upregulated within the *Hand2* null mice, but also ERK1/2 and ER α levels were increased within the uterine epithelium (Li et al., 2011). Due to the upregulated ER α levels, it was not surprising that estrogen targets such as *Muc1* remained aberrantly upregulated in the epithelium, demonstrating that *Hand2* is critical for mediating the downregulation of estrogen target genes. Therefore, *Hand2* is an important downstream target of PR that mediates the inhibition of estrogen signaling and specifically, the inhibition of estrogen-induced epithelial proliferation in the uterus. Hence, inhibition of epithelial proliferation by PR signaling may be acting via the Hh pathway signaling downstream to COUP-TFII. COUP-TFII then acts as a mediator to activate genes necessary for implantation and decidualization which may include *Hand2*, causing the inhibition of estrogen signaling and subsequent allowance for proper embryo implantation. A summary of the proposed paracrine regulation of PR signaling regulating embryo implantation is illustrated in Fig. 1.

3.1.5. The IHH–COUP-TFII Axis in the Human—In the human, *IHH* was found to be expressed in the healthy endometrium, peaking specifically in expression at the secretory phase (Wei et al., 2010). Also, *GLI1* was observed to mirror the expression pattern of *IHH* suggesting that it is likely activated downstream of *IHH* within the human endometrium as is demonstrated in the murine system. Within the human uterus, *IHH* was determined to be dependent on progesterone which was evidenced by increased *IHH* protein and mRNA levels during the proliferative phase upon treatment with a selective progesterone receptor modulator (SPRM). Another study demonstrated that *IHH* expression is dysregulated in patients with endometriosis (Burney et al., 2007), while COUP-TFII was found expressed within endometriotic and eutopic endometrial stromal cells (Zeitoun et al., 1999). A recent study using human endometrial stromal cells observed increased *HAND2* expression upon induction of decidualization *in vitro* (Huyen and Bany, 2011). Also, this study provided evidence to suggest that *HAND2* may regulate *FOXO1* and *IGFBP1*, critical genes involved in human decidualization (reviewed in Gellersen and Brosens (2003)). Therefore, the *Ihh*–COUP-TFII axis that was evidenced to be critical in the murine system has been identified to be conserved in humans.

4. Progesterone receptor regulation of post-implantation support of the developing embryo

Successful embryo implantation requires proper attachment of the embryo and subsequent differentiation of the surrounding stroma. Both processes require paracrine signaling to occur within the uterus. Activation of paracrine signaling within the stroma has demonstrated to be necessary to initiate differentiation and promote proliferation and vascularization in order to support the implanted embryo. Therefore, paracrine signaling is not only required for proper embryo attachment, but also is important in the support and development of the implanted embryo in decidualization. Knock out mice have been pivotal in demonstrating that members of the bone morphogenetic protein (BMP) and wingless-related MMTV integration site (Wnt) family are critical for these processes within early pregnancy.

4.1. Bmp2

Bmps are growth factors that are part of the transforming growth factor-beta (TGFB) superfamily. Numerous members of the Bmp family have been identified as expressed in the peri-implantation mouse uterus (Paria et al., 2001; Ying and Zhao, 2000). One member of the BMP family that is expressed upon embryo attachment and throughout the post implantation decidua is bone morphogenetic protein 2 or *Bmp2*. *Bmp2* is known to play a critical role in early development. In fact, *Bmp2* knock out mice are embryonic lethal due to cardiac developmental defects and a failure of proamniotic canal closure (Zhang and Bradley, 1996). In order to study the functions of *Bmp2* in the uterus while avoiding the developmental defects associated with *Bmp2* knock out mice, conditional knockouts using the *PR^{cre}* model were implemented. In mice in which *Bmp2* is conditionally ablated in cells specifically expressing the PR (*PR^{cre/+}Bmp2^{fl/f}*), embryos have shown to successfully implant in the uterine luminal epithelium, yet the stroma fails to undergo a decidual response in order to support the developing embryo (Lee et al., 2007). Although embryo implantation occurs, activation of decidualization fails to transpire resulting in infertility. However, upon injection of recombinant BMP2 within the uterus of *PR^{cre/+}Bmp2^{fl/f}* mice undergoing a manually-induced scratch to mimic implantation, the BMP2 injection was observed to rescue the absent decidual response. This demonstrated that *Bmp2* is necessary and sufficient for decidualization and likely acts as a paracrine signaling factor for the initiation of the proliferative response after embryo implantation within the uterine stroma. Additionally, through the use of the *Bmp2* conditional knock out mouse model, *Bmp2* was demonstrated to be responsible for the upregulation of *Wnt4*, *Wnt6*, and *Fkbp3*, -4, and -5 (Lee et al., 2007). Thus, *Bmp2* acts via a paracrine mechanism to initiate decidualization after embryo implantation, yet also plays a fundamental role in preparing the epithelium for implantation through the regulation of Fkbp3 and Wnt ligands. In the human, *BMP2* was also identified to be important in human decidualization as it was observed to be robustly expressed in human endometrial stromal cells undergoing decidualization in culture (Li et al., 2007). Therefore, *Bmp2* is a critical gene necessary for decidualization and regulation of Fkbp3 and Wnt ligands within the murine system, and also has been identified to be conserved in human decidualization function.

4.2. Precise regulation of wnt signaling is necessary for uterine function and homeostasis

Although *Bmp2* is a critical paracrine factor in relaying the embryo attachment signal from the epithelium to the stroma to initiate decidualization, Wnt ligands have established themselves as potent activators of their canonical and non-canonical signaling pathways important in development. Wnt signaling operates canonically through the binding of a Wnt ligand to the transmembrane FRIZZLED receptor which signals downstream to facilitate β -CATENIN function and entrance into the nucleus to activate transcription of its target genes. Wnt signaling can also occur via the non-canonical pathway through the binding of non-frizzled receptors such as receptor tyrosine kinase-like orphan receptor 2 (ROR2) and receptor-like tyrosine kinase (RYK) confirming that Wnt signaling pathways are very diverse and can often cross-react in multiple ways (reviewed in Angers and Moon (2009)).

Due to the critical role that this diverse signaling pathway plays in development, tight control of Wnt signaling is critical for proper growth of the developing embryo. In order to study Wnt signaling effectively, this pathway can be efficiently manipulated in the uterus through the use of transgenic mouse models. In β -catenin ex3 mice (*Ctnnb1^{fl(ex3)/+}*), the exon containing the phosphorylation sites on the β -CATENIN molecule necessary for tagging the molecule for degradation by the proteasome is excised upon expression of *Cre* recombinase (Harada et al., 1999). Therefore, when these mice are crossed with *PR^{cre/+}* mice, β -catenin is constitutively active within cells expressing the PR. These mice exhibit subfertility, contain enlarged glands, and display a hyperplastic phenotype within the uterus

(Jeong et al., 2009). Although constitutive activation of β -catenin results in uterine hyperplasia and subfertility, ablation of β -catenin within the uterus leads to the development of metaplasia and causes infertility. In both the β -catenin knockout and constitutively expressed mouse models, the uterus fails to exhibit a decidual response. Therefore, β -catenin is necessary for decidualization and fertility, illustrating that precise regulation of β -CATENIN activity is critical for the maintenance of a healthy endometrium.

4.3. Wnt4

Modulation of actual Wnt ligands, such as *Wnt4*, can also have a detrimental affect on embryo development and uterine function. The wingless-related MMTV integration site 4 or *Wnt4* is a known Wnt ligand and *Bmp2* target that was specifically identified to be necessary for uterine development in mammals. In fact, upon total knockdown of *Wnt4* in mice, genetically female mice develop testis confirming that *Wnt4* is critical for female reproductive tract development (Vainio et al., 1999). To elucidate *Wnt4* function within the uterus, mouse models were utilized to ablate *Wnt4* specifically in cells that express the PR (*PR^{cre/+}Wnt4^{fl/f}*). In these mice, implantation fails to occur due to the inability for the embryo to invade into the luminal epithelium (Franco et al., 2010a). Furthermore, the ablation of *Wnt4* in the uterus results in hypertrophy and pseudostratification of the luminal epithelium. This may account for the difficulty of embryo invasion. In addition, the amount of endometrial glands observed in the *Wnt4* ablated mice is reduced compared to control mice, which can further restrict implantation due to the decrease in the production of LIF (Stewart et al., 1992).

In order to circumvent the attachment defect and evaluate decidualization in these *PR^{cre/+}Wnt4^{fl/f}* mice, artificial induction of the decidual response was performed by steroid hormone treatment and the administration of a mechanical trauma (Franco et al., 2010a). These mice failed to undergo the decidual response. Instead of expected levels of proliferation and differentiation occurring within the decidualized stroma of these mice, *PR^{cre/+}Wnt4^{fl/f}* mice exhibited an elevation of stromal apoptosis and an absence of differentiation. Since apoptosis is contrary to what is expected in decidualization, an investigation was performed to understand the mechanism behind the induction of apoptosis in these mice. *Wnt4* was previously identified to control the nuclear localization of FOXO1, a transcription factor and known promoter of apoptosis (reviewed in Huang and Tindall (2007)). In the *PR^{cre/+}Wnt4^{fl/f}* mice, FOXO1 was determined to be expressed in the nucleus, suggesting that FOXO1 actively promotes the transcription of pro-apoptotic genes (Franco et al., 2010a). A study published previously using human endometrial stromal cells, demonstrated that progesterone signaling induces cytoplasmic localization of FOXO1, thereby inhibiting apoptosis (Labied et al., 2006). Therefore, besides the role of FOXO1 in initiating apoptosis in *PR^{cre/+}Wnt4^{fl/f}* mice, Wnt signaling has also demonstrated to be conserved in the human.

In point of fact, although most of the work on Wnt signaling has been performed in the murine system, studies have shown that Wnt signaling is active in human uterine function. Multiple Wnt ligands and their FRIZZLED receptors have shown to be spatially and temporally expressed within the uterine compartments during the menstrual cycle in women and were reported to be downregulated in patients with endometrial cancer (Bui et al., 1997; Tulac et al., 2003). Additionally, it has been proposed that Wnt signaling likely plays a role in human embryo implantation, yet more studies need to be performed in order to confirm this statement (Liu et al., 2010; Tulac et al., 2003). Therefore, Wnt signaling has demonstrated to play a crucial role in regulating uterine function and homeostasis within the murine uterus as well as the human uterus.

4.4. Fkbp4

Bmp2 was shown to be responsible for the upregulation of not only Wnt ligands, but also *Fkbp4* (*Fkbp52*) (Lee et al., 2007). The Fkbps or FK-506-binding proteins act as co-chaperones of steroid hormone receptors by containing a unique tetratricopeptide repeat domain necessary for binding to heat shock protein 90 (*Hsp90*) which has demonstrated to directly bind to the steroid receptors to modulate receptor activity (reviewed in (Pratt and Toft, 1997)). In relation to progesterone signaling, *Fkbp4* was demonstrated to bind in a complex with *Hsp90* and PR to modulate PR activity within the cytoplasm (Renoir et al., 1990). In order to further understand the function of *Fkbp4* and its affect on PR activation, an *Fkbp4* null mouse on the C57BL/6J background was generated (Tranguch et al., 2005; Yang et al., 2006). Although ovulation proved to be normal in this mouse, sterility resulted from the failure of the embryo to implant. Furthermore, decidualization failed to occur upon administration of a manual scratch to the uterine wall, suggesting that *Fkbp4* is critical for both embryo implantation and the decidual response. The failure of the uterus to implant embryos and to undergo decidualization was not due to decreased progesterone signaling, as the expression levels of PR and the hormone levels of progesterone both were comparable to control mice. Interestingly, the expression patterns of *Fkbp4* and the PR were found co-localized on days 4 and 5 of implantation suggesting that *Fkbp4* plays a critical role in modulating PR activity during implantation. (Tranguch et al., 2005). Also, another *Fkbp4* null mouse was generated on the CD1 background and when treated with progesterone, embryo implantation and decidualization occurred successfully (Tranguch et al., 2007). Surprisingly, these *Fkbp4* null mice were able to carry and deliver a normal litter size when administered elevated levels of progesterone throughout pregnancy, suggesting that genetic background may affect the function of *Fkbp4*. In the *Fkbp4* null CD1 mice, progesterone hormone was identified to be necessary during peri-implantation, but then was needed at elevated levels after implantation during pregnancy, which is likely necessary for the compensation of reduced PR signaling activity. In addition to its role in the murine system, *Fkbp4* was identified to be conserved in the human. Specifically, *FKBP4* was found to be downregulated in endometriotic tissue compared to healthy endometrium, suggesting a role for the modulation of progesterone sensitivity in the human uterus (Hirota et al., 2008). Therefore, *Fkbp4* plays a critical role in embryo implantation and decidualization in the murine uterus and demonstrates a conserved role in the potentiation of PR signaling in both the mouse and human uterus.

5. Progesterone receptor regulation of glandular development

5.1. Introduction

Endometrial glands are located within the stroma and are responsible for secreting growth factors and morphogens needed for uterine function and pregnancy (reviewed in Gray et al. (2001)). These glands have not only demonstrated to be important for the promotion of embryo implantation through the secretion of LIF (Stewart et al., 1992), but also have shown to be critical for decidualization (Chen et al., 2000). Glandular development or adenogenesis initiates within rodents through the invagination, growth, and branching of the luminal epithelium at postnatal day 5 in the mouse (reviewed in Gray et al. (2001)). Glands are critical for pregnancy and without them, embryo implantation fails to occur. The importance of glands in regulating embryo implantation has been evidenced in ovine and murine models in which the glands have been hormonally or genetically ablated. These animal models result in sterility due to an inability to support embryo implantation (Bartol et al., 1988; Franco et al., 2010a,c; Jeong et al., 2009, 2010).

Due to the critical role in which glands play in early pregnancy and the vast regulation of progesterone signaling in the establishment and maintenance of pregnancy, it is only logical

that one would propose a role of PR in regulating glandular development. However, to date, progesterone signaling is verified to not be involved in glandular development as evidenced by the PRKO mouse which consequently exhibits normal glandular formation. Although PR is not necessary for glandular formation, exogenous progesterone signaling may nonetheless have a deleterious impact on gland development. In fact, a study using neonatal sheep demonstrated that early progesterone treatment acts to inhibit gland development (Bartol et al., 1988). In a recent study, progesterone-treated neonatal mice exhibited an absence of glands (Stewart et al., 2011). However, these same progesterone-treated mice at 8 weeks of age displayed glands. Therefore, although gland formation was believed to be a one time occurrence early postnatally, may happen at multiple occurrences within the life of the mouse. Therefore, early progestin treatment may not be as detrimental to gland formation as was once surmised. However, it is clear that PR signaling adversely impacts endometrial gland development. This mechanism warrants further investigation of known PR-regulated signaling pathways that have demonstrated to affect gland development. Currently, top candidate pathways of interest include the Wnt and *Ihh* signaling pathways.

5.2. Wnt signaling

Besides their significant role in reproductive tract development, it has been evidenced that the Wnt pathway is involved in the formation of endometrial glands. With constitutive expression of β -catenin in β -catenin ex3 mice (*Ctnnb1^{f(ex3)/+}*), the uterus displays an increased amount of enlarged glands (Jeong et al., 2009). Conversely, upon ablation of β -catenin in the uterus, the endometrium contains no glands whatsoever. Therefore, the proper regulation including the degradation of β -catenin is critical for gland formation in the murine uterus. Furthermore, the Wnt ligands were also identified to be necessary for the development of endometrial glands. Interestingly, the *Wnt4* conditionally-ablated mice, the *Wnt7a* knock out mouse, and *Wnt5a* ablated uteri all exhibit the failure to develop endometrial glands (Franco et al., 2010a; Mericskay et al., 2004; Miller and Sassoon, 1998). Due to the robust expression pattern of *Wnt5a* within the stroma and the juxtaposed expression of *Wnt7a* within the luminal epithelium during postnatal development, these Wnt ligands may be acting conversely between the uterine compartments in order to promote glandular development (Mericskay et al., 2004). *Wnt5a* is thought to promote the invagination of the luminal epithelium by repressing *Wnt7a* action in order to initiate the formation of an endometrial gland. However, this mechanism of adenogenesis has yet to be confirmed.

5.3. Smo

Modulation of Hh signaling *in vivo* has determined to be detrimental in uterine function and fertility. Mice harboring a conditional *Smo* allele that upon expression of *Cre* recombinase results in the constitutive activation of *Smo* (*SmoM2+*), were utilized to elucidate the role of the Hh pathway in uterine morphology and function (Franco et al., 2010c; Jeong et al., 2004). The *SmoM2+* mice were crossed to *PR^{cre/+}* mice to constitutively activate the Hh pathway in PR positive cells (Soyal et al., 2005). Of the many uterine defects displayed in these *PR^{cre/+}SmoM2+* mice, one of the most interesting defects consisted of the failure to form endometrial glands (Franco et al., 2010c). Although these mice displayed normal levels of the steroid hormone receptors, gland formation was prevented. Therefore, the steroid hormone receptor levels did not affect gland formation, but rather some other signaling pathway, possibly acting through hormone receptor signaling, is responsible for modulating glandular development. Due to the fact that progestin treatment can activate downstream PR targets such as *Ihh* and cause the inhibition of gland formation in neonatal sheep, abnormally active *Ihh* signaling in these *PR^{cre/+}SmoM2+* mice may be responsible for the reduction in gland formation within the murine uterus.

Additionally, the *Ihh* and Wnt pathways may affect glandular development through their control of uterine epithelial cell differentiation. Within both the *PR^{cre/+}Wnt4^{fl/f}* and *PR^{cre/+}SmoM2⁺* mice, not only is there an absence of glands, but also there is a presence of focal p63 positive basal cells (Franco et al., 2010a,c). p63 is a transcription factor that is important for the formation of the first basal layer in the embryonic epidermis (reviewed in Koster and Roop (2007)). Therefore, these p63 positive cells may play an initiating role in glandular development. Furthermore, upon estrogen treatment for three months, *Wnt4* conditionally ablated mice display a pseudostratified epithelium consisting of a robust layer of p63 positive basal cells (Franco et al., 2010a). Since these mice display a reduced number of glands and glands are known to develop from the luminal epithelium, it may be possible that the Wnt pathway is regulating the appropriate differentiation of the luminal epithelium. *Wnt4* likely functions to induce glandular formation via elongation and budding from the luminal epithelium, while causing the inhibition of basal cell formation at the sub-epithelia. Due to the similar basal cell positive phenotypes between the *PR^{cre/+}Wnt4^{fl/f}* and *PR^{cre/+}SmoM2⁺* mice, it is probable that both the Wnt and *Ihh* pathways play a role in controlling epithelial cell differentiation.

6. Discussion: the complex and diverse progesterone signaling network

Progesterone signaling through active PR has been identified to regulate many genes including growth factors, transcription factors, and morphogens in a variety of different signaling pathways (Wang and Dey, 2006). PR is of paramount significance for the implantation of the fertilized embryo and the subsequent decidualization of the uterine stroma during post-implantation (Mulac-Jericevic et al., 2000). It is also apparent that the regulation of PR signaling and its downstream targets are important for glandular formation (Bartol et al., 1988; Franco et al., 2010a,c; Jeong et al., 2009, 2010). It is through the further elucidation of this complex PR signaling network acting through paracrine signaling mechanisms within the murine endometrium that we can understand what is necessary for the establishment and continuation of pregnancy in the human uterus. Not only does this work have far-reaching implications in the scope of increased fertility and the development of safe contraceptives, but also in the understanding of human endometrial carcinoma and endometriosis in which the PR signaling pathway is often dysregulated. Therefore, the continued study of genetically engineered mouse models will help us to further comprehend the complex PR signaling network critical for human pregnancy.

The PR knockout mouse models have proven to be important in elucidating PR regulation within implantation, decidualization, and also mammary gland development. In particular, the isoform specific knockouts of PR, PRAKO and PRBKO, have assisted in the delineation of the separate roles of PRA and PRB. As PRA has demonstrated to play a critical role in uterine function in pregnancy, PRB primarily has shown to be responsible for mammary gland development (Mulac-Jericevic et al., 2000, 2003). Although these models have demonstrated their significance in defining PR isoform function, there is still knowledge to be gained. While PRB was demonstrated to play a major role in branching morphogenesis in the mammary gland and was thought to contribute no function in the uterus, PRB is the only PR isoform found in the uterine luminal epithelium in cycling mice (Mote et al., 2006). Therefore, the exclusive expression of PRB in the luminal epithelium may act as a reservoir of PRA heterodimerizing partners, or PRB may homodimerize and regulate epithelial target genes. Therefore, studies still need to be performed to assess the function of the PRB isoform in the uterine epithelium during the estrus cycle. It is clear, however, that the PR isoforms have exhibited different functional roles in the past. Indeed, PRA is suggested to have an inhibitory affect on proliferation within the uterus, while PRB displays more of a pro-proliferative role within the mammary gland (Conneely et al., 2002). Therefore, the expression ratio of the PR isoforms within the uterine compartments could potentially

regulate the gene expression profile observed. Furthermore, the dysregulation of the PR isoforms has been shown to occur in patients with endometriosis and breast cancer, demonstrating the integral requirement for proper balance of these isoforms in hormone responsive tissues (Attia et al., 2000; Mote et al., 2002). Therefore, comprehension of the separate roles of the PR isoforms will help give insight to treating human disease.

However, the identification of the specific roles of the PR isoforms represents only a small facet in understanding the complexity of the PR signaling network. True appreciation of PR signaling occurs through the identification of PR target genes and their signaling mechanisms that bring about regulation of uterine function. In this review, the *Ihh*-COUP-TFII axis was described as an important paracrine signaling network that not only provides an epithelial to stromal signal necessary for embryo implantation and decidualization, but also likely participates in a stromal to epithelial signal critical for embryo attachment. This specific stromal to epithelial signal is thought to operate through the transcription factor *Hand2* which was identified to be critical for the inhibition of ER α signaling and therefore, epithelial proliferation (Li et al., 2011). Indeed, ER α signaling was upregulated in the *Ihh* and COUP-TFII conditionally ablated mice providing further testament to the role of *Hand2* in the inhibition of estrogen signaling. (A description of this complex network is illustrated in Fig. 1). Future work includes confirming that it is indeed the *Ihh*-COUP-TFII signaling axis that activates *Hand2* to inhibit epithelial proliferation during pre-implantation. This complicated yet precise paracrine network governed by PR has been determined to be critical for the implantation of the embryo at the window of receptivity and subsequent activation of decidualization (Kurihara et al., 2007; Lee et al., 2006). The *Ihh* pathway has further shown to be conserved in humans and was identified to be dysregulated in endometriosis (Burney et al., 2007; Wei et al., 2010). Furthermore, COUP-TFII was found expressed in endometriotic and eutopic endometrial stromal cells (Zeitoun et al., 1999), providing witness to the significance of this pathway and conservation within humans.

Although the initiation of embryo implantation by paracrine signaling is critical for pregnancy, the involvement of paracrine factors in decidualization is equally important. *Bmp2* was found to be robustly expressed in the stroma during peri-implantation and is thought to be responsible for activating decidualization once the embryo has attached (Lee et al., 2007). This was evidenced by the rescue of the *Bmp2* conditionally ablated mice by an injection of recombinant BMP2 combined with a manual scratch to mimic implantation. Of course, BMP2 was also observed to be highly expressed in the stroma of human endometrial cells, confirming that this gene is important for decidualization and is conserved in the human (Li et al., 2007). Additionally, *Fkbp4* was identified to be a crucial modulator of implantation and decidualization through the potentiation of PR signaling (Tranguch et al., 2005; Yang et al., 2006). A similar role in the regulation of progesterone signaling activity was thought to occur for *FKBP4* as it was found downregulated in human endometriotic tissue (Hirota et al., 2008). Through the treatment with progesterone, the implantation and decidualization failure observed in the *Fkbp4* null CD1 mouse was rescued, suggesting that *Fkbp4* function may be affected by genetic makeup (Tranguch et al., 2007). Furthermore, the Wnt signaling pathway was identified as a significant pathway in the maintenance of uterine architecture and the regulation of pregnancy. Modulation of α -catenin levels were determined to be critical for the establishment of appropriate uterine pathology while also being important for the activation of decidualization (Jeong et al., 2009). The Wnt ligand, *Wnt4*, also demonstrated to be necessary for decidualization by likely modifying cell survival mechanisms through localization of FOXO1 (Franco et al., 2010a). Interestingly, the Wnt pathway was found to be expressed in humans in pregnancy and in different stages of the menstrual cycle (Bui et al., 1997; Tulac et al., 2003). Therefore, Wnt signaling is likely critical in human pregnancy and further work is needed to fully assess the role of Wnts in the human uterus.

Lastly, this review highlighted the important process of glandular development in ovine and murine models which demonstrated the opposing role of PR signaling in the formation of glands neonatally. Early progestin treatment in neonatal sheep was shown to inhibit gland formation (Bartol et al., 1988). However, recent studies suggest that early progestin treatment may not completely inhibit the ability to form glands later in life, proposing that adenogenesis may take place over time rather than occurring once neonatally (Stewart et al., 2011). In murine models, Wnt signaling proved to be important for glandular development. Ablation of *Wnt5a*, *Wnt7a*, or *Wnt4* in the murine uterus resulted in the absence of glands (Franco et al., 2010a; Mericskay et al., 2004; Miller and Sassoon, 1998). Also, α -catenin activity determined to be critical for gland formation as well (Jeong et al., 2009). Of most interest were the *PR^{cre/+}Smom2+* mice that failed to form glands upon constitutive activation of *Smo* within the uterus (Franco et al., 2010c). Due to the PR signaling pathway's history in inhibiting glandular formation and since *Ihh* is a known target of PR, inhibition of gland development could be operating via the *Ihh* pathway or possibly through its downstream target, *Hand2*. This requires further investigation in order to support that it is indeed activation of the *Ihh* signaling pathway that is causing gland inhibition. It was also noted that the formation of p63 positive basal cells occurred in the *PR^{cre/+}Smom2+* mice and *Wnt4* conditionally ablated mice (Franco et al., 2010a,c). Interestingly, interruption of these signaling pathways leads to the build up of basal cells in the luminal epithelium and inhibition of glands. These pathways may be involved in the differentiation of progenitor cells to become basal cells or glandular epithelial cells. Further studies are needed to confirm the roles of *Smo* and *Wnt4* in the modulation of epithelial cell differentiation.

Although much progress has been made in the field of female reproduction utilizing the murine system, there is still a large void in our knowledge of human reproduction. Through the implementation of new techniques that allow for the study of human tissue, new advancements in the field of human female reproduction can be made. Two methods that have recently been employed in the field include the implementation of xenograft models and the culturing of human endometrial stromal cells (Masuda et al., 2007). In xenograft models, human or mouse tissue can be grafted under the kidney capsule, allowing the tissue to develop similarly to how it would normally develop in an *in vivo* environment. Since these models are viable, we can also treat these mice with exogenous hormones in order to elicit a response from the implanted tissue, making these models critical tools for the study of reproductive tissues. Also, through the use of human endometrial stromal cells treated with exogenous hormones, gene expression analysis and changes in cellular architecture can be easily assessed in important reproductive processes such as decidualization. Through the implementation of these new techniques, we can further our understanding of female reproduction in the human.

Progesterone signaling through the PR is responsible for activating a complex paracrine signaling network that is imperative in pregnancy and is necessary for the development of healthy offspring and the survival of a species. Not only is the PR important in pregnancy, but the PR is also found to be dysregulated in many malignancies such as endometriosis, endometrial carcinoma, and breast cancer (Attia et al., 2000; Mote et al., 2002). Therefore, through an enhanced understanding of PR targets and their roles in paracrine signaling within the uterine compartments, we can identify and develop preventative interventions and better therapies for patients with these debilitating and life-threatening diseases.

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Fig. 1.

The progesterone regulated paracrine signaling model depicted within the uterine compartments at the time of embryo implantation. *Ihh* is a progesterone target activated within the epithelium which signals downstream to COUP-TFII in the stroma establishing the *Ihh*–COUP-TFII axis within the dual uterine compartments. COUP-TFII has demonstrated to be a critical effector of decidualization and the maintenance of pregnancy during post-implantation through the activation of *Bmp2* and *Wnt4*. COUP-TFII may also play a role in the activation of *Hand2* in the stroma leading to the inhibition of the FGF pathway, a pathway known to be involved in the promotion of epithelial proliferation by estrogen signaling. The arrows represent activation. The bars denote inhibition. The question marks next to arrows represent an activation that is lacking substantial evidence.