Role of nuclear progesterone receptor isoforms in uterine pathophysiology

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TABLE OF CONTENTS

- Introduction
- The human progesterone receptor
- Progesterone receptors in normal uterine function
- Progesterone receptors in uterine pathophysiology
  - Endometriosis
  - Leiomyoma
  - Endometrial cancer
  - Cervical cancer
  - Recurrent pregnancy loss
- Conclusions

BACKGROUND: Progesterone is a key hormonal regulator of the female reproductive system. It plays a major role to prepare the uterus for implantation and in the establishment and maintenance of pregnancy. Actions of progesterone on the uterine tissues (endometrium, myometrium and cervix) are mediated by the combined effects of two progesterone receptor (PR) isoforms, designated PR-A and PR-B. Both receptors function primarily as ligand-activated transcription factors. Progesterone action on the uterine tissues is qualitatively and quantitatively determined by the relative levels and transcriptional activities of PR-A and PR-B. The transcriptional activity of the PR isoforms is affected by specific transcriptional coregulators and by PR post-translational modifications that affect gene promoter targeting. In this context, appropriate temporal and cell-specific expression and function of PR-A and PR-B are critical for normal uterine function.

METHODS: Relevant studies describing the role of PRs in uterine physiology and pathology (endometriosis, uterine leiomyoma, endometrial cancer, cervical cancer and recurrent pregnancy loss) were comprehensively searched using PubMed, Cochrane Library, Web of Science, and Google Scholar and critically reviewed.

RESULTS: Progesterone, acting through PR-A and PR-B, regulates the development and function of the endometrium and induces changes in cells essential for implantation and the establishment and maintenance of pregnancy. During pregnancy, progesterone via the PRs promotes myometrial relaxation and cervical closure. Withdrawal of PR-mediated progesterone signaling triggers menstruation and parturition. PR-mediated progesterone signaling is anti-mitogenic in endometrial epithelial cells, and as such, mitigates the toxic effects of estrogen on eutopic normal endometrium, and on ectopic implants in endometriosis. Similarly, ligand-activated PRs function as tumor suppressors in endometrial cancer cells through inhibition of key cellular signaling pathways required for growth. In contrast, progesterone via PR activation appears to increase leiomyoma growth. The exact role of PRs in cervical cancer is unclear. PRs regulate implantation and therefore aberrant PR function may be implicated in recurrent pregnancy loss (RPL). PRs likely regulate key immunogenic factors involved in RPL. However, the exact role of PRs in the pathophysiology of RPL and the use of progesterone for therapeutic benefit remains uncertain.

CONCLUSIONS: PRs are key mediators of progesterone action in uterine tissues and are essential for normal uterine function. Aberrant PR function (due to abnormal expression and/or function) is a major cause of uterine pathophysiology. Further investigation of the underlying
mechanisms of PR isoform action in the uterus is required, as this knowledge will afford the opportunity to create progestin/PR-based therapeutics to treat various uterine pathologies.

**Key words:** progesterone / progesterone receptors / uterine pathophysiology

### Introduction

Progesterone is an essential hormone in the female reproductive system. In conjunction with estrogen (mainly in the form of estradiol), it controls uterine function to facilitate reproduction. The central role of these hormones is reflected in the fact that progesterone/estrogen therapy alone is sufficient to produce a receptive uterus capable of supporting a viable pregnancy in post-menopausal women receiving donor embryo transfer. The principal uterine targets for progesterone are stromal and epithelial cells in the endometrium, smooth muscle cells in myometrium, and stromal fibroblasts and glandular epithelial cells in the cervix. Effects of progesterone in these cells are mediated by its interaction with specific progesterone receptors (PRs), and its pleiotropic actions are due to cell type-specific variations in PR signaling. The etiology of uterine pathologies, including endometriosis, leiomyoma, endometrial cancer, cervical cancer and recurrent pregnancy loss has been associated with aberrant PR signaling. Consequently, therapies targeted to correct problems with PR-mediated progesterone signaling have promise for the treatment of multiple uterine disorders. The goal of this review is to synthesize the current understanding of how PRs mediate progesterone actions in the endometrium, myometrium and cervix to facilitate normal uterine function, and how PR dysregulation may contribute to uterine pathophysiology.

### The human progesterone receptor

Effects of progesterone on target cells are mediated by cellular PRs whose function is altered by progesterone binding. To date two groups of PRs have been identified: (i) the nuclear PRs that function as ligand-activated transcription factors and mediate genomic actions (i.e. affect gene expression) (Evans, 1988) and (ii) a family of PRs that reside at the cell surface and are structurally related to G-protein ligand-activated transcription factors and mediate non-genomic actions of progesterone (Gerdes et al., 1998; O’Brien et al., 1998; Saner et al., 2003; Welter et al., 2003; Zhu et al., 2003a,b; Price et al., 2005; Younlai et al., 2006; Behera et al., 2009; Lee et al., 2010). Actions of progesterone in the female reproductive system are thought to be primarily mediated by the nuclear PRs. The physiologic relevance of the membrane PR is unclear since their capacity to bind progesterone is relatively low, compared with the nuclear PRs, and some studies suggest that they are not activated by progesterone (Krietsch et al., 2006). In light of this controversy, the following discussion focuses on the role of nuclear PRs in uterine pathophysiology.

The human nuclear PRs are encoded by a single gene (PGR) located on chromosome 11 (11q22-q23). Expression of PGR is controlled by two promoters to produce two major mRNA transcripts that encode two proteins: the full-length PR-B (116 kDa) controlled by the distal PR-B promoter region and initiated from the first AUG (492 bases upstream) translational start codon (Kastner et al., 1990; Sartorius et al., 1994; Wen et al., 1994; Leonhardt et al., 2003) (Fig. 1). Other PR isoforms are thought to be generated by the initiation of translation from further downstream AUG start sites (e.g. PR-C), exon splicing and exon insertions (Fig. 1) (reviewed by Hirata et al., 2003; Cork et al., 2008); but their physiologic relevance is uncertain, and for some of these variants, especially the putative PR-C, their production in vivo is unclear since the natural AUG start sites lacks an upstream Kosak sequence needed for translation initiation (Samalecos and Gellersen, 2008). The following discussion will therefore be limited to PR-A and PR-B.

PR-A and PR-B belong to a family of ligand-activated transcription factors and share common structural and functional elements (i.e. regulatory region, DNA binding domain, hinge region and ligand binding domain) with other steroid hormone receptors (Fig. 1) (Evans, 1988; Mangelsdorf et al., 1995; Escriva et al., 2004; McEwan, 2009). The DNA binding domain, hinge region and ligand binding domain are identical in PR-A and PR-B with the difference between the two PRs being in the N-terminal regulatory domain that is truncated by 164 amino acids in PR-A. Differences in the activities of PR-A and PR-B are thought to be conferred by the N-terminal segment (B-upstream segment) unique to PR-B.

Effects of progesterone are generally considered to represent the combined activities of PR-A and PR-B. Upon ligand binding, PR-A and PR-B affect cellular function by altering gene expression via two modes of action: (i) the direct genomic mode, whereby the PRs function as ligand-activated transcription factors to directly interact with specific DNA promoter/enhancer elements and transcriptional co-regulators to modulate the expression of downstream genes; and (ii) the indirect extranuclear mode whereby the PRs interact with Src tyrosine kinases in the cytoplasm to activate mitogen-activated protein kinases (MAPKs) which then affect gene expression (Boonyaratankomkit et al., 2001; Leonhardt et al., 2003; Boonyaratankomkit and Edwards, 2007) (Fig. 2).

PR-A and PR-B are co-expressed in varying relative amounts depending on the cell type and pathophysiologic condition. Studies to assess the abundance and cellular localization of PR-A and PR-B are complicated by technical limitations due to the common sequence of PR-A and PR-B. Consequently, antibodies specific for PR-A are not available, and PR-A mRNA abundance cannot be directly measured by quantitative RT–PCR-based techniques. The abundance of PR-A and its level relative to PR-B can only be determined by immunoblotting, which is semi-quantitative at best. In vitro approaches, however, using various cell types genetically modified to express PR-A and/or PR-B in conjunction with PR-reporter systems, have revealed key functions of PR-A and PR-B, and how they interact to affect transcription in specific cell types. In addition, significant progress in understanding PR function has been gained from studies of mice genetically modified to abolish the PR-A and PR-B isoforms together or individually (see below).

Initial studies of PR transcriptional activity were performed using artificial reporter genes controlled by canonical progesterone responsive
elements (PREs). In that assay, PR-B is a strong transactivator in response to progesterone, whereas PR-A is less active and in most cases inhibits the transcriptionally active PR-B, especially when its level exceeds that of PR-B (i.e. PR-A:PR-B ratio > 1) (Tung et al., 1993; Giangrande et al., 1997, 2000; Pieber et al., 2001; Richer et al., 2002; Condon et al., 2006; Merlino et al., 2007). Those observations led to the concept that PR-A and PR-B have opposing transcriptional activity and that as such net progesterone responsiveness is inversely related to the PR-A:PR-B ratio. Such a mechanism permits control of progesterone responsiveness by the target cell via modulation of isoform levels. Recent studies, however, using global gene expression analyses, show that both isoforms are transcriptionally active at diverse sets of endogenous promoters, most of which lack a canonical PRE. Importantly, the repressive activity of PR-A was found to be minimal at endogenous genes.

Figure 1  Structure of the human PR isoforms produced from the PGR gene. The major mRNA transcripts are derived from translational start sites controlled by the PR-B (distal) and PR-A (proximal) promoters. The major proteins products (boxed) are the full-length PR-B produced from PR-B mRNA and initiated from the first AUG, and the PR-A which is produced from PR-A mRNA and initiated from the second AUG. The receptors contain functional domains that are typical of the nuclear receptors family. The structures of other putative splice variants are shown below the boxed area.
including serine phosphorylation, ubiquitination and sumoylation of the PRs that affects their stability, trafficking, transcriptional activity and target gene selectivity (Abdel-Hafiz and Horwitz, 2012, 2014; Knutson et al., 2012) (Fig. 4). Regulation of numerous coregulators (coactivators and corepressors) is pivotal for modulation of PR-mediated gene transcription leading to activation or repression of specific target genes (Xu and Li, 2003). For example, the p160 steroid receptor coactivator-1 (SRC-1) is a transcriptional coactivator for PR, which interacts with liganded-PR and serves to recruit histone acetyl transferases and methyltransferases to specific gene promoter regions to facilitate transcription by altering chromatin structure (McKenna et al., 1999). These factors account for most of the cell- and context-specific pleitropic actions of progesterone.

PGR expression by uterine cells is stimulated by estrogens via estrogen receptor-α (ERα) and consequently progesterone responsiveness is dependent on the presence of an estrogenic drive (Tsai et al., 1998). The effect is especially relevant in the endometrium where estrogen exposure during the follicular phase promotes PR expression in endometrial cells to augment progesterone responsiveness during the luteal phase. In fact, low levels of estrogen are required for progesterone responsiveness throughout the luteal phase (Janne et al., 1975; Kreitmann et al., 1979; Katzenellenbogen et al., 1980; Bergeron et al., 1988; Lessey et al., 1988; Press et al., 1988; Okulicz et al., 1989; Savouret et al., 1990; Fung et al., 1994; Ingamells et al., 1996; Moutsatsou and Sekeris, 1997). Conversely, ERα expression in uterine cells is inhibited by progesterone via PRs (Haluska et al., 1990). This functional feedback interaction between the progesterone and estrogen hormonal systems is crucial for normal uterine function and for balancing the often-opposing actions of the progesterone/PR and estrogen/ER systems. Steady state levels of PR are decreased by progesterone through ligand-induced phosphorylation of the PR, which increases transcriptional activity but also induced PR ubiquitination that targets the protein for degradation through the proteosome (Lange et al., 2000; Shen et al., 2001; Abdel-Hafiz and Horwitz, 2014). Similar to ligand-dependent PR down-regulation through the ubiquitin-proteosome pathway, the presence of ligand also contributes to down-regulation of specific PR coregulators, including SRC-1 (Amazit et al., 2011). Thus, in the presence of ligand, levels of PR (especially PR-B) are inversely correlated with transcriptional activity.

Other PR-independent mechanisms could also operate to facilitate target cell control of progesterone responsiveness. One important mechanism is via the intracrine mode of hormonal control whereby a hormone is metabolized to a more or less active form within the target cell prior to its interaction with a cognate receptor. In the case of progesterone, target cells may express enzymes such as 5α-reductase and 20α-hydroxysteroid dehydrogenase that metabolize progesterone to less active forms prior to its interaction with intracellular receptors. This PR-independent regulatory mode may be critical for controlling progesterone actions in the cervix during pregnancy and especially at parturition (Mahendroo et al., 1999). Intracrine mechanisms also may control progesterone actions on the brain (Mellon, 2007).
Progesterone receptors in normal uterine function

Effects of progesterone on uterine physiology occur during the post-ovulatory luteal/secretory phase of the menstrual cycle and during pregnancy; i.e. when circulating levels of progesterone are sufficient to activate PRs in uterine cells. Progesterone affects cells in each of the functional tissue types of the uterus, and PR-A and PR-B are detectable in epithelial and stromal/decidual cells in the endometrium (Mote et al., 1999), smooth muscle cells in the myometrium (Fig. 5) (Mesiano, 2007), and stromal fibroblasts in the cervix (Cowan et al., 2004). The overall scope of progesterone action in the uterus has been delineated through studies utilizing PR antagonists such as mifepristone (RU486) for emergency contraception. While administration of high doses of RU486 in the mid- or late follicular phase delays the luteinizing hormone (LH) surge and inhibits ovulation, low doses can cause infertility by delaying endometrial maturation (Spitz et al., 1994; Gemzell-Danielsson and Marions, 2004; Lakha et al., 2007). Similarly, if administered during pregnancy, RU486 induces abortion, fetal loss or parturition depending on the gestational age (Chwalisz, 1994; Wildschut et al., 2011; Shaw et al., 2013). Thus, PR-mediated progesterone actions that are disrupted by RU486 are essential for normal uterine function.

The endometrium is a remarkably dynamic uterine tissue that exhibits dramatic steroid hormone dependent changes in morphology and function during the menstrual cycle and during pregnancy. It is composed of stromal and epithelial cells arranged into two morphologically and functionally distinct zones: the inner basalis, made up mainly of stromal cells, and the outer functionalis, which contains stromal and epithelial cells. Progesterone exerts specific effects on endometrial epithelial and stromal cells. During the pre-ovulatory follicular phase, the functionalis thickens in response to estrogens (mainly estradiol) by epithelial and stromal cell proliferation. Cell proliferation is then inhibited by progesterone during the post-ovulatory luteal phase and a morphologic and functional change occurs to establish a glandular secretory endometrial epithelium and a vascular stroma conducive for blastocyst implantation and the establishment of pregnancy. In endometrial stromal cells, progesterone increases proliferation during the follicular phase and during the peri-implantation phase via activation of the ERK/AKT pathway.

Figure 3  Functional interaction between PR-A and PR-B. Upon ligand binding, the receptors form transcriptionally active homo- and hetero-dimers that affect the expression of specific and common gene sets. PR-A also acts as a trans-repressor of PR-B at some promoters.
Figure 4 (A) Sites for known serine phosphorylation and ubiquitination/sumoylation in PR-A and PR-B that are induced in response to ligand binding and/or in response to growth factors and other kinases. (B) It is proposed that post-translational modifications (PTMs) affect the targeting of ligand-activated PRs to specific gene promoter classes. This mechanism explains how the PRs mediate pleiotropic actions of progesterone in different cell types and different physiologic conditions.

Figure 5 Immunohistochemical localization of PR-A and PR-B at the human myometrial/endometrial interface during the secretory phase of the menstrual cycle. Total PR (PR-A and PR-B) was detected with antibody PgR1294 (Dako Corp) that reacts with an epitope in the N-terminal region common to both PR-A and PR-B. PR-B was detected with antibody C1A2 (Cell Signaling, Inc.) that reacts with an epitope in the N-terminus unique to PR-B. IgG represents the negative control using the non-immune immunoglobulin of the same isotype as the PgR1294 (mouse) and C1A2 (rabbit) primary antibodies. Positive staining is indicated by brown coloration. Sections were counterstained with hematoxylin (stains nuclei blue). Magnification = × 200.
(Vallejo et al., 2014). This effect highlights the complex functional interaction between the ER and PR systems especially via their extranuclear modes of action (see Fig. 2). Importantly, progesterone induces stromal cell decidualization in the late luteal phase and is essential for maintenance of the decidual phenotype (Lydon et al., 1995; Das et al., 2009). In the decidualization process, which initiates prior to implantation and is independent of the presence of a blastocyst, endometrial stromal cells proliferate, become rounded, and accumulate cytoplasmic glycogen (Bergeron et al., 1988; Lessey et al., 1988). In non-conception cycles, the functional lifespan of the corpus luteum is limited, and within 14–18 days after its formation, it undergoes apoptosis leading to a rapid fall in circulating progesterone levels. If pregnancy is not established, falling progesterone levels due to luteolysis reverse decidualization and induce the expression of chemokines, pro-inflammatory cytokines and matrix metalloproteinases that cause endometrial inflammation, cell death and extracellular matrix degradation leading to shedding of the functionalis, i.e. menstruation (Gellersen and Brosens, 2003; Jabbour et al., 2006; Gellersen et al., 2007). The functionalis then regenerates from the remaining basalis layer in response to estrogen during the proliferative phase of the following cycle (Ferenczy et al., 1979; Graham et al., 2005). If conception occurs, the steroidogenic life span of the corpus luteum is prolonged for up to 10 weeks by chorionic gonadotrophin (CG) secreted by trophoblast cells of the successfully implanted blastocyst. The extended time of progesterone exposure causes the endometrium to undergo complete decidualization within the first week of pregnancy. Complex paracrine signaling between the decidua and trophoblast cells in the chorionic membrane is critical for establishing and maintaining pregnancy. The appropriate role of decidual cells in that dialogue is dependent on progesterone. PR is expressed in endometrial epithelium immediately prior to blastocyst implantation but decreases markedly during the implantation process. At the same time PR expression in stromal cells increases and remains high during the decidualization process (Tan et al., 1999; Spencer et al., 2004). Specific ablation of PR expression in endometrial epithelial cells showed that expression of the receptors in these cells is critical for embryo attachment, stromal decidualization and the inhibition of estrogen-induced epithelial hyperplasia (Fernandez-Valdivia et al., 2010). Loss of PR expression in decidual cells at term is thought to cause functional progesterone withdrawal that triggers inflammation at the maternal–fetal interface leading to parturition (Lockwood et al., 2010).

Differential effects of progesterone on endometrial epithelial and stromal cells are thought to be due to cell type-specific differences in PR-A and PR-B expression and function. PR-A and PR-B are present in endometrial epithelium during the proliferative phase and increase coincidentally with estrogen levels, consistent with the known induction of PR expression by estrogen. Late in the secretory phase, PR-A levels decline, whereas PR-B levels remain constant in the epithelial cells, suggesting that it is involved in the control of glandular secretion. Stromal cells, in contrast, exhibit a predominance of PR-A throughout the menstrual cycle, which likely reflects the need for prolonged progesterone–PR-A signaling in this compartment to support the establishment of pregnancy (Mote et al., 1999, 2000; Attia et al., 2000).

Studies of mice genetically modified to alter PR expression demonstrated fundamental roles of the PRs in mediating progesterone actions on the uterus. Animals lacking PR expression develop normally to adulthood regardless of sex (Lydon et al., 1995; Fernandez-Valdivia et al., 2010). Female PR-null homozygous mice, however, have multiple defects in uterine growth and function, the most notable being hypertrophy and inflammation of the glandular epithelium and failure to exhibit decidualization in response to a traumatic stimulus. PR-null mice fail to reproduce due to defects in ovulation and implantation. Studies targeting PR-A or PR-B demonstrated specific roles of each PR isoform in mediating progesterone actions on the murine uterus. Progesterone actions mediated by PR-A (i.e. phenotype of PR-B-knockout mice) were sufficient to restore normal uterine function. Ovarian function, implantation, pregnancy and parturition are normal in mice expressing only PR-A (Mulac-Jericevic et al., 2003). Progesterone actions mediated by PR-B (i.e. reproductive phenotype of PR-A-knockout mice) lead to increased hyperplasia of the endometrial epithelium and inflammation, and no decidualization in the endometrial stroma (Mulac-Jericevic et al., 2000). Taken together the data from PR knockout mice show that PR-A is critical for normal function of the endometrial epithelium and stroma, and that PR-B functions to promote hyperplasia of the epithelium, an effect which is repressed by PR-A. Both receptors appear to mediate anti-inflammatory actions of progesterone on the endometrium. Studies in mice over-expressing PR-A show that the relative levels of PR-A and PR-B are critical for normal response to progesterone (Fleisch et al., 2009). Over-expression of PR-A was associated with enlargement of the uterus, and hyperplasia of the endometrium that included atypical lesions, endometritis and pelvic inflammation.

The myometrium and cervix also undergo changes in response to estrogen and progesterone during the menstrual cycle, albeit less dynamic compared with those in the endometrium. The myometrium, which forms the bulk of the uterus, is composed of myometrial smooth muscle cells arranged into randomly orientated interlacing bundles. Progesterone and estrogen promote myometrial growth mainly by stimulating hyperplasia and hypertrophy of myometrial cells. After menopause, the myometrium becomes atrophic and the size of the uterus decreases to about half its size. Myometrial cells contain PR-A and PR-B throughout the menstrual cycle and during pregnancy. Progesterone affects the contractile activity of myometrial cells. During the estrogen-dominated proliferative phase, peristaltic waves of myometrial contractions gradually increase in frequency and intensity and at the time of ovulation the direction of the waves is predominantly from cervix to fundus (Chalubinski et al., 1993; Kunz et al., 1996). The waves decrease during the progesterone-dominated post-ovulatory secretory phase presumably due to the relaxatory actions of progesterone, and contractions increase in association with the decrease in progesterone levels late in the secretory phase and during menstruation. These effects are mediated by the nuclear PRs since administration of the nuclear PR antagonist, RU486, increases uterine contractions during the secretory phase (Bygdeman et al., 1993; Gemzell-Danielsson et al., 1993). Likewise, during pregnancy, nuclear PRs expressed by myometrial cells mediate relaxatory actions of progesterone and inhibition of this activity by RU486 treatment increases myometrial contractility and excitability and, in most cases, induces labor and delivery (Avrech et al., 1991). Withdrawal of progesterone in human pregnancy is thought to be mediated by increased expression of PR-A, possibly due to altered methylation of the PR-A promoter region (Chai et al., 2014), leading to a switch in the PR-A:PR-B ratio to PR-A-dominance, which is thought to inhibit PR-B-mediated pro-gestational actions (Mesiano et al., 2002). The withdrawal or disruption of PR-mediated progesterone actions generally leads to the uterine emptying, i.e. menstruation or parturition, that involves increased myometrial contractility and tissue level inflammation.
within the endometrium, myometrium and cervix (Thomson et al., 1999; Jabbour et al., 2006).

The cervix is composed of stromal fibroblasts and squamous epithelial cells and can be divided into three anatomically distinct compartments: (i) the ectocervix that projects into the vagina and is lined by a thick stratified squamous epithelium, (ii) the endocervix that forms the lining of the cervical canal and comprises single layer of columnar mucus-secreting epithelial cells that form deep furrows and tunnels; and (iii) the stroma which comprises the bulk of the cervix and is composed of cervical fibroblasts that produce a tough collagenous extracellular matrix (ECM) (Bathgate et al., 2006). PRs localize to the nucleus in stromal fibroblasts and basal squamous epithelial cells, but are absent in intermediate and superficial squamous epithelial cells (www.nordiqc.org/Run-18-B2/assessment/assessment-PR.htm). During the proliferative phase of the menstrual cycle, the endocervical epithelium, in response to estrogen, produces a thin watery mucus that is conducive to the passage of sperm into the uterus. In contrast, during the secretory phase, progesterone promotes cervical ripening (Dai et al., 2001; Jaffe et al., 2007; Ward et al., 2008; Neubauer et al., 2011). Progesterone also antagonizes estrogen-induced collagenase expression (Shiozawa et al., 1998; Kyo et al., 2011) and inhibits hyaluronate synthesis in human endocervical fibroblasts (Uchida et al., 2005, 2007). A decrease in hyaluronate prevents water imbibition and collagen dissolution and, therefore, maintains ECM rigidity. In all species studied so far, treatment with PR antagonists at any stage of pregnancy promotes cervical ripening (Dai et al., 2001; Smid-Koopman et al., 2003; Gielen et al., 2006; Paulsson et al., 2008; Moe et al., 2009), which demonstrates that PR signaling is essential for progesterone-induced cervical competence. Sensitivity of the cervix to PR antagonist-induced ripening increases as pregnancy nears term, suggesting that the capacity for progesterone to maintain cervical competence wanes and prosoftening influences increase as pregnancy progresses (Orbo et al., 2009).

In the gravid uterus, progesterone promotes relaxation of the myometrium, closure and rigidity of the cervix, and inflammatory quiescence in the chorion/decidua. The progestational actions of progesterone are mediated by PRs expressed in myometrial, cervical and decidual cells (Merlino et al., 2007). Progesterone promotes pregnancy, in part, by decreasing responsiveness of myometrial, cervical and decidual cells to pro-inflammatory/pro-labor stimuli (Hardy et al., 2006; Tan et al., 2012). In myometrial cells, the anti-inflammatory effects of progesterone are mediated by PR-B and inhibited by PR-A (Tan et al., 2012).

In all viviparous species studied to date, the process of parturition is triggered by withdrawal of the progesterone block to labor, and depending on the species, occurs by a either a decrease in circulating progesterone levels (referred to as systemic progesterone withdrawal) or desensitization to PR-mediated progesterone action (referred to as functional progesterone withdrawal). Human parturition is triggered by a functional progesterone withdrawal mediated, in part, by changes in the transcriptional activities of PR-A and PR-B such that the capacity for progesterone to exert anti-inflammatory actions via PR-B is inhibited by transrepressive actions of PR-A (Mesiano et al., 2011; Tan et al., 2012). Transcriptional activity of PR-B is further impaired at parturition by decreased expression of progesterone-PR coregulators such as cAMP-response element-binding protein (CREB-binding protein) and steroid receptor coactivators 2 and 3 (Condon et al., 2003). Secondary to their inherent histone acetytransferase activity, decreased expression of these coactivators may result in the decline of histone acetylation, and therefore, chromatin inaccessible and transcriptional repression during parturition (Chen et al., 1997; Condon et al., 2003).

Thus, a common theme of PR-mediated progesterone actions in the human uterus (gravid and non-gravid) is that PRs mediate pro-gestational and anti-inflammatory actions and withdrawal of this activity, either by functional inhibition of PR-B signaling or a systemic decrease in progesterone levels, leads to tissue level inflammation, which causes menstruation in the non-gravid uterus and parturition and involution in the gravid uterus. In both contexts, the key physiologic consequence of progesterone/PR withdrawal is the resumption of the menstrual cycle.

**Progesterone receptors in uterine pathophysiology**

The following discussion focuses on the role of PR-A and PR-B in the development and progression of endometriosis, uterine leiomyoma, endometrial cancer, cervical cancer, and recurrent pregnancy loss. As discussed above, progesterone affects normal uterine function via a finely tuned and tissue/cell type specific balance between PR-A- and PR-B-mediated transcriptional activities. Most pathophysiologies of myometrium, endometrium and cervix are responsive to progesterone, albeit in an abnormal manner. PR-mediated progesterone actions vary according to the cell type. In the breast, PR (mainly PR-B) mediates proliferative actions of progesterone, whereas in the uterus progesterone stimulates growth of leiomyomas but inhibits growth of the endometrium. In general, studies examining the role of PRs in the etiology of uterine pathophysiologies have assessed only the expression level and cellular location of PR-A and PR-B, without considering net transcriptional activity. Clearly, it is now evident that functional pleiotropy in PR activity can arise not only through cell type-specific differences in PR-A:PR-B levels, but also by changes in PR PTMs, and differences in transcriptional co-regulators and target gene promoter structure. Nonetheless, temporo-spatial information of PR isoform expression has provided a better understanding of PR function in uterine pathophysiology.

**Endometriosis**

Endometriosis is the presence of glandular and stromal endometrial implants at an extruterine (ectopic) site. The disease affects 5–10% of women of reproductive age and is markedly influenced by estrogen and progesterone. Inflamed endometriotic lesions are usually found in the peritoneal cavity and on the ovaries, and it is generally considered that they derive from abnormal endometrial cells that access the peritoneum by retrograde menstruation (Sampson, 1927; Giudice and Kao, 2004; Giudice, 2010). Lymphatic/hematogenous dissemination of abnormal endometrial cells and metaplastic transformation of native peritoneal tissue may also be responsible for implants at distant sites (Giudice, 2010).
Endometriotic implants undergo estrogen- and progesterone-induced changes in growth and morphology during the menstrual cycle in parallel with the eutopic endometrium (Jiang et al., 2002). Consequently, the clinical severity of endometriosis is affected by estrogens and progesterone, with estrogen exposure being a major endocrine risk factor for disease development and progression (Halme et al., 1995), whereas progesterone has inhibitory effects on endometriotic implants and is associated with disease regression (Kaunitz, 1998; Olive et al., 2004). This pattern of response is consistent with estrogen-induced proliferation of endometrial epithelial cells during the proliferative phase and the inhibition of proliferative activity by progesterone during the secretory phase.

Whether aberrant PR signaling in endometrial cells plays a role in the etiology of endometriosis is unclear. Endometriosis appears to be associated with decreased progesterone responsiveness in endometrial stromal cells (Bulun et al., 2006; Yin et al., 2007, 2012) that may be due to decrease in PR expression. Studies of PR-A and PR-B levels and cell localization in endometriosis however are equivocal. Most studies using assays that have not discriminated between PR-A and PR-B have reported lower PR levels in ectopic compared with eutopic endometrium (Lyndrup et al., 1987; Prentice et al., 1992; Bergqvist and Ferno, 1993a,b; Bergqvist et al., 1993), with one study (Nisolle et al., 1994) reporting no difference. Immunoblot analysis of PR-A and PR-B shows that peritoneal endometriotic tissue does not express PR-B and has reduced levels of PR-A compared with eutopic endometrium (Attia et al., 2000). In contrast, mRNA analysis of ovarian endometriotic tissue shows dominant expression of PR-B (Misao et al., 1999), whereas studies of PR gene structure in endometriosis show hypermethylation of the PR-B promoter consistent with decreased PR-B expression (Wu et al., 2006).

A key unanswered question regarding the etiology of endometriosis is why it affects 5–10% of women even though retrograde menstruation occurs in most women (Eskenazi and Warner, 1997). One explanation is that endometriosis arises due to changes in eutopic endometrial cell progesterone responsiveness, especially decreased responsiveness, that confers a predisposition to form ectopic implants. To test this hypothesis, several studies analyzed PR expression in eutopic endometrium from women with and without endometriosis. Outcomes, however, conflict with some studies reporting dysregulation of PR isoform expression associated with the presence of disease (Kao et al., 2003; Burney et al., 2007) and others reporting no difference (Prentice et al., 1992; Attia et al., 2000; Igarashi et al., 2005; Aghajanova et al., 2009). Taken together, published data regarding the role of PRs in the etiology of endometriosis produce no clear consensus. Nonetheless, aberrant PR signaling, especially reduced capacity for progesterone to oppose estrogen-induced endometrial cell proliferation and/or responsiveness to pro-inflammatory stimuli in eutopic endometrium may contribute to the development of endometriosis once the cells relocate to the peritoneum (Osteen et al., 2005). Progesterone is clearly a central player in the disease, and it is likely that further studies using more specific and sensitive assays for PR-A and PR-B expression, PTM forms, and transcriptional activity will elucidate the role of the PRs in the pathophysiology of endometriosis.

Leiomyoma

Uterine leiomyoma, also known as uterine fibroids, are benign monoclonal smooth muscle cell tumors thought to arise from a genetically modified myometrial cell. The tumors comprise modified myometrial smooth muscle cells, known as leiomyoma cells that overproduce ECM (Williams et al., 1997; Sumitani et al., 2000; Barbarisi et al., 2001; Park et al., 2008). Leiomyoma is the leading indication for hysterectomy occurring in ~70–80% of reproductive age females and causes multiple gynecologic symptoms including pelvic pain and dysfunctional uterine bleeding (Flynn et al., 2006; Parker, 2007).

Clinical observations provide strong circumstantial evidence that leiomyoma is estrogen and progesterone responsive. The disease has not been reported in pre-pubescent girls but the incidence and burden of tumors increase in association with high estrogen and progesterone levels, especially during the reproductive years, and decrease after menopause when ovarian steroids are low. Moreover, treatment with gonadotrophin releasing hormone (GnRH) agonist, which causes hypogonadism, suppresses leiomyoma growth, whereas estrogen and progesterone replacement therapy increases the tumor size in menopausal women (Sener et al., 1996; Palomba et al., 2001; Yang et al., 2002). The mitogenic potential of estrogen and progesterone on leiomyoma cells is curbed by concurrent treatment with GnRH agonists (Chegini et al., 2002). As side effects related to hypoestrogenism preclude long-term treatment with GnRH analogs, low doses of estrogen and progesterone can be utilized as add-back therapy without stimulation of leiomyoma growth (Thomas, 1996; Takeuchi et al., 2000).

Clinical evidence shows that progesterone stimulates leiomyoma growth. An elevated proliferative index (assessed by Ki67 staining) and epidermal growth factor receptor expression were detected in leiomyoma cells compared with adjacent myometrium, and are highest during the progesterone-dominant secretory phase (Tiltman 1985; Kawaguchi et al., 1989; Brandon et al., 1993; Harrison-Woolrych et al., 1994). In post-menopausal women, the leiomyoma proliferative index is higher in women receiving combined estrogen and progesterone replacement therapy compared with those receiving estrogen alone (Lamminen et al., 1992). Progestin (synthetic progestogen) therapy decreases the efficacy of GnRH agonist treatment to reduce leiomyoma size (Friedman et al., 1993) and increases leiomyoma size in post-menopausal women (Lamminen et al., 1992; Palomba et al., 2002). Importantly, RU486 and other selective progesterone receptor modulators (SPRMs) significantly reduce the leiomyoma tumor burden, suggesting that targeting progesterone signaling in leiomyoma is an effective strategy for reducing the burden of this disease (Murphy and Mahesh, 1985; Murphy et al., 1993, 1995; Cermik et al., 2002; Eisinger et al., 2003, 2005; Steinauer et al., 2004; Fiscella et al., 2006; Xu et al., 2006, 2008; Chwalisz et al., 2007; Williams et al., 2007; Wilkins et al., 2008; Donnez et al., 2012a, b).

Actions of progesterone in leiomyoma are thought to be mediated by PR-A and PR-B. Both receptors have been detected in leiomyoma tissue and in immortalized leiomyoma cell lines (Kawaguchi et al., 1991; Nisolle et al., 1999; Carney et al., 2002), and some studies show increased expression of PR-A and PR-B in leiomyoma compared with normal myometrium from the same subjects (Brandon et al., 1993; Viville et al., 1997; Fujimoto et al., 1998). Interestingly, PR-A and PR-B expression in leiomyoma is decreased by GnRH agonist therapy (Nisolle et al., 1999), which is consistent with estrogen-induced PR expression. ExTRANuclear actions of PRs, especially via the PI3K/AKT signaling cascade, which augments cell viability and prevents apoptosis, may contribute to progesterone/PR-induced leiomyoma growth (Hoeckstra et al., 2009). In addition, progesterone via the PRs may inhibit apoptosis by directly augmenting
expression of Bcl-2 (Yin et al., 2007) and promote proliferation by increasing expression of EGFr (Shimomura et al., 1998; Matsuo et al., 1999).

Leiomyoma are characterized as benign fibrous tumors with excessive deposition of disorganized ECM. The composition of the leiomyoma ECM, and in particular the relative levels of specific proteoglycans such as decorin and versican, are thought to affect tumor growth by modulating the activity of growth factors, especially transforming growth factor-β (TGF-β), that bind to proteoglycans (Harper et al., 1994). Compared with unaffected tissues, leiomyoma contain higher relative amounts of versican and lower relative amounts of decorin (Carrino et al., 2012). Higher TGF-β levels have been found in leiomyoma, and decorin has been shown to antagonize TGF-β signaling. A decrease in decorin would be expected to increase TGF-β activity in the leiomyoma microenvironment. Importantly, progesterone and estrogen increase ECM production and TGF-β expression in leiomyoma cells (Arici and Sozen, 2000; Joseph et al., 2010) and potentially initiate a positive feedback interaction whereby excessive deposition of the decorin-deficient ECM leads to excessive TGF-β signaling which increases the proliferation of cells that produce the aberrant decorin-deficient ECM and overexpress TGF-β in response to progesterone. Although studies assessing the link between proteoglycan synthesis and PR transcriptional activity in leiomyoma are lacking, the use of SPRMs targeting this interaction to treat leiomyoma disease has been proposed (Kim et al., 2013).

To date, numerous SPRMs have been investigated as potential treatments for symptomatic leiomyomas and two, asoprisnil (DeManno et al., 2003) and ulipristal acetate (Yoshida et al., 2010), have been examined in detail. Asoprisnil reduces leiomyoma volume and associated symptoms (Chwalisz et al., 2007) possibly by inhibiting proliferation and inducing apoptosis of leiomyoma cells without effecting normal myometrial cells (Chen et al., 2006; Wang et al., 2006; Sasaki et al., 2007). Asoprisnil also decreases leiomyoma collagen synthesis and enhances the expression of matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs), thereby reducing collagen deposition into the leiomyoma ECM (Stewart et al., 1994; Morikawa et al., 2008). Ulipristal acetate also has demonstrated anti-proliferative, anti-fibrotic and pro-apoptotic effects on cultured leiomyoma cells, but not on normal myometrial cells (Yoshida et al., 2010) and has recently received approval by both the US Food and Drug Administration and the European Medicine Agency for preoperative treatment of leiomyoma (Talaulikar and Manyonda, 2012; Biglia et al., 2014). Ulipristal has been shown to reduce both leiomyoma and uterine size in women with symptomatic leiomyoma (Levens et al., 2008; Nieman et al., 2011). Repeated doses of ulipristal acetate at 3-month intervals are effective in treating symptomatic leiomyoma, with induction of amenorrhea and reduced leiomyoma volume (Donnez et al., 2014). Ulipristal may inhibit the proliferation of leiomyoma cells and induce apoptosis through increased expression of pro-apoptotic caspase 3 and decreased expression of anti-apoptotic Bcl-2 (Maruo et al., 2000; Maruo, 2007; Biglia et al., 2014). Ulipristal also down-regulates expression of key angiogenic growth factors including vascular endothelial growth factor (VEGF), and similar to asoprisnil, may reduce deposition of collagen in the extracellular matrix (Spitz, 2009; Talaulikar and Manyonda, 2012; Biglia et al., 2014). Overall, SPRMs are associated with fewer adverse effects compared with pure progesterone receptor antagonists. In some studies, however, SPRM use has been associated with adverse endometrial effects, including endometrial hyperplasia. Endometrial hyperplasia is classified by the World Health Organization (WHO) based on cystic glandular dilation/crowding of the epithelium and a gland to stromal ratio of >50% in the absence of secretory endometrial changes (Owings and Quick, 2014). Simple hyperplasia is associated with few mitoses and glands with minimal outpouching. In contrast, complex hyperplasia is associated with numerous mitoses, disorganized glands with luminal outpouching. Additionally, nuclear atypia can accompany either of these classifications. As such, endometrial hyperplasia is a precursor to endometrial adenocarcinoma. These endometrial effects associated with SPRMs appear to be related to the dose of PR ligand utilized, hormonal status at the time of treatment, and the animal model used in previous studies. For instance, mifepristone (RU486), a PR antagonist, has both anti-proliferative and hyperplastic effects depending on the dose and subject. In premenopausal subjects, low dose mifepristone has an anti-proliferative effect, while higher doses result in various degrees of hyperplasia (Chwalisz et al., 1998, 2000; Spitz and Chwalisz, 2000; Chabbert-Buffet et al., 2005). The exact mechanism of endometrial hyperplasia with SPRM use remains unclear. SPRMs bind minimally to the estrogen receptor (Chabbert-Buffet et al., 2005). Like mifepristone, the hyperplastic effect of SPRMs on the endometrium may be related to their antiglucocorticoid effects. The ensuing increase in adrenocorticotropic hormone (ACTH) may increase production of androstenedione and testosterone from the adrenal cortex, which are then aromatized to estrone and estradiol respectively, thus resulting in an overall increase in estrogen-drive in the endometrium (Lamberts et al., 1991; Heikinheimo 1997; Heikinheimo et al., 2000; Chabbert-Buffet et al., 2005).

However, histological endometrial changes noted with SPRMs are distinct from estrogen-associated endometrial hyperplasia or disordered proliferative endometrium (Biglia et al., 2014). The term ‘progesterone receptor modulator-associated endometrial changes’ (PAECs) has been used to describe various histological patterns noted in the endometrium of women receiving SPRMs that do not include malignant or premalignant changes (Mutter et al., 2008; Biglia et al., 2014). Patients receiving ulipristal have demonstrated PAECs, with noted cystic changes in the endometrium and increased endometrial thickness. Despite the clinical finding of increased endometrial thickness in these patients, PAECs are distinct from endometrial hyperplasia as there is pronounced dysynchrony of endometrial and stromal proliferation resulting in cystic dilatation admixed with both estrogen-induced (mitotic) and progesterone-induced (secretory) epithelial changes (Mutter et al., 2008). Overall, PAECs differ from classic unopposed estrogen effects in demonstrating lower or absent mitotic activity, exhibiting varying levels of epithelial secretory change, and having occasional stromal pseudodeciidualization. Additionally, these changes are reversible after cessation of ulipristal therapy, with restoration of endometrial thickness to baseline levels. It is important to note, however, that these endometrial changes are of unknown pathologic significance as biopsies from both control subjects and patients prior to treatment have demonstrated these endometrial changes at times (Ioffe et al., 2009). Thus, taken together, the current data regarding SPRM use for the treatment of leiomyoma are promising and further studies are needed to determine their exact mechanism of action and especially their effects on PR-A and PR-B activity in the leiomyoma cell context.

**Endometrial cancer**

Endometrial cancer is the most common gynecologic malignancy, accounting for 6% of all cancers in women (Siegel et al., 2011; American
The majority of endometrial cancers occur in postmenopausal women, and 80% of patients are diagnosed when the tumor is confined to the uterus (stage I disease). As described above, endometrial hyperplasia is a precursor to endometrial adenocarcinoma and is characterized by disordered proliferation of endometrial glands, resulting in a greater gland-to-stroma ratio than observed in normal endometrium (Kurman et al., 1994).

In normal endometrium, expression of PR is induced during the estrogen-dominated proliferative phase. During the secretory phase, when circulating concentrations of progesterone are maximal, activation of PR results in reduced proliferation of the endometrial epithelium, and its differentiation into a secretory phenotype. If progesterone effects are disrupted, as might occur during anovulatory cycles when its production by the ovary is reduced, the epithelium can become hyperplastic in response to unopposed estrogen. Indeed, many of the established risk factors for developing endometrial cancer (most notably type I) are associated with excess exposure to estrogen unopposed by progesterone. For instance, risk of developing endometrial cancer is increased by estrogen-only hormone replacement therapy (Beral et al., 2005) and a high body mass index (BMI), which is associated with higher estrogen levels and anovulation (Rieck and Fiander, 2006). Polycystic ovary syndrome (PCOS), a condition characterized by hyperandrogenism and chronic anovulation, is associated with approximately a 2.7 fold increase in endometrial cancer (Fauser et al., 2012; Barry et al., 2014). In addition, endometrial adenocarcinomas express enzymes involved in estrogen biosynthesis (Bulun et al., 1994; Utsunomiya et al., 2001), which may further increase the local estrogenic drive. Thus, progesterone plays a critical role in restricting the tropic actions of estrogen on the endometrium, and as such PR expression status in endometrial carcinoma is considered to be an independent prognostic factor (Ballester et al., 2013; Zhang et al., 2013). While positive immunohistochemical staining of ER in endometrial adenocarcinoma is one of the most important prognostic factors for survival, PR expression may also predict survival (Ballester et al., 2013; Zhang et al., 2013; Carlson et al., 2014). However, controversy around this issue exists. While some small studies have not shown specific advantages with respect to PR expression status and survival (Thigpen et al., 1999; Singh et al., 2007; Carlson et al., 2014; Gunderson et al., 2014), a recent prospective multicenter trial including specimens from 832 women with endometrial carcinoma found decreased survival in patients with ER and PR receptor loss (Trovik et al., 2013).

The understanding that progesterone exerts PR-mediated antimitotic effects on the endometrium has led to the use of various progestins as therapeutics for low-grade endometrial adenocarcinoma (Lentz et al., 1996; Thigpen et al., 1999; Fiorica et al., 2004; Whitney et al., 2004; Decruz and Green, 2007). Progestin therapy markedly affects the histopathologic characteristics of endometrial cancers (Wheeler et al., 2007). Gland-to-stroma ratio, glandular cellularity, mitotic activity and cytologic atypia are each decreased by progestin therapy, and these changes are associated with complete resolution of disease after 12 months of treatment in some patients. Additionally, the anti-gonadotropic activity of progestrone suppresses endogenous estrogen production by the ovaries (Banno et al., 2012). Progesterone also decreases ER expression in endometrial cells and activates enzymatic pathways that inactivate estradiol by its conversion to estrone by the 17-β hydroxysteroid dehydrogenase type 2 and sulfotransferase within endometrial cells (Banno et al., 2012).

Although increased PR-A expression has been found in ER positive endometrial cancer, the specific roles of PR-A and PR-B in the etiology of endometrial cancer are unclear (Singh et al., 2007). Endometrial cancer is associated with mutations in progesterone/PR responsive genes whose products are thought to mediate anti-proliferative and immunosuppressive actions of progesterone (Shiozawa et al., 1998; Di Nezza et al., 2003; Jaffe et al., 2007; Ward et al., 2008; Kyø et al., 2011). One such PR-responsive gene, forkhead box protein O1 (FOXO1), is emerging as a key mediator for cellular senescence in endometrial carcinomas (Kyo et al., 2011). The transcription factor FOXO1 is a downstream target of the phosphatidylinositol-3-kinase/Akt signaling pathway (Goto et al., 2008) and, as such, FOXO1 is a regulator of the cell-cycle and plays a role in cellular apoptosis. FOXO1 expression is decreased in the majority of endometrial cancers, and treatment with progesterone increases its expression, an effect which is mediated by PR-B (Ward et al., 2008). Progesterone, through PR-B but not PR-A, also induces expression of the anti-mitogen, insulin-like growth factor binding protein 1 (IGFBP-1), in endometrial cancer cells (Nakamura et al., 2013). IGFBP-1 requires upstream binding of FOXO1 to its promoter in order to exert progesterone-induced anti-proliferative effects in endometrial cells, thereby lending credence to the concept that progesterone-induced FOXO1 is key to tumor suppression in endometrial cells.

Progesterone and PRs also may mediate anti-proliferative effects through regulation of cell-cycle dependent kinases (CDKs) (Banno et al., 2012). CDKs advance the cell cycle through functional interactions with other transcription factors including PR (Hagan et al., 2011). This has been proposed as a key mechanism for progesterone/PR induced proliferation of breast epithelial cells and the etiology of breast cancer (Hagan et al., 2011). Progesterone induces expression of p27, a cyclin E/CDK2 inhibitor, thereby suppressing the cell cycle (Banno et al., 2012).

Some endometrial cancers exhibit a complete loss of PR expression. In a recent study, Yang et al. examined the possibility of restoring PR expression in endometrial cancer cells by epigenetic modulation treating cells with histone deacetylase inhibitors (Yang et al., 2014). Importantly, they found that treatment of endometrial cancer cells with a histone deacetylase inhibitor increased PR expression and conferred progesterone responsiveness at various target genes relevant to endometrial cancer biology, including FOXO1, p21 and p27. This innovative therapeutic approach may be used to sensitize endometrial tumors to progestin therapy. Further understanding of how PGR is controlled in endometrial cancer and how PR gene targets are affected by PR-A and PR-B in response to various progestins, and of their roles in disease etiology and progression, will increase the therapeutic arsenal to treat this disease specifically by exploiting the protective action of progesterone.

### Cervical cancer

Cervical cancer is a consequence of infection with human papilloma virus (HPV) (Van Ranst et al., 1996). Specifically, viral gene E2, an important regulator of protooncogenes E6 and E7, is deleted in malignantly transformed cells. Oncogenes E6 and E7 interact with cellular proteins that regulate the cell cycle and apoptosis (zur Hausen, 2002). Notably, E6 promotes proteosome-mediated degradation of tumor suppressor protein p53 and E7 inactivates the tumor suppressor retinoblastoma protein.
The link between sex steroids and cervical cancer surfaced when a higher incidence of cervical carcinoma was noted among long-term oral contraceptive users (Franceschi, 2005). This relationship appears to have temporal effects as cervical cancer risk increases 4-fold in women who have used oral contraceptives for > 10 years (Moreno et al., 2002). Estrogen and progesterone appear to facilitate HPV DNA integration into the host cell genome (Webster et al., 2001), and progesterone facilitates the oncogenic transformation of HPV DNA with the ras oncogene (Pater et al., 1990) and increases expression of the E6 and E7 oncogenes (Chen et al., 1996; Michelin et al., 1997; Yuan et al., 1999). These effects are likely mediated by PRs whose expression is increased in stromal cells of both squamous cell and adenocarcinoma cervical lesions compared with normal controls (Bodner et al., 2010; Kwasniewska et al., 2011). Intensity of PR staining also directly correlates with grade of precancerous cervical lesions, with high-grade precancerous lesions staining intensely for PR (Monsonego et al., 1991).

Despite much of this evidence, it is important to note that the exact role of progesterone and the PRs in cervical cancer still remains unclear. Although most studies have shown a positive correlation between progesterone and the development of cervical cancer, epidemiological data regarding the use of medroxyprogesterone acetate (MPA), a potent progestational agent, and cervical carcinoma have yielded conflicting results. While many studies have suggested that MPA use increases the risk of neoplastic disease in the cervix (Thomas et al., 1985, 1995; McFarlane-Anderson et al., 2008), several case–control studies show that MPA use is not a risk factor for the disease (Thomas and Ray 1995; Kaunitz 1996), and may, in fact, inversely correlate with disease (Harris et al., 2009). Furthermore, from studies in a transgenic mouse model expressing E6 and/or E7, high doses of MPA may actually cause regression of cervical dysplasia (Yoo et al., 2013). Interpretation of these effects is complicated by the fact the MPA also activates the glucocorticoid receptor. Prospective studies are required to provide clear insight into the clinical outcomes of cervical cancer with progesterone use and the possible utility of various SPRMs as therapeutic measures for cervical carcinoma.

Recurrent pregnancy loss

Recurrent pregnancy loss (RPL) is defined as two or more clinically recognized failed pregnancies and affects ~0.5 – 1% of couples (Baek et al., 2007). Embryonic and parental cytogenetic abnormalities, anatomic malformations, thrombophilic disorders, and hormonal aberrations have been implicated in this condition. Progesterone deficiency and aberrant PR-mediated signaling may play a role in RPL. As discussed above, progesterone induces the endometrial phenotype that is conducive to embryo implantation and to the establishment and maintenance of pregnancy (Norwitz et al., 2001). Progesterone deficiency and a shortened luteal phase may result in retarded endometrial development, which has been associated with RPL. While progesterone acting through PRs undoubtly plays a pivotal role in implantation and maintenance of pregnancy, progesterone supplementation for patients with sporadic miscarriage does not improve pregnancy outcomes (Haas and Ramsey, 2013). However, a small number of studies involving patients with RPL have shown that administration of progesterin may be of some benefit in preventing miscarriage in this population (Oates-Whitehead et al., 2003). Larger prospective clinical trials are needed to definitively elucidate the benefits of progestin therapy in patients with RPL. Clearly, in most cases the problem lies with progesterone responsiveness rather than hormone availability. In these cases the abnormal expression and/or function of the PRs is implicated. RPL is associated with decreased PR expression by the embryo (Hickman et al., 2002) and in the endometrium (Carranza-Lira et al., 2000). Specific PGR polymorphisms have been reported in patients with idiopathic RPL (Su et al., 2011). Of particular interest is a 306 base pair insertion polymorphism in intron G of the PGR gene that correlates with RPL and is linked to implantation failure in in vitro fertilization cycles (Cramer et al., 2003). Interestingly, the polymorphism also segregates with progesterone-dependent neoplasms (Romano et al., 2006).

The establishment of pregnancy involves a complex hormonal dialogue between the mother and the fetus that modulates the maternal immune system such that it does not reject the fetal allograft. Progesterone is thought to play a key role in this process through multiple effects on the maternal immune system. Progesterone has been reported to reduce natural killer (NK)-cell activity (Hansen et al., 1992), increase HLA-G production in trophoblast cells (Yie et al., 2006b), increase suppressor-cell levels (Brierley and Clark, 1987), inhibit cytotoxic T-cell activity (Mannel et al., 1990), induce the production of lymphocyte-blocking proteins (Barakonyi et al., 1999), and modify the cytokine response from the Th-1 to the pro-pregnancy Th-2 pattern (Piccinni et al., 1995; Choi et al., 2000). The mechanism for this effect is unclear and the involvement of the nuclear PRs is controversial (Van Voorhis et al., 1989; Szekeres-Bartho et al., 1990, 2001; Mansour et al., 1994; Schust et al., 1996). Indeed, the effects may be indirect via increased trophoblast HLA-G production (Yie et al., 2006a) or via production of a factor in lymphocytes, referred to as progesterone-induced blocking factor (PIBF) (Szekeres-Bartho et al., 1985, 2001). Interestingly, progesterone-treated lymphocytes of pregnant women showing clinical symptoms of threatened preterm delivery fail to release PIBF (Szekeres-Bartho et al., 1990; Yie et al., 2006a). The uncertainty of the role of progesterone and PRs in RPL underscores the need for further research to delineate the exact role of endometrial PR modulation in RPL.

Conclusions

As its name implies, progesterone is a pro-gestation hormone and has long been considered the master hormone of pregnancy. Its effects on the uterus are essential for the establishment and maintenance of pregnancy, and as such it plays a central and critical role in the viviparous reproductive cycle. The discovery of PR antagonists such as mifepristone (RU486) and onapristone demonstrated that the nuclear PRs mediate most, if not all, actions of progesterone on the uterus. Understanding of PR molecular biology has advanced significantly in the last 20–30 years, and it is now clear that progesterone actions in the human uterus are mediated by the combined, and sometimes opposing, effects of the two PR isoforms, PR-A and PR-B. The dual PR isoform systems allow for qualitative and quantitative control of progesterone responsiveness through modulation of relative PR-A and PR-B levels and transcriptional activities and by the presence and activity of transcriptional co-regulators in a cell-specific manner. This paradigm together with cell- and context-specific PR post-translational modifications, explains the pleiotropic actions of progesterone in the various uterine cell types, and allows for control of progesterone action secondary to the presence of hormone. Given the essential role of progesterone in human reproduction and the fact that its levels can vary markedly...
between menstrual cycles, such a system may have evolved to favor the establishment of pregnancy despite a wide range of circulating progesterone levels. It is not surprising therefore that the etiology and clinical trajectory of multiple uterine pathophysiologies involves defects in PR-A/PR-B signaling and its relationship with the estrogen/ER system. Thus, PR expression and activity in conjunction with the associated signal transduction systems are a key element in progesterone actions in the human uterus. A clear understanding of the molecular biology of progesterone/PR signaling in each of the uterine target cells will be essential for the development of SPRM-based therapies to treat uterine pathophysiologies.

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