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Progesterone receptor signaling in the initiation of pregnancy and preservation of a healthy uterus

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ABSTRACT Infertility and reproductive-associated disease are global problems in the world today affecting millions of women. A successful pregnancy requires a healthy uterus ready to receive and support an implanting embryo. As an endocrine organ, the uterus is dependent on the secretions of the ovarian hormones estrogen and progesterone which signal via their cognate receptors, the estrogen and progesterone receptors. The progesterone receptor not only functions using classical nuclear receptor signaling, but also participates in non-genomic signaling at the cellular membrane. The complexity of progesterone signaling is further enhanced by the existence of multiple isoforms and post-translational regulation via kinases and transcription coregulators. This dynamic means of regulation of the progesterone receptor is evidenced in its necessary role in a successful pregnancy. Within early pregnancy, the progesterone receptor elicits activation of its target genes in a spatio-temporal manner in order to allow for successful embryo attachment and uterine decidualization. Additionally, appropriate progesterone signaling is important for the prevention of uterine disease such as endometrial cancer, endometriosis, and leiomyoma. The utilization of progesterone receptor modulators in the treatment of these devastating uterine diseases is promising. This review presents a general overview of progesterone receptor structure, function, and regulation and highlights its important role in the establishment of pregnancy and as a therapeutic target in uterine disease.

KEY WORDS: *progesterone signaling, progesterone receptor, early pregnancy, mouse model*

Introduction

According to the National Survey of Family Growth, 6.7 million women in the United States are unable to become pregnant or carry a pregnancy to term (Stephen and Chandra, 2000). This inability to reproduce affects the survival of the human species and can disturb the overall well-being of an individual. Furthermore, women are plagued by devastating reproductive tract-associated diseases such as endometrial cancer, endometriosis, and uterine fibroids. Today, there is a limited understanding of uterine disease, including how it originates and the importance of hormone signaling in the persistence of the disease. The increased understanding of these diseases, including the molecular mechanisms that initiate and perpetuate these disease states, is important for the development of new therapeutic treatments.

The uterus is a hormone sensitive organ that responds to the presence of the female ovarian hormones, estrogen and progesterone. Estrogen and progesterone individually bind to their cognate receptors, the estrogen receptor (ESR1) and the progesterone receptor (PGR). These receptors work in concert to establish and maintain pregnancy. Specifically, progesterone signaling is absolutely necessary for a successful pregnancy, conveyed in its namesake, the "pro-gestation" hormone. The *in vivo* ablation of progesterone signaling results in the inability to establish pregnancy (Lydon *et al*., 1995). Progesterone signaling involves the binding of progesterone hormone to the PGR, to promote the transcription of target genes (as reviewed in (Mulac-Jericevic and Conneely, 2004)). As a nuclear receptor, the PGR binds hormone and enters into the nucleus to bind DNA to initiate downstream functions within the cell. However, the PGR is also able to successfully promote cellular responses independent of nuclear entrance and DNA binding (Gellersen *et al*., 2009). This review will provide a broad overview of the structure and function of the PGR including a description of the different PGR isoforms generated as they are conserved between mouse and human. The PGR isoforms display unique functional abilities due to the presence of multiple

Abbreviations used in this paper: ESR1, estrogen receptor 1; PGR, progesterone receptor.

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activation domains. The activation domains are responsible for the ability of the PGR to bind ligand and DNA and the unique ability to recruit other molecules to modulate transcription efficiency. Additionally, the PGR is regulated by a variety of factors including interior protein domains, cytosolic chaperone proteins, post-translational modifications, and the presence or absence of ligand. Upon understanding the structure and function of the PGR and how it is regulated, this review will provide a snapshot of the target genes the PGR regulates within early implantation and uterine stromal cell decidualization.

Progesterone signaling is a highly regulated cellular pathway due to its critical role in the initiation and maintenance of pregnancy (reviewed in (Wang and Dey, 2006)). Upon fertilization of the embryo, the murine embryo travels down the oviduct and implants into the uterine epithelium. The surrounding uterine stroma then undergoes what is known as decidualization in which stromal cells proliferate and differentiate into decidual cells to promote the growth of the embryo (reviewed in (Cha *et al.*, 2012)). The current knowledge regarding progesterone signaling during implantation has been successfully attained via the generation of genetically engineered murine models. These models have provided a wealth of knowledge that was impossible to obtain using human endometrial cell culture *in vitro*. Utilizing murine ablation techniques, genes critical for pregnancy, many of which are progesterone target genes, have successfully been identified.

Finally, this review will briefly describe the role of progesterone signaling in uterine disease. Although much knowledge has been gained concerning the nature of uterine disease, there are many unknowns regarding how uterine disease initiates and how it can be prevented or effectively treated. Furthermore, synthetic hormones and hormone receptor modulators have been utilized as treatment therapy for these diseases, yet additional studies of their clinical effectiveness is required. Through the increased understanding of how progesterone functions, we can better understand the involvement of progesterone in the initiation, progression, and treatment of these devastating reproductive tract diseases. Additionally, through the utilization of ligands that promote or abrogate progesterone signaling, we can identify new treatment options for women suffering from reproductive disease.

The progesterone receptor: structure, function and regulation

The progesterone receptor as a nuclear receptor

The PGR is a member of the nuclear receptor family, characterized by its unique ability to bind ligand within the cytoplasm, dimerize, and enter into the nucleus to bind DNA resulting in the activation of target genes (reviewed in (Mulac-Jericevic and Conneely, 2004)). Nuclear receptors are able to function appropriately as they harbor conserved protein activation domains, known as AF-1 and AF-2 (Meyer *et al.*, 1990). AF-1 is located in the amino terminus of the protein and is responsible for functions independent of ligand binding (reviewed in (Brosens *et al.*, 2004, Ellmann *et al.*, 2009)). Between the AF-1 and AF-2 domain reside the hinge region and a conserved DNA binding domain (Kumar and Chambon, 1988). This binding domain allows for the nuclear receptor to bind to DNA as a dimer and subsequently recruit transcription machinery to begin transcription of the target gene. The DNA binding domain specifically recognizes a particular DNA sequence or motif, known as a response element, which functions as a sort of genomic address to elicit transcription of the specified gene (Ham *et al.*, 1988). In contrast to the AF-1 domain, the AF-2 domain confers the ability to bind ligand (Gronemeyer *et al.*, 1987). Furthermore, this domain also contains a nuclear localization sequence (Guiochon-Mantel *et al.*, 1989) and a sequence region allowing for efficient homodimerization or heterodimerization (Kumar and Chambon, 1988). Therefore, the AF-2 functional domain is responsible for ligand binding, dimerization, and also the translocation of the dimer into the nucleus. The presence of these two functional domains defines the family of nuclear receptors and allows for their unique ability to act as ligand-binding transcription factors.

Progesterone receptor isoforms

In addition to its nuclear receptor function, the PGR demonstrates increased complexity and specificity as it functions via two distinct isoforms, the PGR-A and PGR-B isoforms. These isoforms are transcribed from the same gene (Conneely *et al.*, 1989, Kastner *et al.*, 1990). The PGR isoforms are highly similar except that the PGR-B isoform exhibits an extra 164 amino acids at the amino terminus (see Fig. 1). Within this sequence resides an extra activation domain known as AF-3 which bestows unique functions to the PGR-B isoform (Sartorius *et al.*, 1994). The human PGR isoforms exhibit different transcription abilities within *in vitro* cell culture experiments. The PGR-A isoform was identified to exhibit a trans-dominant repressive role on gene transcription, while PGR-B often promoted the transcription of genes (Vegeto *et al.*, 1993). Although the human PGR isoforms are highly conserved, this repressive activity of the PGR-A isoform is unique to the human and has not been observed in all species (Giangrande *et al.*, 1997). To further understand this unique function of PGR-B compared to PGR-A, a scrutinized investigation of the activation domains was performed. Indeed, a transcription inhibitory domain was identified within the AF-1 domain of both isoforms (Giangrande *et al.*, 1997). The presence of this domain was identified to result in transcription repression, as demonstrated by the PGR-A isoform. However, since both of the PGR isoforms contain the inhibitory domain, the question left to ask was, "Why are the transcriptional activities of the isoforms so different? Could the extra sequence within PGR-B affect the inhibitory domain found in the AF-1 domain?" Although both PGR isoforms contain the inhibitory domain, the presence of the AF-3 domain successfully prevents the functioning of the inhibitory domain, rendering PGR-B more transcriptionally active (Giangrande *et al.*, 1997, Sartorius *et al.*, 1994). The mechanism of inhibition of the inhibitory domain by the AF-3 domain was identified as a single phosphorylated serine residing within the AF-1 domain (Clemm *et al.*, 2000). Therefore, the presence of the AF-3 domain proved to be responsible for the positive transcriptional activity of the PGR-B isoform.

Within the mouse, the PGR isoforms have also proven to display distinct functions. *In vivo* studies of the PGR originated with the generation of the PGR ablation mouse model or PRKO mouse. These mice exhibited infertility due to defects in mating behavior, ovulation, and uterine function (Lydon *et al.*, 1995). Furthermore, these mice displayed defects in mammary gland development and ductal side-branching. In order to discern which PGR isoforms were critical for these important reproductive functions, mouse models resulting in the ablation of the individual PGR isoforms were generated. Upon ablation of the PGR-A isoform, the mouse

resembled the PRKO mouse, displaying infertility with defects in uterine and ovarian function (Mulac-Jericevic *et al.*, 2000). However, the PGR-B ablation mouse model was found dispensable for uterine function, yet necessary for normal mammary development and branching (Mulac-Jericevic *et al.*, 2003). Therefore, although highly similar, the PGR isoforms have demonstrated to be responsible for different functions within the murine reproductive system.

In addition to the PGR-A and PGR-B isoforms, it is thought that a third PGR isoform exists, known as the PGR-C isoform (Wei and Miner, 1994). This isoform was identified to be transcribed from a start site 430 amino acids downstream of the PGR-A start site, resulting in the transcription of a 60 kD protein (graphically displayed in Fig. 1). Due to its absent amino terminus, PGR-C lacks the AF-1 and DNA binding domains, but retains ligand binding and dimerization capabilities (Wei *et al.*, 1997). Therefore, although PGR-C is unable to bind DNA to regulate transcription directly, it may play a role in the sequestration of ligand or other PGR isoforms to decrease the functionality of global progesterone signaling. Conversely, PGR-C has been shown to promote the transcriptional activity of the other PGR isoforms (Wei *et al.*, 1996). Indeed, PGR-C was identified to successfully heterodimerize with PGR-B (Wei *et al.*, 1997). Despite the absence of a DNA binding domain, it is hypothesized that PGR-C may functionally bind DNA as a heterodimer as it is found in the nucleus within human tissue (Wei and Miner, 1994). Within *in vitro* studies, the PGR-C heterodimer was recognized to successfully bind DNA, although not as efficiently as the PGR-B homodimer (Wei *et al.*, 1997). In the clinic, PGR-C was upregulated within human myometrium at the time of parturition, suggesting that this isoform may play a specific role in the induction of labor (Condon *et al.*, 2006). Nevertheless, PGR-C has often proven difficult to detect which may be due to the transcription start site residing in an inactive transcriptional region (Samalecos and Gellersen, 2008). As a result, much debate has centered on whether or not the PGR-C isoform exists. Therefore, further experiments are required to confirm the existence and function of the PGR-C isoform.

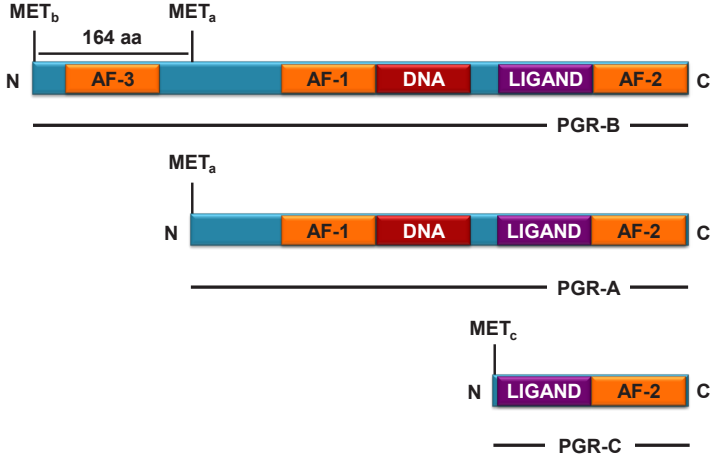

Fig. 1. Progesterone receptor isoforms. *The PGR isoforms are transcribed from the same gene due to three separate start sites located within the PGR locus. The PGR-B isoform is the largest of the three isoforms and exhibits an extra amino transactivation domain known as AF-3. As a nuclear receptor, the PGR harbors DNA binding and ligand binding domains. The presence of these domains and the activation domains (AF-1, AF-2, and AF-3) allow for the unique function of the individual PGR isoforms. aa, amino acid; MET, methionine.*

Recruitment of coregulators by the progesterone receptor

It is known that classical nuclear receptor signaling involves binding ligand, dimerizing with another receptor, and binding to response elements within promoters to recruit or prevent the binding of transcriptional machinery to modulate the transcription of target genes (reviewed in (DeMayo *et al.*, 2002)). Although most transcriptional machinery is generic and can be recruited to multiple sites of transcription, there are molecules recruited along with the generic machinery to promote or repress transcription known as coactivators and corepressors, both types generally termed coregulators (reviewed in (McKenna and O'Malley, 2002)). Interestingly, it was identified that the PGR isoforms recruit specific coregulators to the transcription start site to elicit a transcriptional response (Giangrande *et al.*, 2000). Within the human, PGR-A represses transcription of target genes due to the active inhibitory domain (Vegeto *et al.*, 1993). Therefore, it is not a surprise that the PGR-A isoform preferentially recruits the corepressor, NCOR2 (Giangrande *et al.*, 2000). However, the PGR-B isoform, known to be transcriptionally active in the human, was identified to specifically recruit coactivators such as NCOA1 and GRIP1. Therefore, the PGR isoforms are able to distinctively regulate transcription through their unique structures and preferential recruitment of coregulators.

The nuclear receptor coactivator (NCOA) family is a family of coactivators successfully recruited by a number of nuclear receptors including the PGR (reviewed in (McKenna and O'Malley, 2002)). Through the generation of knockout mouse models, NCOA1 and NCOA2 have proven to be critical for pregnancy. A total ablation model for NCOA1 was generated via gene targeting mechanisms (Xu *et al.*, 1998). Although the *Ncoa1*$^{-/-}$ mice were viable and fertile, they exhibited a decreased decidual response and reduced uterine wet weight when treated with estrogen. Therefore, these mice exhibited decreased sensitivity to treatment with ovarian hormones. In the uterine specific ablation mouse model of *Ncoa2* utilizing a Cre recombinase targeted to the *Pgr* locus (Soyal *et al.*, 2005), NCOA2 demonstrated to be necessary for fertility (Mukherjee *et al.*, 2006). Upon further investigation, these mice were deemed infertile due to the failure of embryo attachment and an impaired decidual response. Interestingly, these mice were crossed to the *Ncoa1*$^{-/-}$ mice, resulting in a double *Ncoa1/Ncoa2* knockout within PGR positive cells. These dual knockout mice completely failed to elicit a decidual response. Therefore, NCOA1 and NCOA2 together play a significant role in the induction of decidualization through the modulation of progesterone signaling at the transcription level.

Progesterone receptor ligand binding

The PGR protein primarily binds progesterone ligand. However, the PGR is able to successfully bind synthesized compounds that mimic the progesterone molecule and fit the PGR binding pocket. These compounds, known as progesterone receptor modulators (PRMs) can act in either an inhibitory or stimulatory manner to PGR function (reviewed in (Spitz, 2003)). PRMs have proven useful in controlling abnormal uterine bleeding, the treatment of endometrial disease, contraception, and hormone replacement therapy. The most well-known PRM is RU486 or mifepristone. RU486 was first identified as a PGR antagonist in the early 1980s (Herrmann *et al.*, 1982). However, the use of

PRMs often results in detrimental side effects which can include non-specific androgenic and glucocorticoid activities (Sitruk-Ware, 2004, Spitz, 2003). To avoid these side effects, compounds that have the ability to act in a dual inhibitory and stimulatory manner were synthesized. These complex compounds are known as selective progesterone receptor modulators (SPRMs) (reviewed in (Chwalisz *et al.*, 2005, Smith and O'Malley, 2004)). Since then, multiple agonists and antagonists have been identified for progesterone signaling. Second generation antagonists such as Proellex, Ulipristal, and Lonaprisan have been utilized in clinical trials for the treatment of leiomyoma, endometriosis, and breast cancer (reviewed in (Spitz, 2009)). Additionally, Asoprisnil, a second generation SPRM, exhibits both stimulatory and inhibitory functions that has proven successful in clinical trials for leiomyoma (Chwalisz *et al.*, 2004, Chwalisz *et al.*, 2007) and is promising for the treatment of endometriosis (reviewed in (Chwalisz *et al.*, 2005)). Although PRMs or SPRMs are engineered to interact with the ligand binding pocket, they exhibit no effect on the removal of chaperone proteins, the dimerization of PGR, or the binding of PGR to DNA (reviewed in (Chabbert-Buffet *et al.*, 2005)). Instead, these modulators affect the post-translational modification of the PGR and oversee the recruitment of certain coregulators, whether corepressor or coactivator (reviewed in (Smith and O'Malley, 2004)). The widespread benefit of these compounds can be applied for the efficient means of contraception, however these molecules have consistently proven to be beneficial in the treatment of women with endometrial disease. Increased understanding of how the existing PRMs and SPRMs function as well as the development of new compounds can aid in the progress and evolution of better treatment therapies for uterine disease. Further discussion regarding the treatment of uterine disease using PRMs will be briefly described in the latter part of this review.

Progesterone receptor response elements

Upon binding ligand, dimerizing, and entering into the nucleus, the nuclear receptor dimer binds to recognition sequences known as response elements. Nuclear receptor proteins have their own response elements, but at times, can cross-react with other response elements (reviewed in (Gronemeyer, 1991)). Although response elements for a particular nuclear receptor, such as the PGR have a specific sequence motif, there is room for flexibility within the sequence. The progesterone response elements or PREs usually consist of a palindromic hormone response element of AGAACAnnnTGTTCT (Ham *et al.*, 1988). However, PGR binding is not limited to the full PRE. Indeed, it was determined that PGR can bind to promoters of known progesterone target genes such as *Lifr*, *Gata2*, *Cyp26a1*, and *Ihh* with just half the sequence of the normal PRE (Rubel *et al.*, 2012). Additionally, it was identified that PGR can also bind promoters of known target genes *Egfr* and *Wnt7a* with no PRE present (Rubel *et al.*, 2012). Therefore, the rules governing the binding of the PGR at specific sites are ambiguous. Further work is required to identify other response elements in which PGR is able to successfully bind.

Non-genomic functions of the progesterone receptor

Canonical progesterone signaling requires the presence of nuclear localization and export sequences on the PGR protein for movement in and out of the nucleus (reviewed in (Mulac-Jericevic and Conneely, 2004)). Therefore, the PGR protein is able to readily participate in non-canonical activity within the cytoplasm. Utilizing human and monkey cells *in vitro*, both PGR isoforms were identified to uniquely interact with SH3 domains found on Src kinase-containing membrane receptors (Boonyaratanakornkit *et al.*, 2001). This unique ability of the PGR to interact with SH3 domains is due to the presence of a polyproline motif that lies upstream of the AF-1 domain. Upon treatment with progesterone, the PGR was identified to bind to the SH3 domain-containing protein, c-Src kinase. The interaction between PGR and c-Src kinase resulted in the efficient activation of MAPK. Therefore, addition of progesterone can result

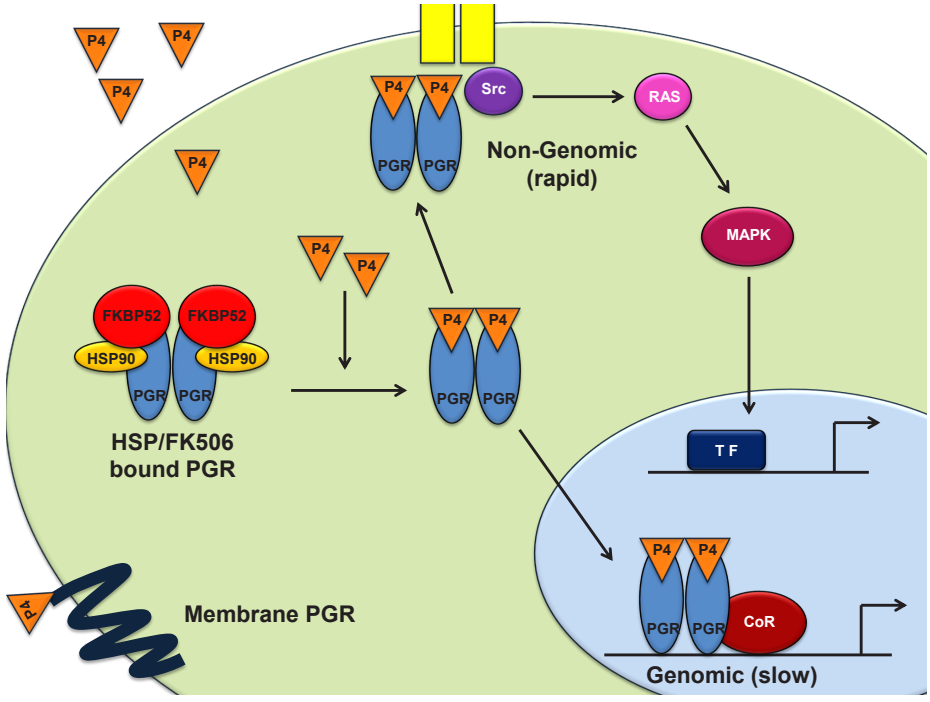

Fig. 2. Progesterone receptor signaling. *The PGR operates via multiple signaling pathways within the cell. Canonical progesterone signaling requires the presence of progesterone ligand and the release of the PGR by chaperone proteins. Upon release and binding of progesterone ligand, the dimerized PGR enters the nucleus and binds to response elements within promoter regions and governs the recruitment of transcription coregulators. This genomic signaling method is the slowest of the progesterone signaling mechanisms. The PGR can also function in a non-genomic context through the binding of SH3-domain containing proteins such as Src kinase on specific membrane receptors. The binding of the PGR to Src kinase elicits rapid activation of the RAS/RAF/MAPK pathway within the cell. Also, progesterone receptors integrated into the membrane were found to elicit rapid activation of cellular signaling pathways upon binding of extracellular ligand. Abbreviations: P4, progesterone; PGR, progesterone receptor; CoR, coregulator; TF, transcription factor; MAPK, mitogen activated kinase-like protein; HSP90, heat shock protein 90; FKBP52, FK506 binding protein 4.*

in the rapid promotion of Src kinase, RAS, and the MAPK pathway resulting in a pro-growth cellular signal, depicted in Fig. 2.

Besides the typical nuclear PGR, previous investigations have described the existence of progesterone receptors spanning the cellular membrane, known as membrane progesterone receptors (not to be confused with PGRMC1 and PGRMC2). There are three speculated membrane progesterone receptor isoforms: mPRα, mPRβ, and mPRγ (Zhu *et al.*, 2003a, Zhu *et al.*, 2003b). Although structurally different from nuclear PGR, these receptors share many commonalities with the GPCR family of transmembrane proteins. Interestingly, the mPRα and mPRβ forms were identified to bind to progestins and rapidly promote the MAPK pathway in mammalian breast cancer cells (Hanna *et al.*, 2006). Furthermore, when expressed in yeast, the membrane progesterone receptors successfully elicit a response upon treatment with progestins (Smith *et al.*, 2008). These membrane proteins are also thought to play a role in eliciting contraction in parturition (Karteris *et al.*, 2006). However, many of these studies have not been corroborated. Therefore, future work is required to confirm the existence, ligand binding ability, and functionality of these membrane progesterone receptors (further discussion found in (Gellersen *et al.*, 2009)).

Ligand independent functions of the progesterone receptor

Canonical progesterone signaling states that a dimerized receptor and ligand is necessary for active signaling. However, previous studies have shown that the PGR can function in elaborate ways independent of ligand. In the absence of ligand within human breast cancer cells, the PGR was shown to interact with a complex of proteins to collectively repress chromatin (Vicent *et al.*, 2013). Upon addition of ligand, the repressive complex breaks free of the chromatin, allowing for immediate transcription to take place. Also within human breast cancer cells, the PGR-B isoform was found to specifically promote cell migration in the absence of ligand (Bellance *et al.*, 2013). Surprisingly, upon the addition of progesterone, PGR-B ceased exhibiting a pro-migratory effect. The induction of migration resulted from the activation of focal adhesion kinase by the PGR-B isoform in the absence of ligand. Interestingly, the PGR-A isoform was shown to promote PGR-B's role in the induction of migration, yet the PGR-A isoform alone was unable to induce migration. Lastly, utilizing the established PRKO mouse model as a control (Lydon *et al.*, 1995), the PGR in the absence of ligand successfully promoted a lordosis response upon treatment with a dopamine agonist *in vivo* (Mani *et al.*, 1996). Therefore, the involvement of the PGR in the promotion of cell migration, repression of chromatin, and induction of lordosis in the absence of ligand are novel functions in global progesterone signaling and validate the PGR as a supervisor of many diverse signaling mechanisms.

Regulation of progesterone receptor activity

Prior to the presence of progesterone ligand in the extracellular space, the PGR protein resides within the cytoplasm. As the PGR waits for the ligand, it is bound by multiple proteins to preserve its activity, functionality, and rapid response (reviewed in (Mulac-Jericevic and Conneely, 2004)). These proteins consist of heat shock protein, HSP90, a p23 chaperone protein, and one of four chaperones containing a tetratricopeptide repeat (TPR) domain (reviewed in (Pratt and Toft, 1997)). One of these TPR-containing domain chaperone proteins is FKBP52, known to bind and promote the activity of the PGR (Barent *et al.*, 1998). The importance of FKBP52 function in the activity of the PGR was demonstrated via murine gene ablation techniques. A mouse model was designed to completely ablate the *Fkbp4* gene which encodes the FKBP52 protein utilizing gene targeting strategies (Cheung-Flynn *et al.*, 2005). Upon total gene ablation of *Fkbp4*, the female mice displayed complete infertility due to the inability for embryos to attach (Tranguch *et al.*, 2005). Further investigation at the time of implantation revealed that less progesterone ligand was bound by PGR and progesterone target genes were decreased. Furthermore, estrogen target genes were aberrantly upregulated due to impaired progesterone signaling. This suggested that these mice exhibited a dominance of estrogen signaling which prevented normal embryo implantation.

Later experiments were performed to ablate *Fkbp4* across two different mouse backgrounds, the C57BL/6J and CD1 (Tranguch *et al.*, 2007). Interestingly, upon treatment with progesterone, embryo implantation was rescued in mice from the CD1 background. Furthermore, mice from only the CD1 background were able to decidualize properly and carry pups to term when supplied with progesterone daily from day 2 of pregnancy until day 17. Therefore, despite the lack of FKBP52, the excessive administration of progesterone ligand was able to rescue the infertility of mice from the CD1 background. This confirms the important role of FKBP52 in the potentiation of PGR activity. This novel finding also suggests the importance of genetic background in the regulation of progesterone signaling via the modulation of FKBP52. The presence of genetic variation may shed more light on women suffering from infertility in the clinic.

To add to the complexity of progesterone signaling, the activity of the PGR protein is often regulated via post-translational modifications. Phosphorylation of the PGR protein has been extensively studied in human breast cancer cells. According to these studies, the PGR protein contains 7 confirmed phosphorylation sites (Zhang *et al.*, 1997, Zhang *et al.*, 1994, 1995). Of these 7 known serine phosphorylation sites, 3 are located exclusively in the PGR-B protein region (Zhang *et al.*, 1994, 1995). The remaining 4 sites are found in both PGR-A and PGR-B (Zhang *et al.*, 1997, Zhang *et al.*, 1995). Of the 7 total PGR phosphorylation sites, 4 of these sites are basally phosphorylated at all times, yet can be induced by the administration of hormone (Zhang *et al.*, 1997). The other 3 sites are exclusively induced by the presence of hormone. The presence of phosphorylated serines can modulate the activity of the PGR protein in a stimulatory or inhibitory manner. Interestingly, the presence of phosphorylation site Ser294, found within both PGR isoforms, was only phosphorylated in the PGR-B protein (Clemm *et al.*, 2000). This serine was found to reside within the PGR inhibitory domain. Therefore, it was concluded that the phosphorylated Ser294 on the PGR-B protein resulted in the inhibition of the inhibitory domain and active transcription ability of human PGR-B over PGR-A. In addition to phosphorylation, an acetylation site was identified within the hinge region of the PGR protein (Daniel *et al.*, 2010). The presence of this particular acetyl group was identified to regulate nuclear shuttling efficiency and phosphorylation rate, ultimately affecting the transcriptional response of the PGR. Additionally, depending on the promoter context, the PR-B isoform is negatively regulated

by SUMOylation resulting in decreased hormone sensitivity and a decrease in overall transcriptional activity (Abdel-Hafiz *et al.*, 2009). Therefore, post-translational modifications of the PGR are important for the regulation of transcription activity and can be induced upon addition of ligand. (A recent detailed discussion of the post translational modifications of the PGR can be found in the following: (Abdel-Hafiz and Horwitz, 2014)).

In conclusion, progesterone signaling is governed by the highly specialized PGR protein consisting of multiple domains, activated by ligand. The ligand-bound PGR protein functions to bind DNA and activate or inhibit the transcription of target genes. To add further specificity to progesterone signaling, at least two isoforms are transcribed from the *Pgr* gene, resulting in completely different transcriptional functions due to dimerization status, recruitment of specific coregulators, and an active inhibitory domain. Also, the PGR protein is able to bind to SH3 domains to rapidly activate signaling pathways irrespective of DNA binding. Furthermore, membrane-spanning versions of the progesterone receptor may exist and demonstrate completely different functions compared to their nuclear counterparts. Recent studies have described multiple ligand independent roles of PGR in the promotion of migration and repression of chromatin. A few of these many PGR mechanisms are graphically depicted in Fig. 2. Finally, PGR activity is regulated by many mechanisms including the binding of chaperone proteins within the cytoplasm and the addition of post-translational modifications.

Progesterone receptor function during early pregnancy

The murine uterus is composed of multiple compartments including the outer myometrium, made up of two muscle layers, the inner stroma containing the endometrial glands, and the inner luminal epithelium. Located within all major compartments of the endometrium, the PGR protein has continually demonstrated to be essential for pregnancy. Without functional progesterone signaling, pregnancy is unable to progress, as was demonstrated in the PRKO mouse which exhibited infertility due to defects in mating behavior, ovulation, and decidualization (Lydon *et al.*, 1995). Through the utilization of genetically engineered mouse models, the PGR has proven itself as a major transcriptional regulator of genes involved in uterine function. Indeed, progesterone signaling is known to regulate pathways involved in postnatal uterine development, implantation, decidualization, and parturition. This portion of the review will provide a brief overview of progesterone regulated pathways during early implantation.

The steroid hormones, estrogen and progesterone, and their cognate receptors work in concert to regulate early pregnancy. Progesterone signaling functions in an inhibitory manner to the estrogen signaling pathway (Hsueh *et al.*, 1975). This inhibitory relationship between the two hormone pathways provides an important regulatory pattern necessary for early implantation. Early ovulatory secretion results in high estrogen levels at day 1 and 2 of pregnancy (reviewed in (Cha *et al.*, 2012)). As the corpora lutea is maintained within the ovary, progesterone is secreted, resulting in the inhibition of estrogen target genes within the uterus, such as mucin 1 (*Muc1*). MUC1 is a glycosylated protein that lines the uterine epithelium and functions as a mucinous barrier to bacteria, pathogens, and other foreign substances (reviewed in (Carson *et al.*, 2000)). With the release of the egg and fertilization of the embryo within the oviduct, this mucinous layer must be removed to allow for the embryo to come into contact with the epithelium. Activated by estrogen early in pregnancy, MUC1 is soon downregulated following the spike in epithelial PGR levels by day 3 of pregnancy, allowing for proper attachment and invasion of the epithelium by the embryo. Although most epithelial estrogen target genes are suppressed at days 2-3 due to high levels of progesterone, the epithelial PGR decreases in expression in the epithelium by day 4, initiating the start of the “window of receptivity” and permitting the nidatory estrogen surge. This spike in estrogen secretion promotes the production of leukemia inhibitory factor (LIF) within the endometrial glands and results in the implantation of the embryo via the LIF-STAT3 signaling pathway (reviewed in (Cha *et al.*, 2012)). The progression from estrogen-induced to progesterone-induced genes within early implantation is displayed graphically in Fig. 3.

At the time of implantation, many factors within the epithelium and stroma are coordinated to receive the implanting embryo. These factors, many of which are progesterone target genes, contribute to proper implantation in the uterine epithelium and the resultant decidual response in the surrounding stroma. Genes known to be induced by progesterone, *Hoxa10* and *Hoxa11* were identified to be critical for pregnancy utilizing murine ablation models. Upon ablation of *Hoxa10*, female mice exhibited infertility due to maternal defects in embryo implantation and decidualization (Benson *et al.*, 1996). Upon further investigation, it was identified that prostaglandin receptors Ep3 and Ep4 were downregulated in *Hoxa10* null mice (Lim *et al.*, 1999). Additionally, cyclooxygenase 2 or COX2 expression was impaired in these mice during late post-implantation. COX2 is the rate-limiting step enzyme in the synthesis of prostaglandins (reviewed in (Smith *et al.*, 1996)). These uterine defects and decreased COX2 expression suggest the presence of a dysfunctional prostaglandin pathway leading to the impairment of implantation.

Hoxa11 null mice displayed similar defects in embryo implantation compared to their *Hoxa10* counterparts. HOXA11 is normally expressed in the underlying stroma of the blastocyst attachment site (Gendron *et al.*, 1997). Upon complete ablation of this gene, mice exhibited decreased uterine size, a decreased number of glands, and a defective decidual response. The *Hoxa11* null mice also demonstrated decreased LIF at the time of implantation which was attributed to the reduced number of endometrial glands. Both HOXA10 and HOXA11 have shown to be critically important for early embryo apposition and initiation of the stromal decidual response. It is not a surprise that these HOX genes were found expressed within human endometrium during implantation (Taylor *et al.*, 1998, Taylor *et al.*, 1999).

Decidualization consists of the initial proliferation of stromal cells and differentiation into decidual cells to support the growth of the embryo. This process is controlled by critical target genes downstream of the PGR. Before the time of embryo implantation, epithelial PGR activates the expression of multiple genes in the epithelial compartment allowing for embryo attachment to take place (Franco *et al.*, 2012). One of these critical epithelial targets is indian hedgehog (*Ihh*). Known to be indispensable for embryo attachment (Lee *et al.*, 2006), IHH is first expressed in the epithelium and then signals downstream within the uterine stroma to promote decidualization. One particular stromal gene activated downstream of IHH is the nuclear receptor subfamily 2, group F, member 2 (*Nr2f2*) (also known as COUP-TFII) (Lee *et al.*, 2006). Much of the

information obtained regarding the signaling mechanisms leading up to embryo implantation and stromal decidualization is due to the efficient utilization of genetically engineered mouse models. A total knockout mouse model of *Nr2f2* was generated and the mouse exhibited perinatal lethality due to the necessary role of NR2F2 in cardiac development (Pereira *et al.*, 1999). However, heterozygote knockout mice suggested that NR2F2 may play an important role in the uterus (Takamoto *et al.*, 2005). Upon expression analysis, NR2F2 was observed to be specifically expressed within the uterine stroma and was therefore hypothesized to be critical for decidualization. A conditional knockout allele was soon generated and upon mating to the Pgr^{cre} recombinase mouse (Soyal *et al.*, 2005), *Nr2f2* was efficiently ablated in the uterus (Kurihara *et al.*, 2007). Despite its stromal-specific expression, conditional ablation of *Nr2f2* not only resulted in defects in stromal decidualization, but also in embryo attachment. NR2F2 was identified to regulate stromal decidualization through the activation of critical decidual target genes, *Wnt4* and *Bmp2*. Interestingly, NR2F2 was determined to signal back to the epithelium to inhibit estrogen signaling before the time of embryo implantation (graphically displayed in Fig.3). Therefore, NR2F2 acts as a conduit from epithelial IHH to not only elicit the activation of decidual genes, but also feedback on the epithelial compartment to contribute to the induction of uterine receptivity.

To add further complexity to the regulation of implantation via compartmental cross-talk, a stromal progesterone target, heart and neural crest derivatives expressed 2 or HAND2 was identified to be necessary for embryo implantation (Li *et al.*, 2011). Through the generation of a uterine specific knockout mouse for *Hand2*, it was determined that *Hand2* ablation resulted in infertility with defects in embryo attachment. Upon further investigation, HAND2 was found to inhibit FGF ligands within the stroma which feedback on the epithelium to promote estrogen signaling. The FGFs signal through the MAPK pathway to promote epithelial estrogen signaling resulting in increased expression of MUC1 and induction of epithelial proliferation (depicted in Fig. 3). Therefore, although expressed in the stroma, HAND2 acts as an inhibitor of epithelial estrogen signaling allowing for the preparation of the uterine epithelium for the reception of the embryo.

Within the uterine stroma, NR2F2 promotes the expression of BMP2 in preparation for the start of decidualization. Upon conditional ablation of *Bmp2* in the uterus, the mice exhibited infertility with defects in decidualization (Lee *et al.*, 2007). Interestingly, it was demonstrated that these mice undergo normal embryo attachment, yet exhibit major defects in decidualization. Although proliferation appeared normal within these mice at the onset of decidualization, by 48 hours after the decidual stimulus the stroma exhibited minimal proliferation suggesting that BMP2 is important for the secondary round of proliferation within the decidual response. Furthermore, upon staining with alkaline phosphatase, the uterine stroma displayed minimal levels of differentiation. However, this stromal cell differentiation defect was partially rescued upon intraluminal uterine injection of recombinant BMP2 at the onset of decidualization. This rescue experiment confirmed the important role of BMP2 in the induction of decidual differentiation. Upon performing microarray analysis of this mouse model, it was determined that BMP2 regulates non-canonical WNT ligands, WNT4 and WNT6 at the time of decidualization. Furthermore, BMP2 was also observed to regulate FKBP proteins which function to modulate the activity of the PGR. Therefore, BMP2 signaling is necessary for decidualization through the promotion of non-canonical WNT signaling and the regulation of PGR activity.

Canonical WNT signaling consists of WNT ligands binding to a FRIZZLED membrane receptor, resulting in the sequestration of molecules known to bind and inhibit β-CATENIN (reviewed in (Angers and Moon, 2009)). Due to the sequestration of β-CATENIN inhibitors, β-CATENIN is free to enter the nucleus and promote the activation of transcription factors within the LEF/TCF family. Non-canonical WNT ligands operate via different membrane receptors to elicit cellular mechanisms independent of β-CATENIN (reviewed in (Angers and Moon, 2009)). The method of binding to extracellular

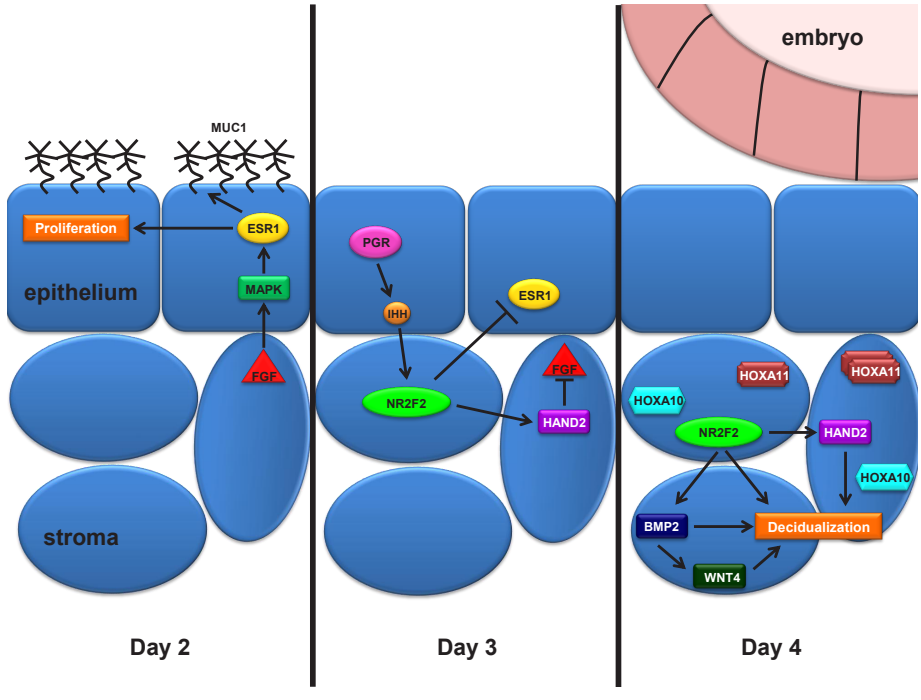

Fig. 3. Progesterone receptor signaling through the implantation window. *On day 2 of pregnancy, MUC1 coats the lining of the proliferative uterine epithelium due to active estrogen signaling. By day 3, the PGR is expressed at high levels within the uterine epithelium activating downstream stromal target NR2F2 through the indian hedgehog ligand (IHH). NR2F2 promotes the induction of HAND2 and inhibits estrogen signaling within the epithelium leading to decreased expression of MUC1 and reduced epithelial proliferation. At the time of implantation on day 4 of pregnancy, NR2F2 activates BMP2 in the preparation of the stroma for decidualization. HOXA11 accumulates in the stroma near the sites of embryo attachment. HOXA10 also increases in the stroma at the start of decidualization. Abbreviations: PGR, progesterone receptor; ESR1, estrogen receptor; FGF, fibroblast growth factor; NR2F2, nuclear receptor subfamily 2, group F, member 2; HAND2, heart and nerual crest derivatives expressed transcript 2; BMP2, bone morphogenetic protein 2; WNT4, wingless-related MMTV integration site 4; MAPK, mitogen activated kinase-like protein; HOXA10, homeobox A10; HOXA11, homeobox A11.*

membrane proteins allows WNT ligands to activate coordinated signaling patterns within large groups of cells in an organ. Indeed, WNT ligands are able to function via crosstalk between the epithelial and stromal compartments of the uterus. Activated downstream of NR2F2 and BMP2 within the uterine stroma, WNT4 was determined to be a critical factor for post implantation uterine function. Upon conditional ablation of *Wnt4* within the mouse uterus, the mice exhibited a failure of embryo attachment and the absence of a decidual response (Franco *et al.*, 2011). Initially, these defects were thought to arise from the absence of glands which are critical for secreting the growth factor, LIF. However, intraluminal uterine injection of recombinant LIF failed to rescue decidualization within the *Wnt4* null mice. Therefore, WNT4 itself is necessary for decidualization and embryo implantation.

Targeting the progesterone receptor for the treatment of uterine disease

This review has examined PGR structure, function, and regulation as a nuclear receptor and briefly described its necessary role in the initiation and continuance of pregnancy. The remainder of this review will switch gears to focus on the role of progesterone signaling in endometrial disease. Expanding our knowledge of basic progesterone signaling within the uterus is important, yet this knowledge generates lasting impact when it is applied to the development of new therapies to help treat women suffering from endometrial disease. Women today suffer from a variety of reproductive tract-associated diseases. This review will describe the most prevalent uterine diseases, specifically endometrial cancer, endometriosis, and leiomyoma.

Endometrial cancer is a detrimental and at times deadly uterine disease with an expected 46,000 new cases and about 8,000 deaths in 2011 (Siegel *et al.*, 2011). Although the most common endometrial cancer is adenocarcinoma, endometrial sarcoma can occur. Due to its prevalence, much knowledge has been gained in the field of endometrial adenocarcinoma. Cases of adenocarcinoma can be divided into two groups (Bokhman, 1983). The first group consists of an estrogen responsive tumor, thought to arise from treatment with unopposed estrogens. The second group is able to grow independently of estrogen while exhibiting poor differentiation (reviewed in (Sherman, 2000)). Women presenting with adenocarcinoma from the second group often fail to respond to hormone therapy and are usually given a poor prognosis. The positive expression of the steroid hormone receptors usually indicates the high differentiation state of the tumor and the ability to effectively respond to hormone treatment. Indeed, high expression of the PGR correlated with low tumor grade and low recurrence rate, while expression of ESR1 also correlated with low recurrence rate (Ehrlich *et al.*, 1988). More recent studies have corroborated this data, reporting that the expression of the PGR isoforms inversely correlates with tumor grade (Arnett-Mansfield *et al.*, 2001). Furthermore, although normal human endometrium expresses both PGR isoforms (Mote *et al.*, 1999), loss of expression of one isoform is considered an early event in the development of endometrial carcinoma (Arnett-Mansfield *et al.*, 2001). Interestingly, studies have provided conflicting evidence suggesting the role of the PGR-B isoform in the exacerbation of endometrial cancer. Expression of the PGR-B isoform was suggested to positively correlate with tumor grade in endometrial, ovarian, and cervical cancers (Fujimoto *et al.*, 1995), yet loss of PGR-B was also identified in poorly differentiated endometrial cancers (Kumar *et al.*, 1998, Saito *et al.*, 2006, Sakaguchi *et al.*, 2004). Therefore, the role of the PGR-B isoform in promoting endometrial cancer is controversial.

Typical treatment for women suffering from endometrial cancer involves a hysterectomy and bilateral salpingo-oophorectomy. However, pre-menopausal women often opt out of this treatment plan in order to maintain their fertility (discussed in (Gunderson *et al.*, 2012)). Therefore, the development and utilization of PRMs or SPRMs is necessary in the treatment of this disease. Clinical trials utilizing progesterone agonists also known as progestins, which function to inhibit the extensive proliferation of the epithelium, have exhibited relative success in younger women with high differentiated endometrial carcinoma, but can result in recurrence in a minimal number of women (Kaku *et al.*, 2001, Ramirez *et al.*, 2004, Thigpen *et al.*, 1999, Ushijima *et al.*, 2007). However, combination treatment of a progesterone agonist with tamoxifen may prove to be a more effective treatment for endometrial cancer (Whitney *et al.*, 2004). Although mifepristone has been tested in the treatment of PGR-positive endometrial adenocarcinoma and sarcoma (Ramondetta *et al.*, 2009), future work is needed to corroborate its efficacy. Therefore, the expression of the PGR isoforms and the progesterone signaling status directly affects the tumor grade and severity of disease and can also govern the possible treatment options available to these women suffering from endometrial cancer (discussed further in (Kim *et al.*, 2013)).

Endometriosis is defined as the presence of endometrial tissue outside of the endometrium, occurring in about 10% of women in the nation today (reviewed in (Giudice and Kao, 2004, Kim *et al.*, 2013)). Endometriosis can occur in different locations throughout the body, but is highly concentrated in the surrounding peritoneal cavity and the ovaries. It is commonly thought that endometriosis initiates upon the exit of endometrial tissue from the fallopian tubes into the peritoneal cavity via retrograde flow of menstrual fluid (discussed in (Giudice and Kao, 2004)). Once the tissue exits the uterus, it attaches and grows on tissues within the peritoneum. Although endometriosis is thought to initiate via retrograde menstrual flow, most women are known to retrograde flow under normal conditions. Therefore, the pathological initiation of endometriosis is still under debate today.

Women with endometriosis experience chronic pain and bleeding which is directly correlated to ovulatory cycling. Since the symptoms and persistence of endometriosis are dependent on the production of the ovarian steroid hormones, anti-hormone therapies such as anti-progestins or inhibitors of estrogen signaling can be utilized to minimize pain and growth of the endometriotic lesions (reviewed in (Olive and Pritts, 2001)). Effective treatment involves the removal of the lesions and inhibition of ovulation or possible oophorectomy. Interestingly, the role of progesterone in the treatment of endometriosis is not clearly defined. Although treatment with mifepristone prevents endometriotic growth (Kettel *et al.*, 1996), clinical trials have identified progestins as an effective pain reducer in women suffering from endometriosis (Vercellini *et al.*, 1997). However, large amounts of progesterone are thought to be secreted by ectopic endometrial lesions (Sun *et al.*, 2003). Therefore, the role of progesterone in the progression of endometriosis is complicated and requires further investigation to determine safe treatment options for women suffering from this detrimental disease.

Leiomyoma or uterine fibroids are the most common endometrial disease affecting about 80% of women with occurrence increasing with age (Cramer and Patel, 1990, Parker, 2007). Uterine fibroids are benign tumors arising from the myometrium resulting in pain and uterine bleeding. Due to the general discomfort of this disease, many women opt for surgery to remove the fibroids. In fact, an average of 40% of all hysterectomies are performed with the intent to remove uterine fibroids (Wilcox *et al.*, 1994). The effect of hormones on the growth of leiomyoma has been controversial. Although estrogen is generally known to potentiate proliferation of uterine tissue, while progesterone often inhibits abnormal growth, it is unclear whether this dogma holds true for leiomyoma (discussed in (Kim *et al.*, 2013)). Treatment with estrogen plus progesterone was found to increase proliferation of leiomyoma over treatment with estrogen alone (Ishikawa *et al.*, 2010, Lamminen *et al.*, 1992). Progesterone treatment alone was identified to both enhance (Hoekstra *et al.*, 2009) and inhibit (Yamada *et al.*, 2004) leiomyoma growth on different occasions *in vitro*. However, multiple clinical trials have proved the efficacy of PGR antagonists such as mifepristone in the decrease of fibroid size and reduction of symptoms (Carbonell Esteve *et al.*, 2008, Fiscella *et al.*, 2006). Furthermore, the SPRM, Asoprisnil, was effective in the reduction of fibroid size, uterine bleeding, and abdominal pain (Chwalisz *et al.*, 2007). Although the effect of hormone therapy on the growth of leiomyoma is complicated and warrants further investigation, the use of PRMs or SPRMs is a promising option for women suffering from leiomyoma.

Conclusion

The PGR functions via a dynamic and highly regulated system of interconnected pathways to govern the success of a pregnancy and the overall health of the endometrium. As a nuclear receptor, the PGR is unique in its ability to bind both ligand and DNA to promote the transcription of target genes at the appropriate time. Multiple isoforms are transcribed from the *Pgr* locus and display unique transcriptional abilities depending on the species. The individual PGR isoforms also exhibit the distinct ability to recruit specific coregulators to sites of transcription. Furthermore, the PGR is able to function rapidly in a non-genomic fashion to promote the MAPK pathway. Additionally, new evidence suggests the presence of distinct membrane progesterone receptors on the cellular surface. Previous studies have also identified unique ligand-independent functions of the PGR. Without ligand, the PGR was identified to increase migration through focal adhesion kinase signaling and successfully repress chromatin through binding to a repressive protein complex. The function and activity of the PGR is regulated by bound chaperone proteins within the cytoplasm that release the PGR only in the presence of progesterone ligand. Furthermore, the PGR isoforms exhibit seven experimentally verified phosphorylation sites which affect the transcriptional ability and recruitment of coregulators to the promoters of genes. This functional diversity and intricate regulation of the PGR provides evidence to its chief role in female reproduction.

The progesterone signaling pathway governs uterine function and fertility via epithelial to stromal crosstalk to initiate embryo implantation and decidualization at the appropriate time. Through the induction of hedgehog signaling via the epithelial PGR, NR2F2 is activated, resulting in the preparation of the stroma for the decidual response and promotion of embryo implantation. At attachment, the HOX proteins promote apposition and implantation of the embryo, allowing for pregnancy to progress. Downstream of NR2F2, BMP2 promotes the differentiation and proliferation of the uterine stromal cells necessary for decidualization and also activates non-canonical WNT ligands to promote stromal cell proliferation. Through these complex mechanisms occurring within both the epithelial and stromal compartment, the PGR tightly regulates the progression of pregnancy.

This knowledge of global progesterone signaling within the uterus is critical for understanding how the PGR functions within endometrial disease. The expression and responsiveness of the PGR within diseased endometrial tissue frequently indicates the prescribed treatment options for the individual patient. An increased understanding of the PGR under normal and diseased states will benefit the development of improved therapies to treat these devastating uterine diseases. Additionally, further identification and utilization of PRMs such as mifepristone and SPRMs such as Asoprisnil will be instrumental in the successful treatment of reproductive-associated disease.

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