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NEW RESEARCH HORIZON ***Review***

Control of ovulation in mice by progesterone receptor-regulated gene networks

Jaeyeon Kim[1], Indrani C. Bagchi[2], and Milan K. Bagchi[1,3]

[1]Department of Molecular and Integrative Physiology, Center for Research in Reproduction and Infertility, University of Illinois at Urbana-Champaign, 534 Burrill Hall, 407 S. Goodwin Ave., Urbana, IL 61801, USA [2]Department of Veterinary Biosciences, Center for Research in Reproduction and Infertility, University of Illinois at Urbana-Champaign, Urbana, IL, USA

[3]Correspondence address. Tel: +1-217-244-5054; Fax: +1-217-333-1133; E-mail: mbagchi@life.uiuc.edu

ABSTRACT: The mid-cycle surge of luteinizing hormone (LH) induces ovulation, a process during which a fertilizable oocyte is released from a mature ovarian follicle. Although ovulation is a physiologically well-characterized event, the underlying molecular pathways remain poorly understood. Progesterone receptor (PGR), which mediates the biological effects of the steroid hormone progesterone, has emerged as a key regulator of ovulation in mice. The development of a progesterone-receptor-null (*Pgr*-null) mouse model confirmed a critical role of this hormone in ovulation because in these mutant mice, mature pre-ovulatory follicles fail to release the oocytes. This animal model has thus presented a unique opportunity to study the molecular pathways underlying ovulation. Gene-expression profiling experiments by several groups, using the ovaries of *Pgr*-null mice, revealed novel gene networks, which act downstream of PGR to control ovulation. These genes encode diverse molecules such as proteases, transcription factors, cell-adhesion molecules, modulators of vascular activities and regulators of inflammation. Functional analyses using gene-knockout mouse models have confirmed that some of these factors play critical roles during ovulation. The knowledge gained from these studies has helped us to understand better the molecular mechanisms that facilitate the release of oocytes from pre-ovulatory follicles. Further analysis of the role of molecular regulators of ovulation will help identify useful molecular targets that would allow the development of improved contraceptives and new therapeutics for anovulatory infertility.

Key words: ovulation / progesterone / luteinizing hormone

Introduction

Ovulation is an essential reproductive event, which involves the breakdown of a mature ovarian follicle to release a fertilizable oocyte. In mammals, it is a highly coordinated process initiated by hormones released from the hypothalamic–pituitary–ovarian axis. In mice and rats, primary follicles are released daily from the primordial follicle pool by mechanisms that are yet to be elucidated but likely involve changes in oocytes and somatic cells. These primary follicles commence to grow in response to the pituitary gonadotrophins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). A much smaller pool is selected from these growing follicles for further maturation and eventually reaches the pre-ovulatory stage. In response to the high levels of estrogen produced from these maturing ovarian follicles, the pituitary releases LH midway through a menstrual cycle in humans or at pro-estrus of an estrous cycle in rodents. This LH surge initiates the ovulatory process that culminates in the release of fertilizable oocyte(s) (Richards, 1994).

The hormonal regulation of ovulation in the mouse is remarkably similar to that of the human (Espey, 1998). In addition, since mice are amenable to genetic manipulation, they serve as an excellent model for investigating the signaling pathways that control follicular growth and release of mature oocytes. Ovulation, controlled primarily by the cyclic changes in FSH and LH, can be experimentally induced in mice by administration of exogenous gonadotrophins in a process known as superovulation. In this procedure, mice are treated with equine chorionic gonadotrophin, which mimics FSH action, for 48 h to stimulate follicular development; subsequent administration of human chorionic gonadotrophin (hCG), which mimics LH action, induces ovulation (Espey, 1994; Robker *et al.*, 2000). Typically, ovulation occurs 11–14 h following the hCG treatment. Researchers in many laboratories have used a superovulation protocol to analyze gene-expression patterns in ovarian follicles during the ovulatory period in mice or rats (Robker *et al.*, 2000; Espey and Richards, 2002).

Although the physiological function of ovulation is well defined, the mechanisms controlling this process have eluded researchers for many decades. Ovulation is broadly understood to be a complex yet timely process, involving the coordinated expression of numerous genes in the ovary (Richards *et al.*, 2002). LH, the critical trigger of ovulation, exerts its effects by acting through the LH receptor, which is

a G-protein-coupled membrane-bound receptor, in granulosa cells. The LH receptor is induced in granulosa cells during the growth of pre-ovulatory follicles, thereby allowing only these follicles to respond to the LH surge (Peng *et al*., 1991). An increase in the LH levels terminates the program of follicular growth and sets off a cascade of gene-expression changes in the ovary that are critical for ovulation and luteinization (Couse *et al*., 2005). A number of genes that are induced in ovarian follicular cells in response to the LH surge have been shown to be critical for ovulation in mice. These include progesterone receptor (*Pgr*), prostaglandin-endoperoxide synthase 2 (*Ptgs2*), CCAAT/enhancer-binding protein β (*Cebpb*) and epidermal growth factor (EGF)-like growth factors (Lydon *et al*., 1995; Lim *et al*., 1997; Sterneck *et al*., 1997; Hsieh *et al*., 2007). In this review, we will focus on the role of PGR during ovulation in mice. We will discuss a few key PGR-regulated pathways and their potential functions during ovulation.

PGR plays an essential role in ovulation

Earlier studies indicated that the steroid hormone progesterone is a key player in ovulation. Inhibition of progesterone synthesis by epostane blocked ovulation in rats (Tanaka *et al*., 1991). The biological effects of progesterone are mediated by PGR, a ligand-activated transcription factor. Ovarian PGR expression is undetectable during follicular development (Park and Mayo, 1991; Robker *et al*., 2000). In mouse and rat ovaries subjected to gonadotrophin-induced superovulation, a rapid and robust induction of PGR expression is observed in the mural granulosa cells of pre-ovulatory follicles following hCG administration. The expression of PGR peaks at 4–8 h and then declines to undetectable levels, which is maintained throughout the rest of the ovulatory period (Robker *et al*., 2000). The induction of PGR, following the LH surge, is accompanied by coordinated expression of progesterone biosynthetic enzymes in granulosa cells. Several proteins essential for steroidogenesis, including steroidogenic acute regulatory protein (*Star*) and cholesterol side-chain cleavage enzyme (*Cyp11a1*; P450scc), are also induced in the ovary during gonadotrophin-induced ovulation (Ronen-Fuhrmann *et al*., 1998).

Several lines of evidence support a critical role of PGR in ovulation. Blockade of PGR function with a selective antagonist RU486 or CDB-2914 reduces the number of ovulated oocytes in mice (Loutradis *et al*., 1991; Palanisamy *et al*., 2006). The development of *Pgr*-null mice by Lydon *et al*. (1995) firmly established the essential role of PGR in ovulation. *Pgr*-null mice fail to ovulate because pre-ovulatory follicles are unable to undergo follicle-wall degradation and the oocytes remain trapped within luteinized follicles. These mutant mice exhibit, however, normal follicular development. Furthermore, the oocytes isolated from the ovaries of these mutant mice mature normally and are fertilizable (Robker *et al*., 2000). Interestingly, the granulosa cells of unruptured follicles in *Pgr*-null mice undergo luteinization, as indicated by the expression of the luteinization marker *Cyp11a1* (P450scc) (Robker *et al*., 2000).

PGR exists in two isoforms, PGR-A and PGR-B, which are generated from a single gene via alternative usage of two distinct promoters or alternative translation initiation from two different AUG initiation codons (Kraus *et al*., 1993). Both PGR-A and PGR-B proteins are expressed in the mouse ovary, although PGR-A is the predominant form (Shao *et al*., 2003). Accordingly, when PGR-A or PGR-B was selectively ablated in mice, PGR-A-null mice exhibited impaired ovulation, resulting in unruptured pre-ovulatory follicles (Mulac-Jericevic *et al*., 2000). In contrast, PGR-B-null mice showed normal ovulation (Mulac-Jericevic *et al*., 2003). These findings established that PGR-A, but not PGR-B, is the key isoform mediating the effects of progesterone during the ovulatory process (Conneely and Lydon, 2000; Conneely *et al*., 2002).

The anovulatory phenotype of the *Pgr*-null mouse with a distinct defect in follicle-wall breakdown presents a unique opportunity to investigate the molecular pathways underlying ovulation. It is generally believed that PGR, a transcription factor, controls the expression of a number of downstream target genes whose products function in an autocrine/paracrine manner within the ovarian tissue to control the ovulatory process. To decipher the precise molecular nature of progesterone action during ovulation, it is thus imperative to understand the identities of the PGR-regulated gene networks and the mechanisms by which these molecules interact with one another to control the biological events leading to follicle-wall degradation.

Identification of PGR-regulated gene networks that control ovulation

After the LH surge, PGR expression is induced primarily in mural granulosa cells of the pre-ovulatory follicles that are destined to ovulate. As a transcription factor, PGR is presumed to regulate the expression of its primary target genes in these cells. PGR modulates the transcription of these primary target genes by binding directly to specific regulatory sequences, termed progesterone response element, or by interacting with other promoter-bound transcription factors via protein–protein interaction (Li and O'Malley, 2003; Li *et al*., 2004; Sriraman *et al*., 2008). Because PGR appears to control the breakdown of the follicle wall leading to the release of oocytes, it is important to understand how the factors produced by its action in the mural granulosa cells mediate the rupture process by helping to break down the follicular wall. It is likely that some PGR-regulated factors are secreted by the granulosa cells and reach other cell types within the ovary, such as cumulus cells, theca cells and endothelial cells of blood vessels. These factors are likely to act as paracrine effectors to induce downstream pathways within the target cells, regulating critical events that facilitate follicle-wall degradation. Research during the past years has identified several genes acting downstream of PGR during the ovulatory process. In this review, we will highlight a few key genes which mediate PGR function during ovulation.

Richards and co-workers identified a disintegrin and metalloproteinase with thrombospondin motifs (*Adamts1*) as the first PGR-regulated gene that is expressed during the ovulatory process (Robker *et al*., 2000). Although its expression is not detectable in developing follicles, *Adamts1* is induced by hCG specifically in the granulosa cells of pre-ovulatory follicles prior to ovulation. This granulosa-cell-specific induction of *Adamts1* expression is impaired in *Pgr*-null ovaries, indicating that *Adamts1* acts downstream of *Pgr*. Mice deficient in *Adamts1* are subfertile due to their inability to ovulate, confirming that ADAMTS-1 is indeed a critical factor

mediating the ovulatory process of PGR (Mittaz *et al*., 2004; Shozu *et al*., 2005). Since the follicle wall needs to break down prior to oocyte release, it was proposed that ADAMTS-1, a protease, plays a key role in the degradation of the collagen layer. Amino acid sequence analysis of the ADAMTS family of proteins revealed a highly conserved metalloproteinase domain harboring a zinc-binding site, consistent with the prediction that the primary function of this protein family is to catalyze proteolysis (Andreini *et al*., 2005). Although it remains to be established that ADAMTS-1 is directly involved in the breakdown of a follicle wall, this protease displayed a capacity to cleave versican (chondroitin sulfate proteoglycan 2: CSPG2), a hyaluronan-binding extracellular-matrix proteoglycan, which accumulates during the expansion of a cumulus cell–oocyte complex (COC) (Russell *et al*., 2003). There is evidence in the literature that ADAMTS proteins are also associated with inflammatory processes and control of angiogenesis (Kuno *et al*., 1997; Porter *et al*., 2005).

A disintegrin and metalloprotease 8 (ADAM8) is another PGR-regulated gene that belongs to the ADAM family. It is primarily expressed in the granulosa cells of pre-ovulatory follicles during ovulation (Sriraman *et al*., 2008). Like other members of the ADAM family, it possesses an intrinsic metalloprotease and disintegrin domain, and is thought to perform proteolytic cleavage of the membrane-anchored precursors of active signaling molecules and release them in soluble forms into the intercellular space (Yamamoto *et al*., 1999). In the context of the paracrine mode of PGR signaling, one may envision that the proteolytic activity of ADAM8 is involved in releasing signaling molecules from mural granulosa cells, enabling them to act on other ovarian cell types to mediate the paracrine effects of PGR action. Further investigations are necessary to verify this plausible mechanism of ADAM8 action.

It is of interest to note that synaptosomal-associated protein 25 (SNAP25), another novel PGR-regulated gene, is a protein involved in membrane fusion mechanisms that control exocytosis (Shimada *et al*., 2007). It is a constituent of a soluble *N*-ethylmaleimide sensitive-factor attachment protein receptor complex, which mediates fusion of cellular transport vesicles with the cell membrane. SNAP25 plays a key role in the release of signaling molecules by facilitating the fusion of the vesicles, containing these molecules, with the plasma membrane (Sorensen, 2005). In the context of the ovary, it is proposed that SNAP25 contributes to the PGR-mediated ovulatory pathways by controlling the release of paracrine mediators from mural granulosa cells (Shimada *et al*., 2007). Consistent with this hypothesis, it was shown that blockade of SNAP25 function in granulosa-cell cultures decreases the secretion of the cytokine interleukin-6 (IL6), along with other cytokines, by these cells (Shimada *et al*., 2007).

That PGR controls a paracrine mode of signaling in the ovary is also supported by a previous study of endothelin-2 (EDN2), a potent vasoactive hormone (Palanisamy *et al*., 2006). Gene-expression profiling using ovaries of mice subjected to gonadotrophin-induced superovulation in the presence or absence of CDB-2914, a synthetic PGR antagonist, revealed that EDN2 is produced downstream of PGR action in mural granulosa cells of pre-ovulatory follicles immediately preceding ovulation (Palanisamy *et al*., 2006). Blockade of EDN2 action using a selective antagonist for endothelin receptor B (ETR-B) resulted in a profound inhibition of ovulation in rats and mice (Ko *et al*., 2006; Palanisamy *et al*., 2006). Based on the observation that ETR-B is expressed in the mural and cumulus granulosa cells of pre-ovulatory follicles as well as the capillaries lining the inner border of the theca interna, it is postulated that EDN2 released by the mural granulosa cells acts, in a paracrine manner, on the cumulus cells of COCs and the endothelial cells of the capillaries of theca interna to control gene expression that in turn contribute to follicle-wall breakdown.

Richards and co-workers identified additional PGR-regulated genes in the ovary, such as cathepsin L (*Ctsl*), cyclic-GMP-dependent kinase II (*Prkg2*; cGKII) and pituitary adenylate-cyclase-activating polypeptide (*Pacap*) (Ko *et al*., 1999; Robker *et al*., 2000; Sriraman and Richards, 2004; Sriraman *et al*., 2005). In a recent study to identify the PGR-regulated genes that participate in the ovulatory process, we compared the global changes in ovarian gene-expression profiles of wild-type (WT) and *Pgr*-null mice during gonadotrophin-induced superovulation. This microarray analysis uncovered ~300 genes whose expression was significantly altered in the *Pgr*-null ovaries compared with the WT ovaries at a time that shortly precedes follicular rupture (J.K., I.C.B. and M.K.B., unpublished results). These genes included several previously reported PGR-regulated genes, namely *Adamts1*, *Edn2*, *Snap25*, *Pparg* and *Adam8*, thereby strengthening the validity of our microarray analysis. When these microarray-derived genes were classified according to their known biological functions, they were found to encode diverse molecules such as proteases, transcription factors, growth factors, cell-adhesion molecules, modulators of vascular activities and regulators of inflammation. The PGR-regulated pathways are, therefore, linked to diverse biological processes, reflecting the overall complexity at the cellular and molecular levels that governs ovulation.

Novel insights into PGR regulation of inflammatory responses during ovulation

Ovulation has long been viewed as an inflammatory process. It has been proposed that the biological events driven by the LH surge in the ovary resemble an acute inflammatory response (Espey, 1980, 1994). Shortly after the pre-ovulatory LH surge, there is a significant rise in local circulation in the ovary, which persists until the time of follicle-wall degradation (Tanaka *et al*., 1989a, b). The LH-mediated pathways increase vasodilation and vascular permeability, creating a hyperemic condition within pre-ovulatory follicles. The increased vascular permeability drives the exudation of serum proteins and allows transmigration of leukocytes, primarily neutrophils and macrophages, from the blood vessels to the interior of the pre-ovulatory follicles (Brannstrom *et al*., 1993). In an inflamed tissue, migrating leukocytes secret proteases, damaging the tissue. Likewise, intrafollicular leukocytes may produce proteolytic enzymes that contribute to the disruption of the follicular wall at the time of ovulation.

Although it is unknown how this inflammatory condition results in the breakdown of the pre-ovulatory-follicle wall, especially at the molecular level, one can envision that the key molecules and signaling pathways implicated in inflammation may also play a critical role in the ovulatory process. Consistent with this hypothesis, recent ovarian gene-expression profiling experiments in our laboratory have indicated that several genes, such as *Edn2*, *Pparg* and *Il6*, are regulated by PGR during the ovulatory process (unpublished). Previous studies

have shown that these genes mediate or control inflammatory response in a variety of tissues (Rose-John *et al*., 2006; Filipovich and Fleisher-Berkovich, 2008; Tontonoz and Spiegelman, 2008).

EDN2 regulates blood-vessel dynamics by controlling the constriction or dilation of the vessels and plays a role in inflammation (Meidan and Levy, 2007; Filipovich and Fleisher-Berkovich, 2008). Identification of *Edn2* as a PGR-regulated gene essential for ovulation strengthened the notion that PGR-driven inflammatory reactions control ovulation (Palanisamy *et al*., 2006). It was shown recently that granulosa cells and COCs of pre-ovulatory follicles express innate immune-related genes, including several members of the Toll-like receptor surveillance system, the inflammatory cytokine IL6 and the scavenger receptor CD36 during the ovulatory process, pointing to an intimate link between ovulation and inflammation-like processes (Shimada *et al*., 2006a, b; Liu *et al*., 2008).

Our recent microarray study and those of others have revealed the induction of interleukin *Il6* in granulosa cells of ovulating follicles (Hernandez-Gonzalez *et al*., 2006; Liu *et al*., 2009; J.K., I.C.B. and M.K.B., unpublished results). Our recent unpublished studies using mouse primary granulosa cell cultures showed that addition of IL6 strongly enhances mRNA expression of *Ptgs2*, a vital inflammatory molecule and a known regulator of ovulation. We further observed that treatment with IL6 also up-regulated mRNA expression of vascular endothelial growth factor A (*Vegfa*), which promotes vascular permeability and, thus, likely contributes to the inflammation process during ovulation (unpublished). Recent studies indicated that IL6 produced during the ovulatory process regulates ovarian cumulus cell function (Liu *et al*., 2009). These results are consistent with the hypothesis that IL6 functions downstream of PGR to induce or modulate inflammatory pathways that contribute to ovulation. It is, however, unclear whether the regulation of IL6 by PGR is direct or indirect.

Recently, we identified peroxisome proliferator-activated receptor γ (PPARγ), a ligand-inducible transcription factor and regulator of inflammation, as a novel PGR-regulated gene with an important role in ovulation (Kim *et al*., 2008). An extensive literature describes PPARγ as a key regulator of adipocyte differentiation, lipid and glucose homeostasis, insulin sensitization and inflammation (Lee and Evans, 2002; Koutnikova *et al*., 2003; Lazar, 2005; Tontonoz and Spiegelman, 2008). There is also accumulating evidence in favor of a role for PPARγ in reproduction (Cui *et al*., 2002; Komar, 2005; Minge *et al*., 2008).

During superovulation, PPARγ is expressed primarily in the granulosa cells of pre-ovulatory follicles in a PGR-dependent manner. To address the functional role of this gene during ovulation, we created a conditional knockout mouse model by crossing mice harboring 'floxed' *Pparg* gene with progesterone receptor Cre knock-in (*Pgr-Cre*) mice. This resulted in the generation of females ($Pparg^{flox/flox}$ $Pgr^{Cre/+}$), in which the *Pparg* gene undergoes Cre-mediated excision in the mural granulosa cells of pre-ovulatory follicles. When *Pparg* conditional-null mice were subjected to gonadotrophin-induced superovulation, there was a drastic reduction in the number of released oocytes in mutant mice compared with $Pparg^{flox/flox}$ (control) mice. Upon histological examination of the ovaries of control and *Pparg* conditional-null mice at 18–19 h post-hCG, numerous corpora lutea were seen in the control ovary, whereas only a few corpora lutea and a large number of unruptured pre-ovulatory follicles were found in the mutant ovary. Loss of PPARγ signaling in the mural granulosa cells of mutant ovaries thus leads to impairment in follicular rupture. Gene-expression analysis identified a subset of PGR-regulated genes including *Edn2*, *Prkg2* and *Il6* as targets of regulation by PPARγ in the ovary. These results supported the concept that PPARγ functions as a mediator of certain of the biological actions of PGR in the ovulatory pathway.

It has been reported that PPARγ can be activated by an array of endogenous metabolites derived from fatty acids such as arachidonic acid and linoleic acid (Forman *et al*., 1995; Kliewer *et al*., 1995; Nagy *et al*., 1998; Schopfer *et al*., 2005). These fatty acid metabolites include prostaglandins, hydroxyeicosatetraenoic acids and hydroxyoctadecadienoic acids, which are not only potent signaling molecules but also inflammatory agents produced during ovulation (Tanaka *et al*., 1989a, b). In the ovary, PTGS2, an LH/hCG-induced enzyme that converts arachidonic acid into prostaglandins, plays a critical role in ovulation (Lim *et al*., 1997; Davis *et al*., 1999). Administration of indomethacin, a non-steroidal anti-inflammatory agent and an inhibitor of PTGS2, to rats undergoing gonadotrophin-induced ovulation, effectively inhibits ovulation (Tanaka *et al*., 1991). Interestingly, suppression of the synthesis of PTGS2-mediated inflammatory molecules by indomethacin or the selective inhibitor NS-398 decreased the expression of PPARγ-regulated genes, such as *Edn2*, *Prkg2* and *Il6*. Since these genes also operate downstream of PGR (Sriraman *et al*., 2005; Kim *et al*., 2008), this study uncovered an important link between PGR- and PTGS2-driven pathways. It is conceivable that metabolism of long-chain unsaturated fatty acids by PTGS2 in mural granulosa cells produces inflammatory molecules that serve as endogenous activating ligand(s) of PPARγ in the ovarian follicles and regulates the expression of its downstream target genes to control ovulation. This finding further supports the inflammation hypothesis of ovulation and offers an insight as to how seemingly distinct pathways regulated by PGR-PPARγ and -PTGS2, respectively, converge functionally to control the events leading to ovulation.

It is important to note that synthetic PPARγ ligands have been clinically used to treat polycystic ovary syndrome (PCOS), which is the most common cause of anovulatory infertility. Recent clinical studies indicated that treatment of subjects with PCOS with rosiglitazone, a potent PPARγ agonist, restores normal ovulation in 55–85% of these patients (Dereli *et al*., 2005; Cataldo *et al*., 2006). Despite the remarkable effectiveness of this treatment, the precise mechanisms underlying the therapeutic effects of the PPARγ agonist have remained unclear. In light of the importance of PPARγ in the ovulatory process, it is tempting to speculate that the treatment of PCOS by rosiglitazone may work via modulation of ovarian functions of PPARγ (Froment *et al*., 2005). This hypothesis, however, needs to be tested by further studies.

PGR regulates hypoxia signaling during ovulation

We recently identified genes encoding the hypoxia-inducible factors (HIFs), HIF-1α, HIF-2α and HIF-1β, as novel PGR-regulated genes in the granulosa cells of the pre-ovulatory follicles (Kim *et al*., 2009). These transcription factors serve as critical regulators of the tissue's response to changes in oxygen levels (Semenza, 2003). They form heterodimeric complexes consisting of one α subunit and one β subunit,

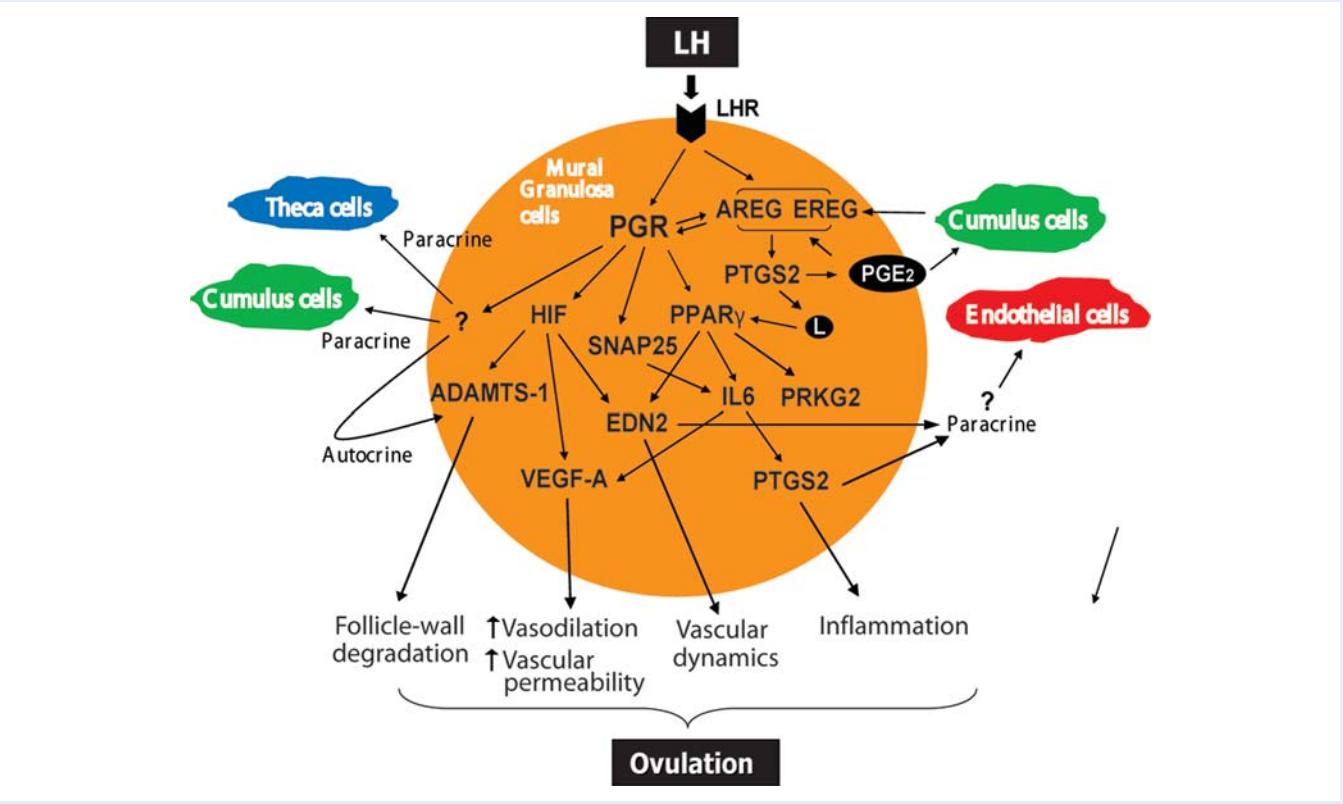

Figure 1: An emerging blueprint of PGR-regulated gene networks that control ovulation in the mouse. The schematic describes the PGR-regulated factors that are discussed in this review. The PGR, expressed in mural granulosa cells of pre-ovulatory follicles, regulates downstream genes such as peroxisome proliferator-activated receptors (PPARγ) and HIFs, which are important mediators of its function during ovulation. These genes in turn regulate the production of critical factors that are secreted and act on various ovarian cell types, such as cumulus granulosa cells, theca cells and vascular endothelial cells. Both PPARγ and HIFs control the production of endothelin (EDN2), which likely contributes to the follicle-wall degradation by influencing the dynamic changes in the vasculature during ovulation. PPARγ also controls the synthesis of IL6, which acts by regulating VEGF-A, which enhances vascular permeability, and PTGS2, which generates inflammation-inducing fatty-acid metabolites. The fatty-acid metabolites produced by PTGS2 promote ovulation by acting as agonists of PPARγ, thereby forming a classical regulatory loop. SNAP25, another PGR-regulated gene, may also participate in the ovulatory process by regulating the release of cytokines, including IL6, from granulosa cells. PGR also mediates the induction of the EGF-like growth factors, such as AREG and EREG, in granulosa cells. These factors in turn induce PTGS2, which generates prostaglandin E_2 (PGE_2). AREG and PGE_2 can then act on cumulus cells, via paracrine mechanisms, to induce AREG and PTGS2. Since ovulation also involves physical degradation of the follicular wall, the actions of proteases, such as ADAMTS-1, are essential during this process. The coordinated actions of the PGR-regulated pathways eventually bring about the breakdown of the pre-ovulatory follicle, releasing the oocyte. PRKG2, cyclic-GMP-dependent protein kinase; L, fatty-acid metabolites.

each represented by three isoforms that are encoded by distinct genes (Semenza, 2003; Pouyssegur *et al*., 2006; Keith and Simon, 2007). Under conditions of abundant oxygen (>8–10%), HIF-α proteins are expressed but rapidly degraded by an oxygen-dependent proteasomal activity. When the oxygen level in the tissue falls below the normal (<5%), the degradation of HIF-α proteins is prevented, leading to the accumulation of these proteins in the nucleus. The active HIF-α protein forms a heterodimeric complex with its binding partner HIF-1β, which is constitutively expressed, independent of oxygen levels. The HIF-αβ heterodimer then binds to the regulatory regions of target genes and controls their expression.

In mice, inhibition of the HIF transcriptional activity by echinomycin, a small-molecule inhibitor that suppresses the binding of HIF-αβ heterodimers to target genes, blocked gonadotrophin-induced superovulation by preventing the follicle-wall breakdown of pre-ovulatory follicles. Since a similar defect in follicular rupture is also observed in the *Pgr*-null mice, our results are consistent with the concept that HIFs are critical mediators of the ovulatory effects of PGR. Consistent with this scenario, the HIF-target genes in the ovary included *Adamts1*, *Edn2*, *Vegfa* and *Cxcr4*, which are also identified as PGR-regulated genes in our microarray analysis. Most importantly, *Adamts1*, *Edn2* and *Vegfa* are known to play critical roles during ovulation (Hazzard *et al*., 2002; Mittaz *et al*., 2004; Fraser *et al*., 2005; Shozu *et al*., 2005; Ko *et al*., 2006; Palanisamy *et al*., 2006).

Interestingly, the exposure of primary granulosa cells to the hypoxia mimetic cobalt chloride enhanced the levels of HIF-1α and HIF-2α proteins, which in turn stimulated the expression of HIF-target genes such as *Edn2*, *Vegfa* and *Cxcr4* (Kim *et al*., 2009). This result raised the possibility that a hypoxic condition is generated within the ovulatory follicles downstream of gonadotrophin action during ovulation. Presently there is no direct evidence indicating that such a condition is actually created *in vivo* within the intrafollicular

environment during ovulation. It is nevertheless conceivable that the rapid proliferation of granulosa cells in the growing follicles in response to gonadotrophin stimulation in conjunction with the lack of vasculature in the interior mural granulosa layers may give rise to a local hypoxic environment within the follicles (Neeman *et al*., 1997). This condition would prevent degradation of HIF-α proteins, leading to accumulation of the HIF-αβ heterodimeric complexes in the nucleus and subsequent induction of HIF-target genes to regulate the ovulatory process.

Is there a role of PGR in COC expansion?

A well-known biological consequence of the pre-ovulatory LH surge is the expansion of the COC (Eppig, 2001; Richards *et al*., 2002). This process involves accumulation of hyaluronan-rich extracellular matrix (ECM), leading to the generation of an expanded COC. The COC expansion is closely linked to ovulation because the lack of key structural components of the expanded COC matrix or factors regulating this process impairs oocyte release from the pre-ovulatory follicle (Richards *et al*., 2002; Richards, 2005). The previous report that versican, which is a hyaluronan-binding proteoglycan in the COC ECM and a potential cross-linker of the matrix components, is cleaved by the PGR-regulated protease ADAMTS-1 raised the possibility that PGR has a role in cumulus expansion that occurs prior to ovulation (Russell *et al*., 2003).

The role of PGR in cumulus expansion, however, remains unclear. Initial morphological examinations indicated that the COCs undergo expansion in *Pgr*-null mice (Lydon *et al*., 1995). However, our recent microarray-based gene-expression analyses at the time of ovulation revealed that the expression of many genes, such as *Tsg6*, *Ptx3* and *Has2*, which are known to be critical for expansion of COC matrix, is reduced in ovaries of *Pgr*-null mice compared with WT mice (unpublished observation). It has also been shown that expression of *Areg* (amphiregulin) and *Ereg* (epiregulin), EGF-like factors critical for COC expansion, is reduced in COCs and ovaries of *Pgr*-null mice (Shimada *et al*., 2006a, b). Moreover, IL6, a PGR-regulated cytokine, induces expansion of COCs and the expression of genes known to be involved in this process (Liu *et al*., 2009). It is thus conceivable that in the *Pgr*-null mice, subtle alterations, which are not discernable by morphological examination, occur in the composition of the COC matrix. These changes may contribute to functional abnormalities in the matrix that is generated, which in turn may hinder the rupture of pre-ovulatory follicles. If confirmed by further analysis, impairment in the formation of a functional COC matrix would represent an additional mechanism by which PGR regulates ovulation.

Conclusions

The failure of *Pgr*-null mice to ovulate in response to a gonadotrophin surge has uncovered an essential role of progesterone-dependent pathways in the regulation of ovulation. The present challenge is to determine how these PGR-controlled pathways work together to bring about follicle-wall breakdown. Gene-expression profiling experiments, aided by functional analysis using various knockout mouse models, have provided an initial blueprint of the signaling network that mediates the effects of PGR during ovulation (Fig. 1). It is increasingly becoming clear that ovulation, which resembles an acute inflammatory response, is associated with the production of several PGR-regulated factors that are potential regulators of inflammation. These include PPARγ, EDN2, ADAMTS-1, IL6, PTGS2, VEGF-A and possibly additional molecules. Continued exploration of these pathways will clarify the precise mechanisms by which the PGR-regulated factors act within the ovarian tissue in concert with each other to direct the program leading to ovulation. Moreover, this research has the potential to help develop novel contraceptives that will target key factors mediating the effects of PGR during ovulation. These contraceptives are likely to be non-steroidal in nature and, therefore, may have lesser side effects than their steroidal counterparts. The knowledge gained from this research may also help advance our understanding of the basis of various anovulatory disorders, such as PCOS, ultimately providing therapies to improve fertility in these patients.

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