Aus der Klinik für Frauenheilkunde und Geburtshilfe
der Heinrich-Heine-Universität Düsseldorf
Direktor: Univ.-Prof. Dr Tanja N. Fehm

Progesterone-induced Progesterone Receptor Membrane Component 1 Rise-to-Decline Changes are Essential for Decidualization

Dissertation

zur Erlangung des Grades eines Doktors der Medizin
der Medizinischen Fakultät der Heinrich-Heine-Universität Düsseldorf

vorgelegt von

Hailun Liu

2025

As an inaugural dissertation printed by permission of the
Faculty of Medicine at Heinrich Heine University Düsseldorf

Signed:
Dean: Prof. Dr. med. Nikolaj Klöcker
First examiner: Prof. Dr. med. Nikolas H. Stoecklein
Second examiner: PD Dr. Rüdiger V. Sorg

Summary

Infertility poses a significant global challenge, affecting above 15% of couples, with female infertility compromises nearly 40% of these cases. During human pregnancy, estrogen and progesterone drive the endometrial stromal cells to undergo a dramatic morphological and functional differentiation event, a process known as decidualization. Impaired decidualization is a major cause of unsuccessful pregnancies and infertility. PGRMC1, a non-classical progesterone receptor, is abundantly expressed in human endometrial stromal cells and has been proposed as a stabilizer in several infertility-related conditions, including endometriosis and repeated implantation failure. PGRMC1 mRNA and protein expression was found elevated during the proliferative phase of the menstrual cycle and subsequently decrease during the late secretory phase. This expression pattern emphasized the importance role of PGRMC1 in decidualization, which must be precisely tuned to promote fertility.

The prohibitin proteins (PHBs), prohibitin-1 (PHB1) and prohibitin-2 (PHB2), are ubiquitously expressed and highly conserved in eukaryotic cells, and primarily function in regulating mitochondrial integrity, cell proliferation, and apoptosis, contributing to cellular homeostasis and longevity(*1*). Besides, the PHBs are required to be maintained at an appropriate protein level, alongside PGRMC1, to support optimal fertility. Progestin-activated PGRMC1 could enhance the cellular proliferation of breast cancer cells by binding PHBs, leading to the activation of ERα signaling. This study aims to elucidate the functional roles of PGRMC1 and PHBs and their interplay in the decidualization process.

Using an *in vitro* decidualization induction model, we observed that PGRMC1 mRNA and protein levels display a characteristic rise-to-decline pattern, with the expression initially increased during the first 6 days post-induction (PGRMC1 increasing phase) and then decreased in subsequent days (PGRMC1 decreasing phase). Silencing PGRMC1 with siRNA prior to induction resulted in a failed decidualization, whereas knockdown post-induction did not disrupt the decidualization, suggesting the crucial nature of the ‘PGRMC1 increasing phase’ for decidualization. Additionally, the interactions between PGRMC1 and PHBs were induced during decidualization. Disruption of either or both PHBs partially hindered decidualization, demonstrating that PGRMC1 cooperates with PHBs to regulate the decidualization.

To the best of our knowledge, this is the first study to reveal that PGRMC1 expression follows a rise-to-decline pattern in an *in vitro* decidualization model and this pattern is necessary for successfully decidualization. The dynamic interplay between PGRMC1 and PHBs offers new perspectives into the mechanisms of decidualization and suggests potential therapeutic targets for maintaining infertility-related conditions.

Zusammenfassung

Unfruchtbarkeit stellt eine erhebliche globale Herausforderung dar, von der mehr als 15 % der Paare betroffen sind, wobei die weibliche Unfruchtbarkeit fast 40 % dieser Fälle ausmacht. Während der Schwangerschaft beim Menschen bewirken Östrogen und Progesteron, dass endometriale Stromazellen eine dramatische morphologische und funktionelle Differenzierung durchlaufen; ein Prozess, der als Dezidualisierung bekannt ist. Eine beeinträchtigte Dezidualisierung ist eine Hauptursache für erfolglose Schwangerschaften und Unfruchtbarkeit. PGRMC1, ein nicht-klassischer Progesteronrezeptor, wird in menschlichen endometrialen Stromazellen reichlich exprimiert und wird als Stabilisator in mehreren mit Unfruchtbarkeit verbundenen Zuständen vorgeschlagen, einschließlich der Endometriose und einem wiederholten Implantationsversagen. Die mRNA- und Proteinexpression von PGRMC1 ist während der proliferativen Phase des Menstruationszyklus erhöht und nimmt anschließend in der späten Sekretionsphase ab, was die wichtige Rolle von PGRMC1 in der Dezidualisierung, die präzise abgestimmt sein muss, um die Fruchtbarkeit zu fördern, unterstreicht.

Die Prohibitin-Proteine (PHBs), Prohibitin-1 (PHB1) und Prohibitin-2 (PHB2), sind ubiquitär exprimiert und in eukaryotischen Zellen hoch konserviert. Sie spielen eine primäre Rolle bei der Regulierung der mitochondrialen Integrität, Zellproliferation und Apoptose und tragen somit zur zellulären Homöostase und Langlebigkeit be. Darüber hinaus müssen die PHBs zusammen mit PGRMC1 auf einem angemessenen Proteinniveau gehalten werden, um eine optimale Fruchtbarkeit zu unterstützen. Progestin-aktiviertes PGRMC1 kann die Zellproliferation von Brustkrebszellen durch Bindung an PHBs verstärken, was zur Aktivierung der ERα-Signalgebung führt. Diese Studie zielt darauf ab, die funktionellen Rollen von PGRMC1 und PHBs sowie ihr Zusammenspiel im Dezidualisierungsprozess zu erläutern.

Unter Verwendung eines *in vitro*-Dezidualisierungs-Induktionsmodells beobach-teten wir ein charakteristisches „Anstieg-Abnahme-Muster“ für die mRNA- und Proteinspiegel von PGRMC1, wobei die Expression während der ersten 6 Tage nach der Induktion zunächst erhöht ist (PGRMC1-Zunahmephase) und dann in den folgenden Tagen abnimmt (PGRMC1-Abnahmephase). Die Suppression von PGRMC1 mit siRNA vor der Induktion inhibierte den Dezidualisierungs-Vorgang, während ein *Knockdown* nach der Induktion die Dezidualisierung nicht störte. Dies deutet auf die entscheidende Funktion der „PGRMC1-Zunahmephase“ für die Dezidualisierung hin. Zusätzlich wurden Protein-Protein-Interaktionen zwischen PGRMC1 und PHBs während der Dezidualisierung beobachtet. Dabei behinderte die reduzierte Expression von einem oder beiden PHBs die Dezidualisierung teilweise, was darauf hindeutet, dass PGRMC1 mit PHBs bei der Regulation der Dezidualisierung kooperiert.

Nach unserem besten Wissen ist dies die erste Studie, die aufzeigt, dass die Expression von PGRMC1 in einem *in vitro*-Dezidualisierungsmodell einem „Anstieg-Abnahme-Muster“ folgt, welches für eine erfolgreiche Dezidualisierung

notwendig ist. Das dynamische Zusammenspiel zwischen PGRMC1 und PHBs bietet neue Perspektiven in die Mechanismen der Dezidualisierung und schlägt potenzielle therapeutische Ziele vor, um Zustände im Zusammenhang mit Unfruchtbarkeit zu behandeln.

List of abbreviations

Abbreviation	Full Name
ATP	Adenosine triphosphate
AKT	Protein kinase B, PKB
ART	Assistive reproductive technology
ANOVA	Analysis of variance
BAP31	B-cell receptor-associated protein 31
CYB5D2	Cytochrome B5 Domain-Containing Protein 2
CD	Cytoplasmic domain
Cytb5	C-terminal cytochrome b5-like domain
CPI	Chronic placental inflammation
Co-IP	Co-immunoprecipitation
cDNA	Complementary DNA
DSCs	Decidual stromal cells
Dap1	Damage-associated response protein 1
DMEM	Dulbecco's modified eagle medium
DNA	Deoxyribonucleic acid
E2	Estrogen
ESCs	Endometrial stromal cells
EMC	Extracellular matrix
ESR1	Estrogen receptor alpha
EC	Extracellular domain
ER	Endoplasmic reticulum
EGFR	Epidermal growth factor receptor
ESE	Early secretory
et al.	*Et alii* (Maskulinum), *et aliae* (Femininum) or *et alia* (Neutrum)
FOXO1A	Forkhead box protein A1
FBS	Fetal bovine serum
GST	Glutathione S-transferase
GEO	Gene expression omnibus
Hpr6.6	Human progesterone receptor 6.6
HCCs	Hepatocellular carcinomas
HESCs	Human endometrial stromal cells
HPRT1	Hypoxanthine phosphoribosyl transferase 1
IGFBP1	Insulin-like growth factor binding protein 1
IZA	Inner zone antigen
ITAMs	Immunoreceptor tyrosine-based activation motifs
Insig	Insulin-induced gene
kDa	Kilodalton
LES	Late secretory
MSE	Mid-secretory
MAPR	Membrane-associated progesterone receptor
MAOB	Monoamine oxidase B

MyoD	Myogenic differentiation
MEF2	Myocyte enhancer factor 2
MMP	Mitochondrial membrane potential
MPA	Medroxyprogesterone acetate
mM	Millimolar
mRNA	Messenger RNA
nM	Nanomolar
PRL	Prolactin
P4	Progesterone
PCOS	Polycystic ovary syndrome
PGR	Progesterone receptor
RPL	Recurrent pregnancy losses
PGRMC1	Progesterone receptor membrane component 1
PGRMC2	Progesterone receptor membrane component 2
PHB1/2	Prohibitin 1/2
PHBs	Prohibitins
PCC	Persister cancer cells
PBS	Phosphate-buffer saline
PLA	Proximity ligation assay
PAR	Proteinase-activated receptor
PE	Proliferative
pRb	Rb tumor suppressor protein
qRT-PCR	Quantitative reverse-transcription polymerase chain reaction
REA	Repressor of estrogen receptor activity
ROS	Reactive oxygen species
RIF	Repeated implantation failure
RNA	Ribonucleic acid
RT	Room temperature
SREBP	Sterol regulatory element binding protein
Scap	SREBP cleavage activating protein
SERBP1	Serpin mRNA-binding protein 1
SH2	Src homology 2
SH3	Src homology 3
STAT3	Signal transducer and activator of transcription 3
siRNA	Small interfering RNA
TM	Transmembrane domain
TP63	Tumor protein 63
T-HESCs	hTERT-immortalized HESCs
WOI	Window of implantation
μM	Micromolar
8-Br-cAMP	8-Bromoadenosine 3', 5'-cyclic monophosphate

Table of contents

1. Introduction

1.1 Human Endometrium

1.1.1 Structure and Function

The endometrium, a complex multicellular steroid-target tissue, lines the inner surface of the uterus(*2*). In humans, the endometrium is composed of luminal and glandular epithelial cells, stromal fibroblasts, vascular smooth muscle cells and endothelial cells, which together build the basal and functional layers. The glandular epithelial layer is formed by epithelial cells, which are considered to be in a highly differentiated state. The stroma, containing cells of mesodermal origin, along with epithelial cells, functions as the basal layer to support the functional layer(*3*). The functional layer is shed during menstruation due to the withdrawal of progesterone (P4). The subsequent tissue repair and regeneration during the proliferative phase, which involves re-growth of the glands, stroma, and blood vessels, are driven by rising levels of estrogen. Following ovulation, the glands enter into the secretory phase and the stromal cells begin to decidualize, a process unique to higher simian primates(*2, 4, 5*). Without pregnancy, the functional layer is shed during menstruation, and the whole cycle recommences(*6*).

Histologically, the endometrium can be distinguished into menstrual, proliferative, early-, mid-, and late-secretory phases(*7*). During the secretory phase, the endometrium enters a narrow window of receptive state that is ideal for fertilization and embryo implantation, termed the window of implantation (WOI)(*8*).

1.1.2 Menstrual Cycle

Menstruation is the endometrial response to progesterone withdrawal following the demise of the corpus luteum in the absence of pregnancy. It is meticulously governed by a synergy of endocrine, autocrine and paracrine factors, which oversee ovarian follicular development, ovulation, luteinization and endometrial remodeling(*9*).

The normal menstrual cycle consists of three phases: the follicular (proliferative) phase, the ovulatory phase, and the luteal (secretory) phase(*10*). The follicular phase,

marking the beginning of the menses and concluding with ovulation, varies from 7 to 22 days, averaging at 14 days, and is characterized by estrogen-driven proliferative changes. During ovulation, the mature follicle ruptures and releases an oocyte, then transforming into a corpus luteum, signifying the onset of the luteal phase. The duration of luteal phase are 14 days and is more constant in length compared to the follicular phase. Progesterone, secreted by the corpus luteum, propels the endometrium from a proliferative to a secretory status, preparing for implantation(*11-13*).

1.1.3 Decidualization

Derived from the Latin verb decider, the term decidua means to die, to fall off, or to detach(*14*). The decidua is the maternal tissue intimately associated with the fetoplacental unit and serves a vital role as an endocrine and immunological organ(*15*). The process of implantation results in the formation of three decidual regions, which are decidua basalis, decidua capsularis, and decidua parietalis. These regions are anatomically and functionally distinct, with the decidua establishing a favorable environment for the embryo and protecting the mother from uncontrolled trophoblast invasion(*16-19*).

Decidualization marks the transition of fibroblast-like endometrial stromal cells (ESCs) into larger, epithelioid-like decidual stromal cells (DSCs), with some becoming polyploid or multinuclear(*20, 21*). This phase is characterized by cellular changes, such as nucleus rounding, increased size of nucleoli, dilation of the endoplasmic reticulum and Golgi apparatus, and the accumulation of glycogen and lipid droplets(*22, 23*), reflecting an extensive cellular reprogramming and overall endometrial remodeling to foster an environment conducive to embryo implantation and maternal immune tolerance(*24-26*). Besides, decidualization encompasses the reprogramming of gene families involved in extracellular matrix (ECM) organization, cell adhesion, cytoskeletal organization, signal transduction, metabolism, stress response, cell cycle progression, and apoptosis(*27, 28*).

Owing to ethical constraints preventing the direct study of human decidualization *in vivo*, *in vitro* cell culture models have been developed. These models began with the isolation and culture of endometrial fibroblasts from women(*29, 30*), and have expanded to include immortalized telomerase-expressing endometrial stromal cell lines(*20, 31-35*). Decidual transformation *in vitro* requires progesterone and extended culture(*34, 36*), but can be enhanced by the addition of cAMP analogs (dbcAMP, 8-Br-cMAP)(*37*) and estrogen(*38*), as measured by increased gene expression of decidualization markers, such as insulin-like growth factor binding protein 1 (*IGFBP1*), forkhead box protein A1 (*FOXO1A*), and prolactin (*PRL*)(*39*).

A well-orchestrated decidualization is a critical determinant of pregnancy success, and its disruption can lead to various clinical issues related to implantation, early pregnancy and late gestation.(*40*).

1.2 Women Infertility

1.2.1 Epidemiology and Pathogenesis

Infertility is a disease characterized by the failure to achieve a clinical pregnancy after one year of regular, unprotected intercourse. It affects an estimated 8 to 12% of reproductive-aged couples worldwide(*41-43*). Infertility can be further categorized as primary or secondary, based on whether a clinical pregnancy has been previously achieved(*44*).

Primary infertility of female origin is attributed to hormonal, functional or anatomical dysfunction of the organs of the reproductive tract. It is a complicated disorder influenced by various factors such as systemic diseases, like diabetes and obesity, endocrine disruptors and lifestyle choices that affect reproductive function(*42, 45*). Besides, up to 30% of cases of infertility cases remain unexplained(*46*). Known reasons include premature ovarian insufficiency, poor egg quality, polycystic ovary syndrome (PCOS), and fallopian tubal blockage.

A healthy endometrium is the prerequisite and guarantee for reproduction, making endometrial-factor induced infertility a significant pathology among fertility disorders.

Endometrial injuries, whether from surgical procedures or other causes, can lead to menstrual irregularities, abortion, amenorrhea, infertility and other gynecological issues. Common suboptimal function of the endometrium is characterized by failure to achieve ideal decidualization, including the thin endometrium or an endometrial atrophy, Asherman's syndrome, and intrauterine adhesions(*47-50*).

Infertility continues to affect roughly 10% of the global female population, with the World Health Organization ranking it as the fifth highest serious global disability among young individuals(*51*). Despite considerable efforts to understand the biological mechanisms underlying female fertility, developing predictive methods for reproductive outcomes or treatments to prevent pregnancy failure remains challenging.

1.2.2 Clinical Consequences of Defective Decidualization

The process of decidualization forms a receptive tissue suitable for implantation, and this process depends primarily on the effect of progesterone on the estradiol-primed progesterone receptors expressed by the endometrial stromal cells(*52*). Estrogen, acting through the estrogen receptor alpha (ERa), enhances the expression of progesterone receptors (PGR), allowing the endometrium to respond to increased progesterone levels to initiate decidualization during the mid-late secretory phase(*19, 53*).

In early pregnancy, decidualization forms the decidua, a tissue crucial for detecting and responding to placental signals, protecting the fetus from the maternal immune responses and oxidative stress(*54*). The main functions of decidualization are: 1) inhibiting epithelium proliferation(*55*); 2) protecting the embryo in the maternal-fetal interface from the maternal immune and oxidative stress(*54*); 3) regulating the immune response to control the trophoblast invasion(*56*); 4) allowing angiogenesis to prepare the maternal vasculature to support embryonic development during pregnancy(*57*).

Failures in decidualization can result in impaired utero-vascular remodeling, observed in intrauterine growth restriction(*58*). Aberrant decidualization is related to premature senescence, recurrent pregnancy loss (RPL), and preeclampsia(*21, 59-62*). Furthermore, decidualization defects are evident in disorders with hormonal and metabolic dysfunctions of the endometrium, such as endometriosis, adenomyosis, and PCOS(*63-67*).

1.3 Progesterone Receptor Membrane Component 1 (PGRMC1)

1.3.1 Discovery and Nomenclature

Progesterone receptor membrane component 1 (PGRMC1) is a highly conserved heme-binding protein found in multiple biological contexts(*68*). It was initially identified in mammalian systems, first purified and cloned from porcine liver membranes in 1996 by Meyer and colleagues. Human PGRMC1 was subsequently cloned in 1998 and designated human progesterone receptor 6.6 (Hpr6.6)(*69-72*). The gene encoding PGRMC1, isolated from the livers of dioxin treated rats, was named 25-Dx due to its induction by non-genotoxic carcinogens. In 2001, PGRMC1 was identified in the rat adrenocortical cells, and referred to as inner zone antigen (IZA). The cDNA encoding IZA was found to encode the previously reported putative membrane-associated progesterone receptor (MAPR) and 25-Dx(*73, 74*). Additionally, yeast homologues of PGRMC1 were identified and cloned from *Saccharomyces* cerevisiae, named Dap1 (Damage-associated response protein 1)(*75, 76*).

1.3.2 Function and Structure

PGRMC1 is a multifunctional protein involved in various processes such as progesterone responsiveness, heme binding, cytochrome P450 enzymes regulation, cholesterol and drug metabolism, steroidogenesis, vesicle trafficking, and cell survival in both normal and cancerous cells(*77, 78*). Besides binding to P450 enzymes, PGRMC1 also interacts with cholesterol-sensing proteins like Insig (insulin-induced gene) and Scap (SREBP cleavage activating protein) in Cos7 cells to form a regulatory complex controlling fatty acid lipogenesis and cholesterol synthesis(*79, 80*). In ovarian cells, PGRMC1 binds to the polypeptide serpin mRNA-binding protein 1

(SERBP1) to form progesterone membrane receptor complex, activating protein kinase G, regulating Ca^{2+} levels, and mediating an antiapoptotic effect(*81*).

PGRMC1 belongs to a family of membrane-associated progesterone receptor (MAPR) proteins, which includes PGRMC2, Neudesin, and Neuferricin (Cytochrome B5 Domain-Containing Protein 2/CYB5D2)(*82*). The PGRMC1 protein consists of 195 amino acids and comprises a short luminal peptide, a single N-terminal extracellular domain (EC), a cytoplasmic domain (CD) and a helical transmembrane domain (TM), a C-terminal cytochrome b5-like domain (cyt*b*5)(*83, 84*). The calculated mass of PGRMC1 is around 21.7 kDa, its molecular weight may vary slightly due to phosphorylation states, typically around 25 kDa(*84-86*). The putative structure of PGRMC1 includes several peptide motifs, include a Src homology 3 (SH3) target sequence at P63, two Src homology 2 (SH2) target sequences at Y139 and Y180, which require Tyr-phosphorylation for interaction with SH2 domains(*77, 84, 87*). Runko et al. revealed PGRMC1 contains several YXXΦ motifs (Φ is a large hydrophobic amino acid), that are also known as immunoreceptor tyrosine-based activation motifs (ITAMs), which are involved in cellular protein trafficking and functions include: internalization of receptors after ligand-binding, targeting membrane proteins to membrane compartments, and interacting with vesicle coat protein adaptor protein complexes(*77, 88*). Crystallographic studies have shown that PGRMC1 can form stable dimers mediated by heme-binding(*89*).

1.3.3 Localization and Interactions

PGRMC1 is expressed highly in tissues such as the liver and kidney, but it is also found in the brain, breast, heart, lung, pancreas, skeletal muscle and many other tissues(*71, 90, 91*). It localizes to multiple subcellular compartments, including the endoplasmic reticulum, Golgi apparatus, nuclear and plasma membranes, endosomes and secretory vesicles(*92-94*). PGRMC1 has also been detected in the nucleus, specifically in the nucleolus(*95*). Its subcellular localization can be regulated by post-translational modifications, including phosphorylation, ubiquitination, and Sumoylation(*86*). Sumoylation was thought to be associated with PGRMC1

translocation to the nucleus(*96*). In granulosa cells, PGRMC1 localization changes during ovulation induction and oocyte maturation(*97, 98*).

In breast cancer, PGRMC1's interaction with prohibitin proteins impacts cancer progression by inhibiting their function as transcriptional repressors(*99*). *PGRMC1* also affects hepatocellular carcinomas (HCCs) development by regulating EGFR (epidermal growth factor receptor)-mediated inflammatory responses(*100*). Moreover, upregulation of PGRMC1 in paclitaxel-tolerant persisted cancer cells (PCC) is related to increased free fatty acids, lipid droplets, and fatty acid oxidation compared to parental cells. PGRMC1 expression substantially enhances ferroptosis by xCT (xc^- cystine/glutamate antiporter) inhibition, mediated by lipophagy and tubulin de-tyrosination in PCC(*68*).

1.3.4 PGRMC1 in the Female Reproductive System

In the female reproductive system, the physiological effects of progesterone on target cells are traditionally mediated by the nuclear progesterone receptor (PGR) and its isoforms (PGRA and PGRB), the mechanism referred to as genomic pathway(*101*). Disruptions in progesterone signaling resulted in a wide range of reproductive conditions, including endometriosis, infertility, pregnancy complications, and endometrial cancers. However, PGR may not be the sole receptor mechanism for eliciting progesterone actions, as cells lacking PGR expression can still respond progesterone(*102*). PGRMC1, as a nonclassical progesterone receptor, mediates antiapoptotic and antimitotic progesterone activities in granulosa cells(*103*), ovarian(*104*), breast(*99*), and endometrial cells(*105*). Some studies have shown that progesterone modulates immune cell functions in cells completely lack of classical nuclear PGR(*106*).

The first report of PGRMC1 expression in the uterus stemmed from a microarray study, revealing down-regulated *PGRMC1* mRNA from the proliferative to the secretory phase of the human menstrual cycle(*107*). This result was corroborated by data from primate tissue microarrays (GSE4888 and GSE56364, https://www.ncbi.

nlm.nih.gov/geo/)(*108, 109*) and proteomics-based research in women(*110, 111*). PGRMC1 cellular distribution was most abundant during the proliferative phase in both epithelial and stromal cells. By the last secretory phase, PGRMC1 expression was restricted to most deeper basal layer (the basalis), formed by the basal region of the glands, a dense stroma, and blood vessels, and scattered stromal cells within the upper layer (the functionalis), which contains glands loosely held together by supportive stroma, with individual luminal epithelium cells also expressing PGRMC1(*101*). PGRMC1 expression is highly regulated at the maternal-fetal interface in humans, showing a distinct pattern compared to PGR expression. During the preimplantation period, while PGR expression is lost in the luminal epithelium, PGRMC1 expression persists, suggesting a distinct uterine role for this progesterone mediator(*81*).

1.3.5 Role of PGRMC1 in Decidualization

In non-decidualized endometrial stromal cells (ESCs), PGRMC1 localizes primarily in the membrane and cytoplasm. However, during decidualization, PGRMC1 tends to concentrate near or at the nucleus. Optimal levels of PGRMC1 are required for the decidualization process, as low levels are necessary for its occurrence, while overexpression inhibits decidualization *in vitro*(*105*).

Salsano et al. using pulldown assays and mass spectrometry identified over 20 new significant GST-PGRMC1-precipitated proteins in decidualization. These proteins mainly function within endomembrane system and mitochondria, involved in activities such as adenosine triphosphate (ATP) generation and transport, protein biosynthesis, vesicle trafficking, and protection against oxidative stress. This suggests PGRMC1's involvement in profound remodeling of ESCs during decidualization. The non-modified form of PGRMC1 interacts with B-cell receptor-associated protein 31 (BAP31) in the endoplasmic reticulum (ER), while post-translational-modification of PGRMC1 may enable interaction with monoamine oxidase B (MAOB)(*112*). Decreased PGRMC1 expression in endometrial stroma cells may expedite decidualization and cellular senescence via the upregulation of FOXO1 expression for

appropriate ESCs remodeling and embryo implantation during the secretory phase(*113*). MicroRNA miR-98 has been implicated as a potential repressor of PGRMC1, upregulated during decidualization. Transfection of ESCs with a miR-98 mimic resulted in decreased PGRMC1 protein levels, revealing that miR-98-mediated downregulation of endometrial PGRMC1 may promote decidualization(*113*).

1.3.6 PGRMC1 and AG205

AG205, a PGRMC1 antagonist, is a small molecule designed through in-silico modelling targeting the heme-binding pocket of the *Arabidopsis* MAPR protein encoded by the *AT2G24940* gene(*77, 114*). AG205 was demonstrated to inhibit PGRMC1-dependent antiapoptotic effects in cultured lung cancer cells(*115*). Since then, AG205 has been utilized in various studies to investigate or validate PGRMC1 functions(*116-118*). AG205 interacts with the cytochrome b5/heme-binding domain of PGRMC1(*119*). This domain is the site within PGRMC1 that interacts with various proteins(*77*). For instance, disrupting the PGRMC1-PGRMC2 interaction with AG205 can affect the regulation of human granulosa cells' entry into the cell cycle and the survival of spontaneously immortalized granulosa cells(*120, 121*).

In the human endometrium, PGRMC1 inhibition by AG205 did not impede decidualization. AG205-treated ESCs still underwent remodeling, exhibiting polygonal morphology with stress fiber loss from the center of the cell body and even significantly increased prolactin levels(*122*). However, some publications have raised concerns regarding the specificity of AG205 towards PGRMC1. Knocking out *PGRMC1* and/or *PGRMC2* expression did not alter the ability of AG205 to induce large endosome formation in CHO-K1 and HeLa cells(*123*). Isothermal titration calorimetry analysis showed no binding activity of AG205 to apo- or heme-dimerized PGRMC1(*124*). In two endometrial cell lines, Thieffry et al. observed that AG205 significantly increased expression of genes coding enzymes of the cholesterol biosynthetic pathway or of steroidogenesis, which were not reproduced with cells transfected with siRNA against PGRMC1. Furthermore, AG205 retained its ability to

upregulate selected target genes even when downregulated PGRMC1 expression(*125*).

1.3.7 PGRMC1 and Infertility-related Diseases

Beyond the regular menstrual cycle, PGRMC1 expression changes in chronic placental inflammation (CPI) and several infertility-related diseases including repeated implantation failure (RIF), and endometriosis (EMS). To understand these expression changes, investigations into PGRMC1's expression profile were conducted by mining publicly available RNA-sequencing data sets from endometrial biopsies.

Reduced PGRMC1 expression was observed in fetal membranes among women with preterm premature rupture of the membranes(*126*), in ectopic and eutopic endometrial stroma of women with endometriosis(*127*). In a study McCallum *et al*. in 2016, mice with floxed *Pgrmc1* alleles exhibited subfertility and endometrial cysts when deficient in *Pgrmc1* through $Amhr^{2cre/+}$. Further analysis showed differential regulation of PGRMC1 in receptive vs non-receptive endometrial in women undergoing in vitro fertilization therapy(*101, 128*), indicating its significant role in fertility regulation and uterine health. Ablation of this progesterone receptor resulted in subfertility and potentially premature aging of endometrial tissue, earning PGRMC1 the moniker 'fertility stabilizer'.

1.4 Prohibitin proteins

1.4.1 Structure and Function

Prohibitin proteins, encompassing prohibitin 1 (PHB1) and prohibitin 2 [PHB2, also referred to as repressor of estrogen receptor activity (REA) or B-cell receptor associated protein (BAP)-37], are ubiquitously expressed proteins belonging to the stomatin, prohibitin, flotillin, and Hf1K/C superfamily(*129*). The *PHB1* gene – first isolated and characterized to code for a candidate anti-proliferative protein in rat liver cells(*130*) – is spanning approximately 11 kb on chromosome 17q21 with 7 exons, while *PHB2* gene is located on chromosome 12p13, comprising 10 exons with smaller

introns than *PHB1*(*131, 132*). Both *PHB1* and *PHB2* belong to the evolutionarily conserved band-7 family, or prohibitin domain family(*133*).

Both PHB1 and PHB2 proteins are widely distributed in different cellular components, including mitochondria, cell membrane and nucleus(*134*). Notably, their functions differ significantly based on intercellular localization. In the mitochondria, PHB1 forms a heterodimeric ring-like complex to PHB2 to stabilize mitochondria(*135*), crucial for cellular energy production. Alterations in PHB1 or PHB2 protein expression can impact mitochondrial processes(*136*). In the nucleus, prohibitin proteins have a significant role in regulating transcription factors directly and indirectly, such as estrogen receptor (PHB1/2), E2F family members (PHB1), p53 (PHB1), MEF2 (PHB2), MyoD (PHB2), STAT3 (PHB1), and orphan nuclear hormone receptors (PHB2)(*134, 137, 138*). PHB1, expressed in the nucleus of leukemic cells, served as useful biomarker for leukemia subtypes(*139*), while PHB2, phosphorylated by AKT at Ser-91, induces the differentiation of acute promyelocytic leukemia cells(*140, 141*). On the plasma membrane, PHB1 act as a transmembrane adapter, activating downstream signals via interaction with low density detergent-insoluble lipid raft domains(*142, 143*), PHB1 and PHB2 are present on the surface of platelets and lipid rafts, associating with proteinase-activated receptor (PAR)-1. Disruption of the PHB1-PHB2-PAR-1 complex can cause a loss in granular secretion, calcium mobilization and platelet aggregation(*144*). Widely expressed in diverse cancers, both at mRNA and protein levels(*145*), PHB1 and PHB2 contribute to cancer development processes, such as proliferation, apoptosis and metastasis(*129, 146*).

In breast cancer, PHBs localize predominantly to the mitochondria in normal breast epithelium but become nuclear in breast cancer cells(*147, 148*). PHB1 disrupts the E2F1/retinoblastoma protein (pRb) interaction in the nucleus, affecting cell cycle dependent transcription and division(*134, 137, 147*). PHB2, studied as a potential tumor suppressor in luminal breast cancer, suppresses ER alpha signaling when overexpressed(*149*).

1.4.2 Prohibitin Proteins in Endometrium

In patients with endometriosis, PHB1 expression is significantly elevated in granulosa cells. This elevated expression correlates with increased levels of glycolysis-related enzymes, glucose consumption, and lactic acid production. Additionally, down-regulation of PHB1 alters cell proliferation and apoptosis rates, ATP synthesis, reactive oxygen species (ROS) levels, and mitochondrial membrane potential (MMP) in granulosa cells. These findings suggest that PHB1 plays a crucial role in mitigating energy loss due to impaired mitochondrial function in granulosa cells of endometriosis patients, potentially contributing to compromised fertility in these individuals(*150, 151*).

Park *et al*. mentioned that PHB2 (REA) has gene dose-dependent activity impacting uterine development and fertility. Conditional homozygous mutant ($REA^{d/d}$) mice developed to adulthood and showed normal ovarian function, but females were infertile with severely compromised uterine development and function characterized by cell cycle arrest, apoptosis, and altered adenogenesis, resulting in implantation and decidualization failure. In contrast, heterozygous REA mutant ($REA^{f/d}$) female mice display subfertility with reduced litter numbers and sizes(*152*). Physiologically, REA regulates endometrial stromal cell decidualization, controlling its timing and magnitude to ensure proper decidualization with embryo development, essential for successful implantation and optimal fertility(*153*).

1.4.3 Prohibitin Proteins and PGRMC1

In breast cancer, PGRMC1 interacts with ER alpha activation. Prohibitins interact with PGRMC1, particularly dependent on S181-phosphorylation induced by proliferation-promoting progestins. Phosphorylated PGRMC1 associates with PHBs, preventing their association with ERa, which means PHBs play an important role in inhibiting ERa and breast cancer tumorigenesis. However, PGRMC1 hinders their antiproliferative function, highlighting a complex interplay between these proteins in breast cancer development(*99*).

PGRMC1 is regard as a fertility stabilizer. Similarly, optimal levels of PHBs are required for endometrial function and fertility. Currently, researches on the specific role of PGRMC1 and PHBs in decidualization remain fragmented and sporadic, lacking a systemic and comprehensive approach. In breast cancer cells, progestin-activated PGRMC1 seems to associate with PHBs to stimulate cellular, progression. Binding of activated PGRMC1 to PHBs is accompanied by decreased PHBs-ERα-interaction, resulting in elevated expression of ER-dependent genes. But whether the progestin-depended interaction between PHBs and PGRMC1 also occurs during decidualization has never been characterized before. Therefore, the role of their interaction with regards to female fertility remains to be elucidated.

2. Aim of the Study

This study aims to elucidate the functional roles of PGRMC1 and PHBs, as well as their interplay in successful decidualization. This includes profiling the basic expression of PGRMC1 at the protein and mRNA levels, establishing an *in vivo* decidualization model, and utilizing public RNA-sequencing datasets. Furthermore, we investigate the effects of downregulating PGRMC1 and PHBs expression on decidualization.

The following publication, summarizing part of my doctoral work, forms the cornerstone of this dissertation.

3. Publication

Liu *et al.*
Reproductive Biology and Endocrinology (2024) 22:20
https://doi.org/10.1186/s12958-024-01188-9

Reproductive Biology and Endocrinology

RESEARCH Open Access

Progesterone-induced progesterone receptor membrane component 1 rise-to-decline changes are essential for decidualization

Hailun Liu[1], André Franken[1], Alexandra P. Bielfeld[2], Tanja Fehm[1], Dieter Niederacher[1], Zhongping Cheng[3,4], Hans Neubauer[1*†] and Nadia Stamm[1*†]

Abstract

Background Decidualization of endometrial cells is the prerequisite for embryo implantation and subsequent placenta formation and is induced by rising progesterone levels following ovulation. One of the hormone receptors contributing to endometrial homeostasis is Progesterone Receptor Membrane Component 1 (PGRMC1), a non-classical membrane-bound progesterone receptor with yet unclear function. In this study, we aimed to investigate how PGRMC1 contributes to human decidualization.

Methods We first analyzed PGRMC1 expression profile during a regular menstrual cycle in RNA-sequencing datasets. To further explore the function of PGRMC1 in human decidualization, we implemented an inducible decidualization system, which is achieved by culturing two human endometrial stromal cell lines in decidualization-inducing medium containing medroxyprogesterone acetate and 8-Br-cAMP. In our system, we measured PGRMC1 expression during hormone induction as well as decidualization status upon PGRMC1 knockdown at different time points. We further conferred proximity ligation assay to identify PGRMC1 interaction partners.

Results In a regular menstrual cycle, PGRMC1 mRNA expression is gradually decreased from the proliferative phase to the secretory phase. In in vitro experiments, we observed that PGRMC1 expression follows a rise-to-decline pattern, in which its expression level initially increased during the first 6 days after induction (PGRMC1 increasing phase) and decreased in the following days (PGRMC1 decreasing phase). Knockdown of PGRMC1 expression before the induction led to a failed decidualization, while its knockdown after induction did not inhibit decidualization, suggesting that the progestin-induced 'PGRMC1 increasing phase' is essential for normal decidualization. Furthermore, we found that the interactions of prohibitin 1 and prohibitin 2 with PGRMC1 were induced upon progestin treatment. Knocking down each of the prohibitins slowed down the decidualization process compared to the control, suggesting that PGRMC1 cooperates with prohibitins to regulate decidualization.

Conclusions According to our findings, PGRMC1 expression followed a progestin-induced rise-to-decline expression pattern during human endometrial decidualization process; and the correct execution of this expression program

†Hans Neubauer and Nadia Stamm contributed equally to this work.

*Correspondence:
Hans Neubauer
Hans.Neubauer@med.uni-duesseldorf.de
Nadia Stamm
Nadia.Stamm@med.uni-duesseldorf.de
Full list of author information is available at the end of the article

was crucial for successful decidualization. Thereby, the results of our in vitro model explained how PGRMC1 dysregulation during decidualization may present a new perspective on infertility-related diseases.

Keywords Decidualization, Progesterone receptor membrane component 1 (PGRMC1), Endometrium, Telomerase-immortalized human endometrial stromal cells (T-HESCs), Infertility, Prohibitin-1 (PHB1), Prohibitin-2 (PHB2), AG205, Rise-to-decline pattern

Background

Human endometrium tissue is highly dynamic going through proliferative, secretory, and menses phases during a regular menstrual cycle [1–3]. Correspondingly, its functional layer exhibits steroid hormone-dependent proliferation, progesterone-stimulated differentiation, and shedding in the absence of the trophoblast [3]. After the postovulatory phase, the rising circulating levels of progesterone drive human endometrial stromal cells (HESCs) to differentiate into decidual cells, which is referred to as the decidualization process [2–5]. Decidualization is the morphological transformation of HESCs from a proliferating fibroblastic phenotype to an enlarged and rounded epithelial shape, accompanied by secretion of prolactin (PRL) and insulin-like growth factor binding protein-1 (IGFBP-1), which is required for female fertility [2, 3, 5]. In the presence of a trophoblast, the decidualized endometrium will be maintained through the increased level of progesterone. Otherwise, it will be shed away with a rapid drop of the progesterone level [3]. A successful decidualization process is an essential prerequisite for embryo implantation and subsequent placenta formation.

During decidualization, progesterone (P4) classically affects the endometrium through activation of two major well-characterized progesterone receptor PR-A and PR-B [5]. Progesterone receptor membrane component 1 (PGRMC1), one of the non-classical progesterone receptors, also rapidly respond to progesterone during decidualization; however, its function in this process is still being elucidated. In the human endometrium, PGRMC1 is abundantly expressed during the proliferative phase of the menstrual cycle in both endometrial and stromal cells. Whereas, in the secretory phase its expression levels dramatically decreased [6]. Overexpression of PGRMC1 in primary HESCs abrogated decidualization [7] and reduced PGRMC1 expression observed in multiple gynecological and obstetrics diseases [8–10]. Therefore, PGRMC1 was proposed as a fertility stabilizer to decidualization, whose expression must be finely tuned during the entire decidualization to support female fertility [11]. How this is achieved remains an enigma.

The prohibitin proteins (PHBs), prohibitin-1 (PHB1) and prohibitin-2 (PHB2), are ubiquitously expressed and highly conserved in eukaryotic cells [12]. PHBs has been reported to act as transcriptional corepressors for ERα in vitro and in vivo [13–15]. Loss of PHBs led to dysfunctional mitochondria, further resulting in male infertility and ovarian aging in females [16, 17]. Besides, PHB1 is downregulated in the eutopic and ectopic endometrium of patients with endometriosis compared to women without endometriosis [18]. An uterus-selective, conditional PHB2 knockout mouse model showed a subfertility phenotype with litters reduced both in number and size [19]. This implies that appropriate protein levels of PHB1/2 as well as of PGRMC1 are required for optimal uterine function and fertility. In breast cancer cells, progestin-activated PGRMC1 associated with PHBs to stimulate cellular proliferation [20]. Binding of activated PGRMC1 to PHBs was accompanied by decreased PHBs-ERα-interaction, resulting in elevated expression of ER-dependent genes. Whether the progestin-depended interaction between PHBs and PGRMC1 also occurs during decidualization has never been characterized before. Therefore, the role of their interaction with regards to female fertility remains to be elucidated.

In this study, we aimed to explore the functional role of PGRMC1 and PHBs, and their interplay for successful decidualization.

Materials and Methods

Data sources

We collected the associated gene expression profiles in publicly available Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). Samples from different menstruation phases (proliferative/PE, early secretory/ESE, mid-secretory/MSE, late secretory/LES) were chosen from GSE4888 and GSE56364 to detect expression of PGRMC1 [21, 22]. All raw data were background-subtracted and normalized.

Cell culture

The hTERT-immortalized human endometrial stromal cells (T-HESCs) were purchased from abm (T0533). Both the cell lines T-HESCs and St-T1 were maintained in phenol-red free Dulbecco's Modified Eagle Medium// Ham's F12 (DMEM/F12; Gibco, Thermo Fisher Scientific, 11039021) medium supplemented with 10% (*v/v*) charcoal-stripped fetal bovine serum (Thermo Fisher Scientific, 12676029), 100 units/mL penicillin–streptomycin

(Thermo Fisher Scientific, 2321118), 50 µg/ml gentamycin sulfate (Biowest, L0012), 200 µM sodium pyruvate (Biowest, L0624) and 1.5 g/L sodium bicarbonate (Biowest, L0680) (hereafter referred to as complete medium) in a humidified incubator at 37 °C in the presence of 5% CO_2. Cells (passage number < 10) were regularly tested negative for mycoplasma.

Chemical compounds

AG205 (Sigma-Aldrich) was diluted in 2% charcoal-stripped FBS complete medium to 15 mM. Medroxyprogesterone acetate (MPA) and 8-Br-cAMP MPA (cAMP) were prepared from a 10 mM and 5 mM stock solution, respectively.

MTT Assay

We measured activated cellular metabolism as a surrogate for proliferation by performing the MTT assay. Briefly, T-HESCs cells (5 X 10^3 cells per well) were seeded in triplicates in 96-well plate in complete medium and grown for 24 h. After the attachment, cells were either treated with or without induction cocktail in decidualization medium. On the day of assay, cells were incubated with 0.25 mg/ml MTT (Sigma-Aldrich) in decidualization medium for 3 h at 37 oC. Following 1 h of incubation with DMSO at 37 oC and 300 rpm in a microplate shaker, absorption was measured at 540 nm using TECAN Spark® spectrophotometer.

Immunofluorescence staining

Cells were seeded and cultured in chamber slides (Nunc Lab-Tek, Thermo Fisher Scientific C7182-1PAK) fixed with 4% formaldehyde (Sigma-Aldrich, 20649296018) for 10 min at room temperature (RT), washed with washing buffer (Dako, Glostrup, Denmark, S3006) (3×5 min each). Then, cells were permeabilized with 0.1% Triton X-100 (Sigma-Aldrich, T8787) in PBS for 10 min at RT and washed with washing buffer again (3×5 min each). DAKO protein block buffer (Dako, X0909) was added and incubated for 1 h at RT before incubating with primary antibodies specific for PGRMC1 (Abcam, ab48012), PHB1 (Abcam, ab75766), PHB2 (Cell signaling, 14084S) and Vimentin (Abcam, ab02547) overnight at 4 °C. The next day, cells were washed with washing buffer (3×5 min each) and incubated with secondary antibodies (Donkey-anti-goat, Alexa 488: Invitrogen, A11055; Donkey-anti-rabbit, Alexa 488: Invitrogen, A31573) for 1 h at RT in a humidified chamber in the dark. Nucleic acid was stained with DAPI (Thermo Fisher Scientific, 15733122) simultaneously with co-incubated secondary antibodies. After the final wash, the cells were mounted with Fluorescent Mounting Medium (Dako, S3023). Negative controls were prepared for each sample following the same staining procedure with isotype controls instead of primary antibodies. Fluorescence signals were detected with an Axioplan 2 Imaging fluorescence microscope (Carl Zeiss Microscopy GmbH, Jena, Germany).

Proximity ligation assay

The *in-situ* proximity ligation assay (PLA) procedure was performed with the Duolink® PLA Kit (Sigma-Aldrich, DUO92008) and following the manufacturers protocol. The cells were incubated with the primary antibodies i.e., anti-PGRMC1 (Abcam, ab48012) with PHB1 (Abcam, ab75766) and PHB2 (Cell signaling, 14085S) overnight at 4 °C. The slides were washed twice for 5 min with buffer A, followed by incubation with the PLA probes (anti-goat PLUS and anti-rabbit MINUS) in antibody diluent for 60 min at 37 °C. After washing twice for 5 min with buffer A, ligation was performed using ligase diluted in ligation buffer for 30 min at 37 °C. Then the cells were washed with buffer A before incubation for 100 min with amplification stock solution at 37 °C. After washing twice for 10 min with buffer B, nuclear DNA was labeled with DAPI for 10 min and slides were mounted with mounting medium. Negative PLA control was performed using respective isotype control antibodies (isotype goat, Abcam, ab37373; isotype rabbit, Abcam, ab37415). Red fluorescence dots inside the cellular areas representing a single protein–protein interaction were quantified using image J software.

Western blotting

Cell suspensions were washed twice with ice cold PBS (Thermo Fisher Scientific, 2176323) and lysed in RIPA lysis buffer (50 mM TRIS (Sigma-Aldrich, 74,385), 150 mM NaCl (VWR corporation, 16C030032), 1% NP-40 (Sigma-Aldrich, 74,385), 0.5% Sodium deoxycholate (Sigma-Aldrich, D6750), 0.1% SDS (Sigma-Aldrich, S34121136), containing protease inhibitor (Roche, 49,422,800) and phosphatase inhibitor (Roche, 49121300). Protein concentration was determined using Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, 23225). An amount of 20 µg of total protein was supplemented with 4×Laemmli buffer (Bio-Rad, Feldkirchen, Germany, 1610747) containing 2-Mercaptoethanol (Sigma-Aldrich, M6250) and loaded onto Mini-PROTEAN® Precast Gels (Bio-Rad, 4568123) and separated via SDS-PAGE in SDS buffer (25 mM TRIS, 192 mM glycine (Sigma-Aldrich, 50046), 0.1% SDS, pH 8.3) at 100-150 V. Protein was transferred to Immun-Blot® PVDF Membranes (Bio-Rad,1620177) overnight at 4 °C and 10 mA in blotting buffer (20 mM TRIS, 200 mM glycine, 20% (v/v) methanol). Unspecific binding was blocked by incubation of the PVDF membrane with 5% skim milk powder (Sigma-Aldrich, 70166) in TRIS-buffer

(20 mM TRIS, 150 mM NaCl, pH 7.6) containing 0.1% Tween 20 (TBS-T) for 1 h at RT. Primary antibodies including PGRMC1 (Cell signaling, Danvers, MA, USA, D6M5M), PHB1 (Cell signaling, 2426S), PHB2 (Cell signaling, 14085S) and ß-actin (Santa Cruz Biotechnology, sc-4778) were added in 5% skim milk—TBS-T and incubated overnight at 4 oC. Secondary antibodies were applied in 5% skim milk—TBS-T at RT for 1 h. Proteins were detected using Amersham™ ECL™ Western Blotting Detection Reagent (Cytiva, 17190731).

Subcellular protein fractionation

A subcellular protein fractionation kit (Thermo Fisher Scientific) was used to fractionate proteins into cytoplasmic, membrane, and nuclear fractions. Cells were harvested as pellets. The pellet was lysed with cytoplasmic extraction buffer, membrane extraction buffer, and nuclear extraction buffer. Primary antibodies specific for β-actin (Santa Cruz Biotechnology), Calreticulin (Santa Cruz Biotechnology), and Histon H3 (Cell signaling) were used to indicate the purity of the cytoplasmic, membrane, and nuclear fractions, respectively.

Co-immunoprecipitation

Co-immunoprecipitation was performed using the Pierce Co-IP kit (Thermo Fisher Scientific). Briefly, the anti-PGRMC1 antibody (Cell signaling) was first immobilized for 2 h using AminoLink Plus coupling resin. In parallel, cell pellets were resuspended in ice-cold IP Lysis buffer. An amount of 500 µg protein was incubated with resin at 4 °C overnight. After incubation, the resin was washed, and protein complexes bound to the antibody were eluted using elution buffer. Subsequent western blot analyses were performed as described before.

Gene silencing (siRNA Transfection)

To knock down PGRMC1 expression in T-HESCs, FlexiTube GeneSolution (Qiagen) was used, containing four siRNA(s) that specifically target human PGRMC1 mRNAs. Cells were transfected with the final concentration of 10 nM *PGRMC1* siRNA(s) or negative control siRNA (siCTL) (Thermo Fisher Scientific) using Lipofectamine RNAiMAX Transfection Reagent (Thermo Fisher Scientific) according to recommended procedures. Afterwards, cells were treated with decidualization medium containing either induction cocktail or DMSO, and harvested at different time points for downstream experiments. For PHB1 and PHB2 mRNA expression inhibition (siPHB1, siPHB2: Qiagen), the same siRNAs concentration was used.

Quantitative reverse-transcription PCR (qRT-PCR)

RNA was isolated using the RNeasy Mini Kit (Qiagen) according to the manufacturer's specifications. Reverse transcription of RNA into cDNA was performed with the Omniscript RT kit (Qiagen) according to the manufacturer's instructions. Quantitative PCR was performed using QuantiFast SYBR Green PCR Kit (Qiagen) and LightCycler ®480 System (Roche). Primers for *PGRMC1* (Qiagen), *PRL* (Qiagen) and *HPRT1* (Qrigene, Rockville, MD, USA). The delta-delta cycle threshold method was used to normalized expression to the reference gene HPRT1 [23, 24].

Statistical analysis

A two-tailed paired Student's *t*-test was used to analyze experiments comparing two experimental groups or two-way ANOVA for multiple comparisons of more than two groups. A value of $p < 0.05$ was considered significant. All statistical analyses were performed with GraphPad Prism 9.0. Results were reported as means with standard deviation.

Results

PGRMC1 expression profile during regular menstrual cycle

To understand the dynamics of PGRMC1's expression changes during normal decidualization, we initially investigated its expressional profile by mining publicly available RNA-sequencing data sets from endometrial biopsies (GEO accession numbers: GSE6364 and GSE4888). In a regular menstrual period, PGRMC1 mRNA level gradually decreased from the proliferative phase to the secretory (including early-, mid-, and late-) phase, manifesting the highest level in the proliferation phase and the lowest level in the late-secretory phase (Fig. 1A-B), consistent with previously reported data [9, 25]. This indicates that PGRMC1 may have an important role in regulating cellular proliferation and may not be required for decidualization in the secretory phase as it is consecutively decreased at mRNA level after progesterone stimulation. We hypothesized that the dynamic changes of PGRMC1 have an important role during the menstrual cycle that must be finely tuned.

Rise-to-decline trend of PGRMC1 expression during in vitro decidualization

To investigate our hypothesis, we established a hormone-inducible in vitro decidualization model in T-HESCs based on visualizing its morphological changes and by measuring the expression level of the decidual marker prolactin (PRL) (Fig. 2A). After being exposed to the decidualization induction cocktail consisting of the P4 analog MPA plus cAMP for 10 days, morphological

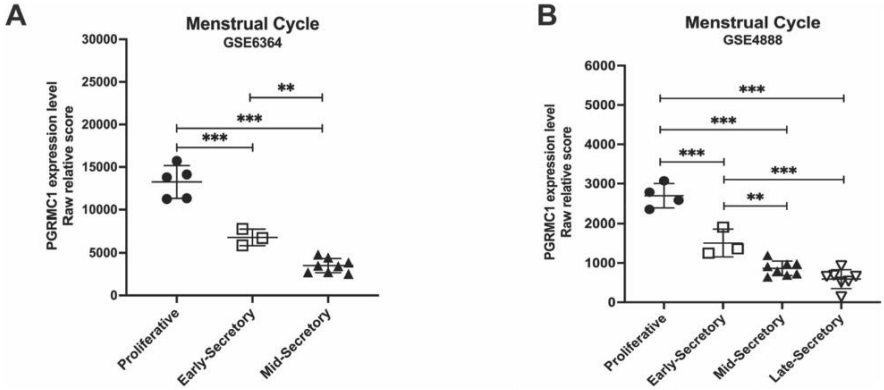


Fig. 1 PGRMC1 expression profile during menstrual cycle. Relative transcript scores of PGRMC1 expression in different stages of a regular menstrual cycle (GSE6364) **(A)** and (GSE4888) **(B)**. Relative transcript scores of PGRMC1 expression level are shown as mean ± SEM. Statistical analysis was performed by two-way ANOVA. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$

changes of T-HESCs were inspected by microscopy in bright-field and by immunofluorescent analysis of the cytoskeletal marker vimentin. With this protocol, T-HESCs underwent a transformation from a fibroblast-like shape to a polygonal epithelial-like shape (Fig. 2B-C) accompanied with a significant increase of PRL mRNA expression compared to non-induced controls (Fig. 2D). Both the morphological changes and enhanced expression of PRL indicate a successfully established the hormone-induced decidualization model, allowing to investigate the role of PGRMC1 in decidualization.

We aimed to determine and modulate the expression level of PGRMC1 in our system to study its impact on the decidualization process. First, we determined the protein expression profile of PGRMC1 during the in vitro decidualization program. Intriguingly, we found that PGRMC1 expression gradually increased from the day the induction cocktail had been added (D0), peaking at day 6 (D6) post-induction, followed by a constant decrease until day 14 (D14) (Fig. 2E-F). This protein expression change could also be observed in the St-T1 cell line (Supplementary Fig. 1A). In contrast with the observation of gradually decreased mRNA levels of PGRMC1 during the secretory phase of the normal menstrual cycle (Fig. 1A-B), our in vitro decidualization model revealed that the promotion of cells into decidualized state comprises a PGRMC1 increasing phase and a PGRMC1 decreasing phase, both on protein and mRNA level (Fig. 2E-G). This data suggests that PGRMC1 was regulated at transcriptional level during decidualization. We termed this PGRMC1 protein dynamic changes as PGRMC1 'rise-to-decline' changes in decidualization.

It is well known that increasing P4 levels initiate decidualization, although the activity of PGRMC1 in decidualization seems to be independent of P4 [3, 5]. Consistently, the T-HESC cells can go through the decidualization process treated with either P4, MPA, or cAMP (Supplementary Fig. 1B-C). Intriguingly, the PGRMC1 expression changes can be observed at each condition, which led us to the conclusion that the PGRMC1 rise-to-decline changes are a universal mechanism within the decidualization program.

The rise-to-decline changes of PGRMC1 are required for decidualization

To explore the potential role of the PGRMC1 rise-to-decline changes during the decidualization, we firstly downregulated its expression before hormone induction with an optimized concentration of an siRNA-mix specific for PGRMC1 mRNA (Fig. 3A). Importantly, PGRMC1 mRNA levels were remained suppressed throughout 10 days post-siRNA-transfection (Fig. 3B). Likewise, expression of PGRMC1 protein was completely abrogated from day 2 (D2) to day 10 (D10) after siRNA transfection (Supplementary Fig. 2A-B).

T-HESCs with suppressed PGRMC1 expression were further treated with the decidualization induction cocktail. As indicated by the lack of morphological transformation and PRL production over 10 days of hormone treatment period (Fig. 3C, Supplementary Fig. 3), these

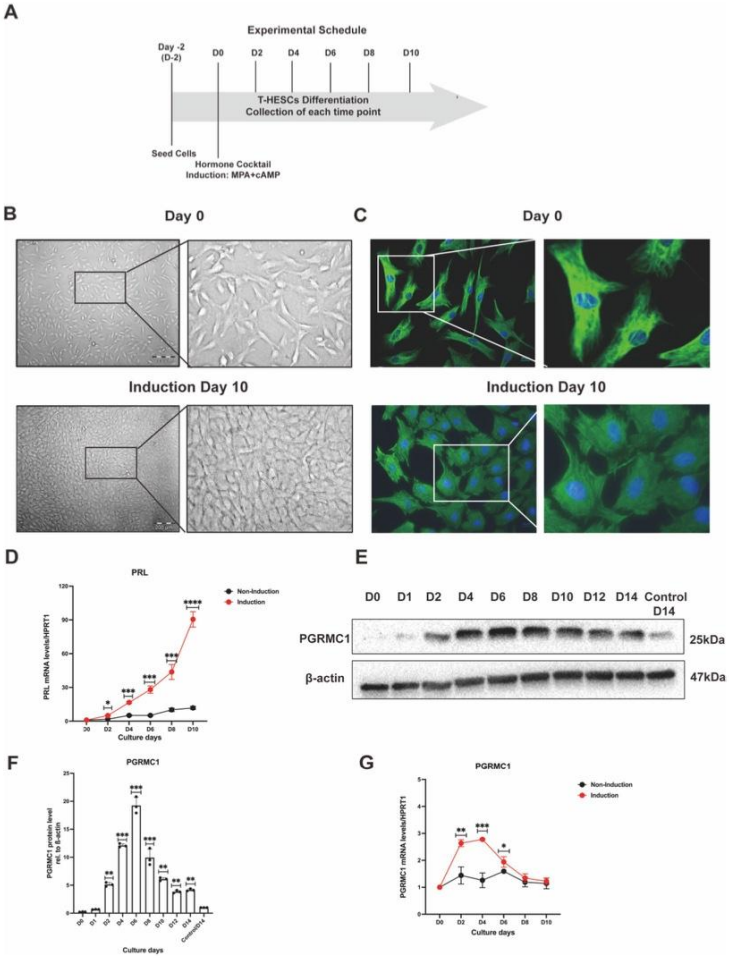


Fig. 2 A rise-to-decline expression pattern of PGRMC1 was revealed by in vitro decidualization. **A** Schematic representation of in vitro decidualization system. The cellular morphology changes of T-HESCs on day 0 and day 10 were imaged with microscopy in bright filed **(B)** or immunofluorescence staining **(C).** PGRMC1 was stained by Alexa Fluor-488 (green), and the nucleus was stained by DAPI in blue. Scale bar: 200 μm. The mRNA expression levels of *PRL* in T-HESCs were analyzed with qRT-PCR when cells were cultured with MPA/cAMP (red line) for decidualization or DMSO (black line) as control **(D)**. The dynamic changes of PGRMC1 protein expression from 1 to 14 days induction and non-induction control (on Day 14) were measured by western blot **(E)** and the bar plot with the relative densitometric analysis of the corresponding PGRMC1 protein level (*p* value calculation based on 'D0') **(F)**. β-actin was used as a loading control. The mRNA expression levels of *PGRMC1* in T-HESCs during decidualization **(G)**. Results are shown as the mean ± SEM from three biological replicates. Statistical analysis was performed by two-way ANOVA. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$

cells did not undergo decidualization. Thus, in the absence of the progestin-induced PGRMC1 increasing phase decidualization failed.

These results prompted us to investigate if expression of PGRMC1 is needed for decidualization at the time point of induction – as a kind of a program switch – or later. To this aim, we postponed the transfection of PGRMC1 suppressing siRNAs to after the induction of decidualization. First, we treated T-HESC cells for 2 days with the combination of MPA and cAMP to induce decidualization followed siRNA transfection (Fig. 3D) and investigated the cells up to day 10 (D10) after induction. As expected, PGRMC1 mRNA levels started to decrease (Fig. 3E, blue line) after 2 days of transfection of PGRMC1-specific siRNAs and the PGRMC1 mRNA levels stayed below mRNA levels reached during normal induction of decidualization (Fig. 3E, red line). Interestingly, in addition to morphological changes (Supplementary Fig. 4), PRL mRNA expression level first dropped, but between D6 and D8 not only recovered to a comparative level to that of normal induction, was even four days earlier compared to the normal induction, indicating a promoted decidualization (Fig. 3F). We further measured the effects of knocking down PGRMC1 after 4 days of induction with MPA/cAMP on the decidualization program. The results are very similar to the outcome achieved when suppressing PGRMC1 after 2 days of induction (Fig. 3G-H). The results could be additionally reproduced in the St-T1 cell line (Supplementary Fig. 5A-D). Taken together, the PGRMC1 rise-to decline changes are required for a proper decidualization.

PGRMC1-signal increases in the peri-nuclear region during decidualization

It has been reported that PGRMC1 translocates from cytoplasmic membranes to the nucleus during decidualization [7]. Recently, PGRMC1-mediated proteomic changes have been well characterized after decidualization, suggesting that PGRMC1 binds to proteins involved in translation, ATP generation, protein maturation, glucose transport, and lipid metabolism [26]. Almost all these proteins locate in the cytoplasm or on membranes, but not in the nucleus. This raises the question of why proteins interacting with PGRMC1 are barely found to be in the nucleus.

To better understand the question, we initially assessed PGRMC1 protein subcellular localization by immunofluorescence. Without induction of decidualization PGRMC1 was essentially located in the cytoplasm, but more intense signals were observed around the nucleus after 10 days induction (Fig. 4A). To further verify these observations, we fractionated the cells into soluble parts containing cytoplasm, membrane, and nucleus and detected the PGRMC1 protein by western blot. In line with the immunofluorescence results, PGRMC1 was only observed in the membrane fraction but not in the nucleus (Fig. 4B). This indicates an accumulation of PGRMC1 in the peri-nuclear region during hormone-induced decidualization.

Interactions of PHB1/PHB2 to PGRMC1 mediate decidualization

We have recently demonstrated in breast cancer cells, that progestin-activated PGRMC1 interacts with PHB1/PHB2 resulting in enhanced ERα-dependent transcription and cell proliferation [27]. In analogy, here we found that PGRMC1 colocalized with PHB1 and PHB2 in the cytoplasm and at the nucleus periphery after induction, whereas barely colocalization signals could be observed without induction revealed by immunofluorescence (Supplementary Fig. 6A-B). This suggests a potential interaction between PHBs and PGRMC1 introduced by progestin treatment. Then, PLA was performed to further explore the associations between PGRMC1 and PHB1/2. Upon induction, a significantly higher PLA signal suggesting the interaction of PGRMC1 to PHB1/2 could be observed compared to the control (Fig. 5A-B, Supplementary Fig. 7A-B).

To explore the function of PGRMC1-PHBs interaction during decidualization, we downregulated PHBs via siRNA transfection, reaching expression levels decreased by 60–80% compared to the control for individual PHBs

(See figure on next page.)

Fig. 3 The rise-to-decline changes of PGRMC1 are required for decidualization. (**A**): Schematic representation of in vitro decidualization system after PGRMC1 downregulation by siRNA. qRT-PCR analysis of *PGRMC1* mRNA expression changes in T-HESCs transfected with either 10 nM of siRNA against PGRMC1 (siPGRMC1) or 10 nM control siRNA (siCTL) for up to 10 days **(B)**. The *PRL* mRNA expression level in T-HESCs after MPA/cAMP-induced decidualization upon transfection with 10 nM siPGRMC1 or siCTL, analyzed with qRT-PCR **(C)**. The workflow for PGRMC1 downregulation after decidualization induction **(D)**. mRNA expression levels of *PGRMC1* **(E, G)** and *PRL* **(F, H)** in T-HESCs treated with MPA/cAMP for decidualization induction (red line) and non-induction (black line). Blue lines indicate the mRNA levels of *PGRMC1* and *PRL* when transfected with 10 nM siPGRMC1 on the second (**E, F**) and fourth (**G, H**) day after decidualization induction, respectively. The statistical analysis of mRNA levels of *PGRMC1* (and *PRL*) between cells with non-induction and induction indicated by red stars, or cells with PGRMC1 knockdown after induction indicated by blue stars. Results are shown as the mean ± SEM from three independent biological replicates. Statistical analysis was performed by two-way ANOVA. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$

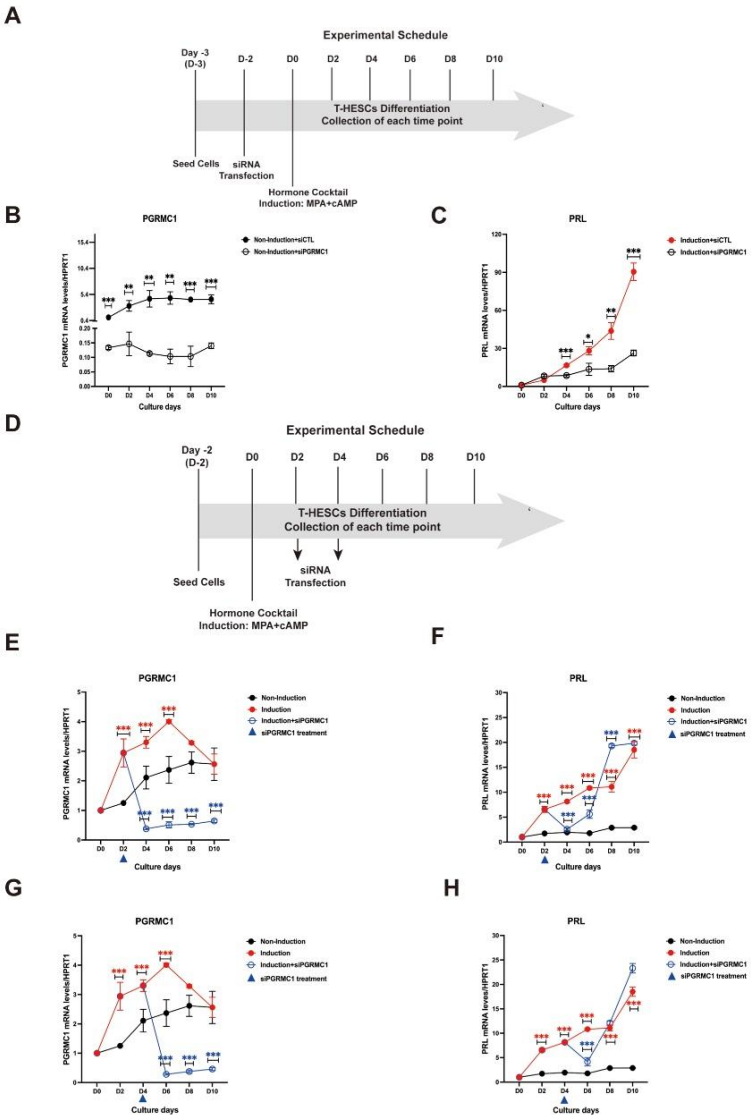


Fig. 3 (See legend on previous page.)

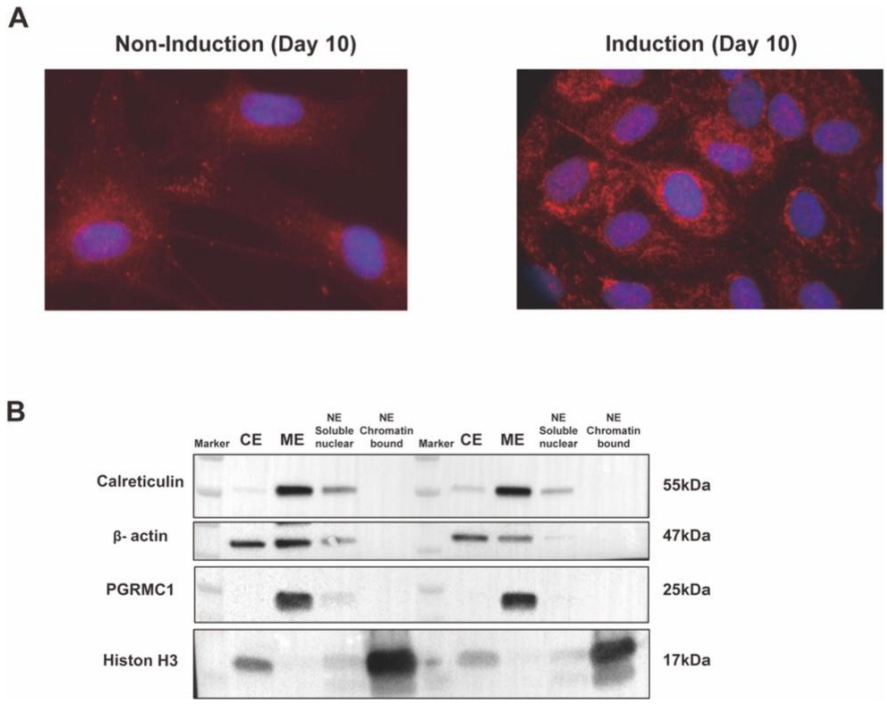


Fig. 4 Prei-nuclear PGRMC1-signal increased during decidualization. (**A**): Immunofluorescence staining of PGRMC1 in T-HESCs treated with DMSO (left) as control or MPA/cAMP (right) for 10 days decidualization induction. PGRMC1 shows in red and the nucleus was stained with DAPI in blue. Scale bar: 200 μm. (**B**) Analysis of PGRMC1 localization by subcellular fractionation in T-HESCs treated with DMSO (left) or MPA/cAMP (right), measured by western blot. PGRMC1 was immunoblotted in equal amounts of cytoplasmic (CE), membrane (ME), and nuclear (NE) biomaterial. Compartment-specific markers: Calreticulin (55 kDa), β-actin (47 kDa), and Histon H3 (17 kDa) were used as loading controls for the membrane, cytoplasmic, and nuclear fractions, respectively

(Fig. 6A-B). Knocking down each of the PHBs before hormone induction partly impaired the decidualization process (Fig. 6C-E), but the cells still could achieve morphological transformation (Supplementary Fig. 9). These effects on decidualization are comparable to the results achieved with suppressed PGRMC1.

AG205 does not affect PGRMC1 rise-to-decline changes and decidualization

AG205 was reported to be a specific inhibitor of PGRMC1 and was broadly used to explore PGRMC1's role in decidualization [28, 29]. Recent data, however, question the specificity of AG205 for PGRMC1 [29–31].

Taking advantage of the critical role of PGRMC1 rise-to-decline changes for decidualization, we tested the effect of AG205 on PGRMC1 and the decidualization process. Since AG205 concentrations used in previous reports were high enough to impair cellular viability [30, 32, 33], we initially determined the appropriate concentration of AG205 that did not affect cell viability. In the MTT assay (Fig. 7A), a concentration below 15 μM had no (or a moderate) effect, whereas a concentration higher than 15 μM had a detrimental effect on cellular viability, which is consistent with previously reported [29]. In addition, decidualization was successfully achieved with T-HESCs treated AG205 with concentrations below 15 μM, as indicated by the increasing

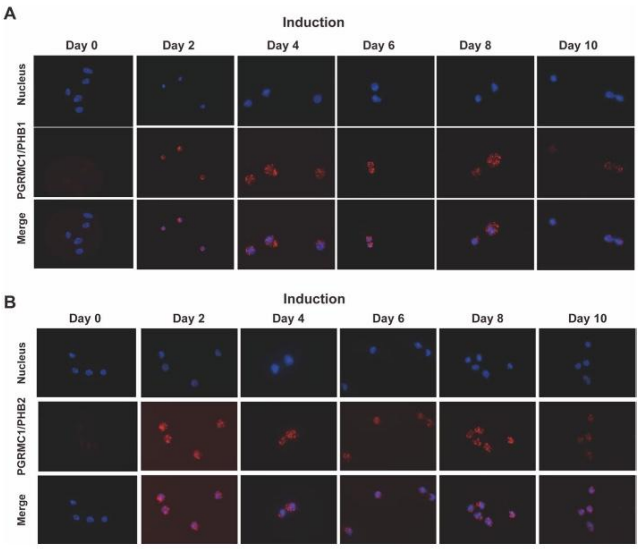


Fig. 5 PGRMC1 interacts with PHBs during decidualization. The interactions between PGRMC1 and PHB1 (**A**) or PHB2 (**B**) in T-HESCs were analyzed with proximity ligation assay upon decidualization induction from day 2 to day 10. 'Day 0' indicates the induction day. Each red spot represents a single interaction. Nuclear stain: DAPI. Magnification 40×

expression of PRL and the change in cell morphology (Fig. 7B-C). Furthermore, AG205 treatment did neither affect PCRMC1 protein level during decidualization, nor its rise-to-decline expression profile (Fig. 7D). Moreover, the interaction of PGRMC1 to PHBs was not disturbed as confirmed by PLA (Fig. 7E-F), which is in line with a previous report [32]. Based on these results, we propose that AG205 (< 15 μM) has no effect on the observed PGRMC1 functions during decidualization.

Discussion

PGRMC1 has been demonstrated to play a role in various reproductive tissues, particularly endometrial stromal cells [27, 34–36]. It influences the decidualization process and female fertility [37]. We revealed that PGRMC1 protein exhibits a rise-to-decline pattern after progestin stimulation, essential for normal decidualization (Supplementary Fig. 10). Additionally, during decidualization, PGRMC1 interacted with PHB1 and PHB2, suggesting their joint contribution to the decidualization program. Despite the unclear mechanisms behind PGRMC1-dependent decidualization failure, PGRMC1 expression profile may serve as a useful fertility indicator.

Previous reports focused on PGRMC1 mRNA profile changes during decidualization, with few investigations into protein level dynamics. We measured both mRNA and protein levels of PGRMC1 after inducing decidualization and observed a rise-to-decline pattern. The observed increase and decrease of PGRMC1 protein expression fits into cyclic changes observed in vivo [25]. The overall dynamic changes of the PGRMC1 protein level during a normal menstrual cycle are composed of two peaks: one occurs in the secretory phase, as revealed in this study and the other one occurs in the proliferative phase as previously reported [9, 25]. It resembles estrogen dynamics during the menstrual cycle, suggesting PGRMC1 expression may be regulated by estrogen concentration or a similar mechanism [1–3]. As PGRMC1 overexpression in breast cancer cells leads to higher E2 secretion, T-HESCs E2 production might depend on PGRMC1 activation. Further research is needed to understand the relationship between estrogen and PGRMC1 expression, including the possibility of estrogen receptor-mediated transcription regulation.

Knocking down PGRMC1 before hormone treatment inhibited decidualization, highlighting its crucial role as a 'switch' at this stage. Appropriate PGRMC1 protein

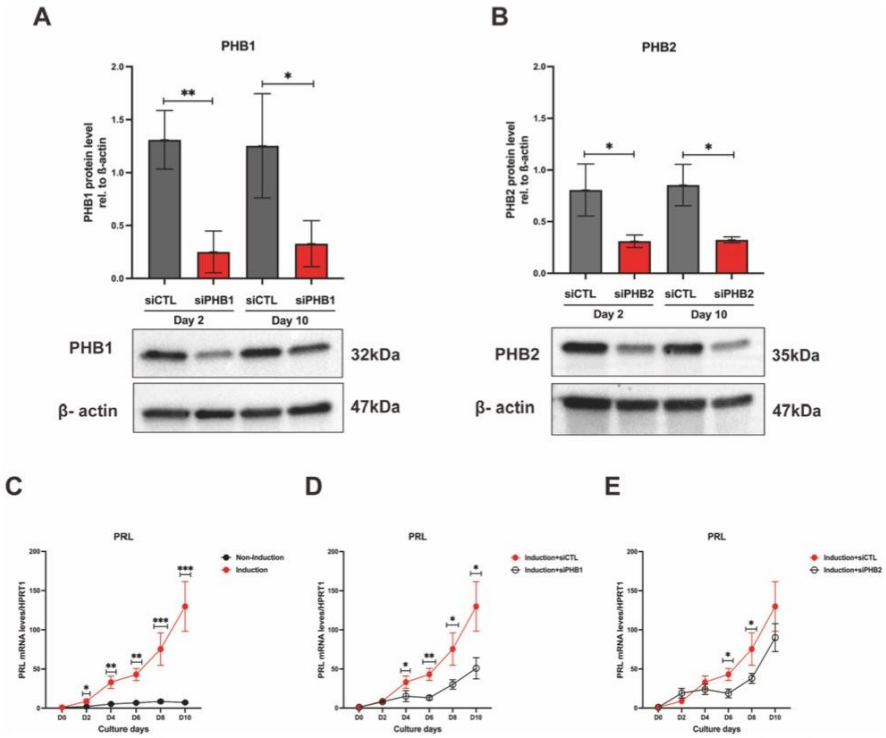


Fig. 6 Downregulation of PHBs partly impairs decidualization. The PHBs protein expression level on day 2 or day 10 after transfection of T-HESCs with 10 nM siPHB1 (**A**), 10 nM siPHB2 (**B**), respectively, was analyzed by western blot. Densitometric analysis was performed with imagej and values were normalized to β-actin. The *PRL* mRNA expression changes in T-HESCs with (red line) and without (black line) induction was determined by qPCR and normalized to *HPRT1* as a reference gene (**C**). The *PRL* mRNA expression changes in T-HESCs transfected with 10 nM siPHB1 (**D**), 10 nM siPHB2 (**E**) upon decidualization induction were determined by qPCR. Results are shown as the mean ± SEM from three biological replicates. Statistical analysis was performed by two-way ANOVA. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$

(See figure on next page.)

Fig. 7 AG205 does not affect decidualization. The influence of AG205 on T-HESCs viability was performed after the cells were incubated with indicated concentrations of AG025 for 10 days and analyzed with colorimetric assay **(A)**. The absorbance values for cultures with AG205 were compared to the DMSO control (0 μM). The *PRL* mRNA expression levels were analyzed after cells were cultured with (black line) or without (red line) 15 μM AG205 **(B)**. Results are shown as the mean ± SEM from three independent biological replicates. Statistical analysis was performed by two-way ANOVA. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. The cellular morphology changes of T-HESCs were imaged with microscopy in the bright field when cells were cultured without (upper panel) or with (down panel) MPA/cAMP upon 15 μM AG205 treatment **(C)**. Scale bar: 200 μm. The PGRMC1 protein expression changes in T-HESCs were analyzed by western blot when cells were treated with DMSO (left panel) or 15 μM AG205 (right panel) upon decidualization induction **(D)**. β-actin was used as a loading control. The interactions between PGRMC1 and PHB1 **(E)** or PHB2 **(F)** in T-HESCs were analyzed by proximity ligation assay when cells were cultured with 15 μM AG205 upon decidualization induction. Each red spot represents a single interaction. Nuclear stain: DAPI. Magnification 40×

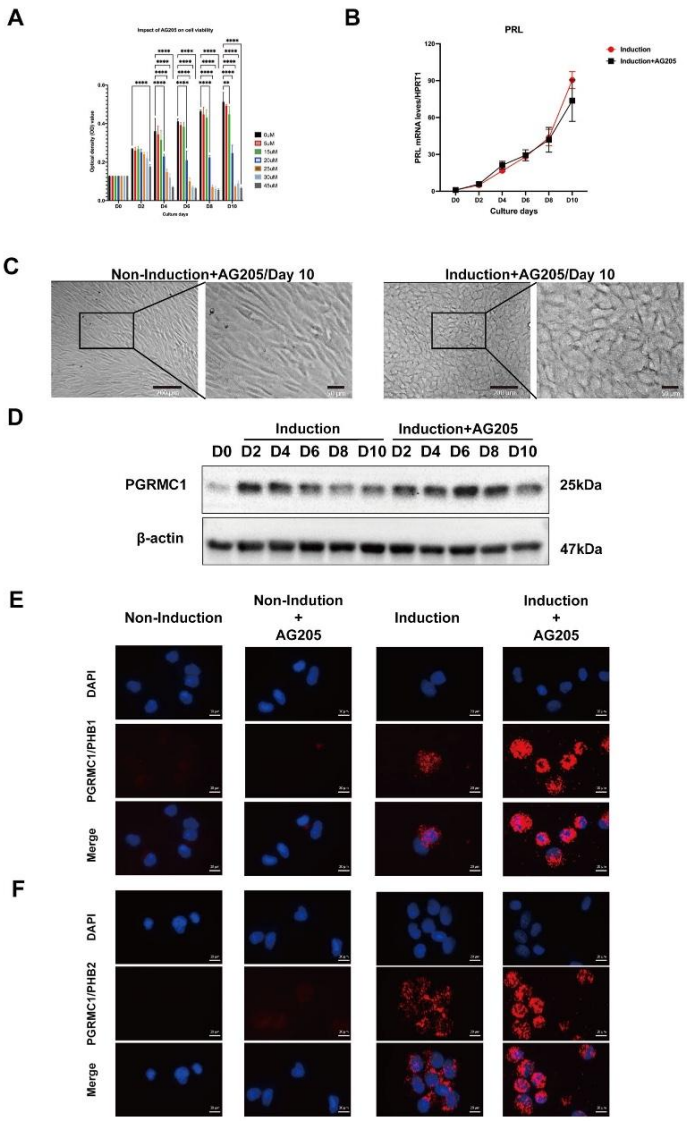


Fig. 7 (See legend on previous page.)

levels are needed to initiate decidualization upon P4/cAMP stimulation. The PGRMC1 rise-to-decline pattern can be induced by various treatments (Supplementary Fig. 1), suggesting a common signaling pathway that correlates with decidualization, which requires further investigation. PGRMC1 seems less necessary after decidualization initiation, as knocking it down either does not affect or even facilitates the process. It is unclear why PRL expression initially drops and then increases when PGRMC1 is knocked down after decidualization induction. Downregulating PGRMC1 after progestin treatment doesn't hamper decidualization, indicating its critical role during the increasing phase and induction. This aligns with observations that PGRMC1 downregulation in the secretory phase promotes decidualization [28]. Overall, PGRMC1 activation by P4 may facilitate the switch from cellular proliferation to decidualization initiation through various biological processes, while the mechanism of how downregulated PGRMC1 promotes decidualization warrants further investigation.

PGRMC1 has been known to occupy multiple subcellular locations, from endoplasmic reticulum, cytoplasm, plasma membrane, nucleus, and mitochondria, and its localization is regulated by including phosphorylation, ubiquitination, and sumoylation [34, 36]. In our study, we noticed an induction-associated peri-nuclear phenomenon, presenting as more intense signals in the peri-nuclear region at 10 day's induction (Fig. 4A). PGRMC1's peri-nuclear expression has been observed in various cells, suggesting its involvement in processes near or within the nucleus [7, 38, 39]. In our current study, we did not detect any nuclear PGRMC1 under the explored conditions, as demonstrated by subcellular fractionation analysis (Fig. 4B).

PGRMC1 associates with proteins involved in protein biosynthesis, intracellular transport, and mitochondrial activity to promote decidualization [26, 35]. However, little is known about how PGRMC1 interacts with these proteins to regulate decidualization. We found that PGRMC1 binds to PHBs at the nucleus periphery after P4 treatment, suggesting it may function as a scaffold protein for decidualization in endometriosis stromal cells. PGRMC1 could be anchored on the membrane of various organelles, co-transporting with them during decidualization-related morphological changes [40]. PHBs form a super complex in mitochondria, playing roles in lipid biogenesis, ATP generation, and more [12, 41]. Knocking down PHBs partially impaired decidualization, similar to PGRMC1 knockdown, suggesting PGRMC1-PHBs interactions may influence decidualization as a complex, requiring further investigation. We speculate that PGRMC1 binding to PHBs may inhibit cellular proliferation and facilitate differentiation, acting as a proliferation-differentiation switch.

We found that the small molecule AG205 neither affect PGRMC1-PHBs interaction, nor decidualization in our study (Fig. 7). Although AG205 has been shown to interact with PGRMC1 in vitro, its in vivo interaction remains unknown. Our data align with a recent study demonstrating that AG205 concentrations over 15 μM reduce cell proliferation, and concentrations above 30 μM result in cell death in HEC-1A and T-HESC cells [29]. Furthermore, our findings are consistent with a previous report indicating that a high concentration (50 μM) of AG205 did not affect decidualization [30].

This study on PGRMC1-PHB association, while informative, presents several limitations. Firstly, while PLA data suggests a close proximity and potential functional interaction between PGRMC1 and PHB, confirming a physical interaction necessitates additional in vivo interaction detection methods. Co-immunoprecipitation (Co-IP) has significant limitations, as it disrupts cellular integrity and loses crucial information about protein localization and physiologically relevant interactions due to cell lysis and potential interference from the buffer system, particularly for detecting weak, transient interactions or those confined to specific cellular compartments. Thus, in vivo crosslinking, which enables interaction detection in their native environment, should be considered. Secondly, the efficiency of PHB1 and PHB2 silencing via siRNA is lower compared to the almost complete knockdown of PGRMC1, suggesting the need for PHB knockout or stable knockdown models for clearer background results. Finally, a general limitation needs to be taken into account. The in vitro experiments performed in cell lines assured reproducibility within the established system, while primary patient endometrium tissues are highly heterogeneous and, in general, require analysis of a large cohort in order to obtain a statistically significant result. Consequently, this study primarily analyzed publicly available datasets and two cell lines. However, to validate the switch-like rise-to-decline expression pattern of PGRMC1 in vivo, future studies could utilize a mouse model with inducible PGRMC1-downregulation.

Conclusion

Based on the results of our study, we postulate that P4/progestin-induced PGRMC1 rise-to-decline expression is essential to start the decidualization program, but, once decidualization started, PGRMC1 is not needed to drive it. Our PGRMC1-knockdown experiments demonstrated that PGRMC1 expression is specifically important at decidualization induction, leading to decidualization failure upon disruption. Taken together, we explained how dysregulated PGRMC1 expression could impact endometrial stromal cell decidualization, which may provide a new perspective on infertility-related diseases.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12958-024-01188-9.

Additional file 1: Supplementary Fig. 1. Rise-to-decline expression pattern of PGRMC1 is linked to the decidualization program. **(A)** PGRMC1 protein expression changes during 9 days of decidualization were measured by western blot in the St-T1 cell line. **(B)** PGRMC1 protein expression changes during 10 days of stimulation with MPA, cAMP, and DMSO, respectively, were measured by western blot in T-HESCs. **(C)** PGRMC1 protein expression levels on day 6 and day 10 when cultured with DMSO, nomegestrel (NOM), P4, cAMP, MPA/cAMP (M + A), and MPA, respectively, measured by western blot in T-HESCs. **Supplementary Fig. 2.** PGRMC1 is effectively downregulated by siRNA on protein level. **(A)** The PGRMC1 protein expression on day 2 and day 10 after transfection of T-HESCs with either 10 nM anti-PGRMC1 siRNA (siPGRMC1) or unspecific scrambled-control siRNA (siCTL). **(B)** A comparison of the PGRMC1 protein expression changes within 10 days after transfection of T-HESCs with either 10 nM siPGRMC1 or 10 nM siCTL. **Supplementary Fig. 3**. PGRMC1-downregulation before decidualization induction impairs morphological remodeling of T-HESCs. The cellular morphology changes of the T-HESCs induced with either DMSO (upper panel) or MPA/cAMP (down panel) after 10 days of siRNA treatment (siCTL, left panel; siPGRMC1, right panel). Scale bar: 200 µm. **Supplementary Fig. 4.** PGRMC1-downregulation after decidualization induction does not impair morphological remodeling of T-HESCs. The cellular morphology changes of the T-HESCs induced with either DMSO (non-induction, column 1) or MPA/cAMP (Induction, columns 2–4). I (column 3) and II (column 4) indicate that siRNA treatment on T-HESCs was conducted on day 2 or day 4 of decidualization induction, respectively. Scale bar: 200 µm. **Supplementary Fig. 5.** PGRMC1-downregulation after progestin induction does not impair decidualization in the St-T1 cell line. The mRNA expression levels of *PGRMC1* **(A, C)** and *PRL* **(B, D)** in St-T1 treated with MPA/cAMP for decidualization induction (red line), and non-induction (black line). The mRNA expression levels of *PGRMC1* and *PRL* in St-T1 cells transfected with 10 nM siPGRMC1 (blue line) on the second (**A, B**) and fourth day (**C, D**) after decidualization induction, respectively. Results are shown as the mean ± SEM from three independent biological replicates. Statistical analysis was performed by a two-way ANOVA test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. The red * indicates the comparison between the red and black lines. The blue * indicates the comparison between the red and blue lines. **Supplementary Fig. 6.** PGRMC1 and PHB1/PHB2 co-localize in T-HESCs. Double Immunofluorescence staining for PGRMC1 (red) and PHB1 (green) or PHB2 (green) in T-HESCs treated with DMSO (**A**) as control or MPA/cAMP **(B)** for decidualization induction. Magnification: 40x. Scale bar: 20 µm. **Supplementary Fig. 7.** PGRMC1 does not interact with PHBs without induction. The interactions between PGRMC1 and PHB1 **(A)** and PHB2 **(B)** in T-HESCs without induction were analyzed with proximity ligation assay from day 2 to day 10. Each red spot represents a single interaction. Nuclear stain: DAPI. Magnification 40X. **Supplementary Fig. 8.** PGRMC1 co-precipitate with PHB1/PHB2 upon decidualization induction. PGRMC1 was immunopurified from native whole cell lysates of T-HESCs using anti-PGRMC1 antibody. Western blot analyses of co-immunoprecipitated PHB1 (upper) and PHB2 (bottom) in T-HESCs with and without decidualization induction. **Supplementary Fig. 9.** Morphological changes during decidualization upon PHBs downregulation. The cellular morphology changes of the T-HESCs induced with either DMSO (non-Induction) or MPA/cAMP (Induction) upon either PHBs knockdown alone or both. Scale bar: 200 µm. **Supplementary Fig. 10.** Overview over the role of PGRMC1 in human endometrial decidualization. Upon stimulation with progesterone or MPA, the PGRMC1 rise-to-decline changes are essential for successful decidualization of the human endometrial cells (upper panel). With downregulated PGRMC1 expression before induction, the decidualization program cannot be carried out, leading to decidualization failure (bottom panel).

Acknowledgements
We are grateful to our colleagues in the Department of Gynecology and Obstetrics, Heinrich-Heine University Duesseldorf for their helpful comments.

Authors' contributions
Conceptualization, H.L., H.N.; Investigation, H.L., N.S.; Methodology, H.L., A.P.B. and N.S.; Supervision, T.F., D.N., Z.C., N.S., H.N.; Project administration, T.F., H.N.; Writing-original draft, H.L.; Writing-review and editing, A P.B., A.F., N.S. and H.N. All authors have read and agreed to the published version of the manuscript.

Funding
Open Access funding enabled and organized by Projekt DEAL. This project was funded by the "German Research Foundation (Deutsche Forschungsgemeinschaft, DFG, NE 805/6–1)", and "The Brigitte and Dr. Konstanze Wegener Foundation (#51, Duesseldorf, Germany)", and "The Medical Faculty of the Heinrich-Heine University Duesseldorf (#48/2018)", and "The Shanghai Shenkang Hospital Development Center (SHDC12021113)".

Availability of data and materials
The datasets analyzed in this study are available at Gene Expression Omnibus (GEO) database: https://www.ncbi.nlm.nih.gov/geo/ with the GEO accessions GSE6364 and GSE4888.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Obstetrics and Gynecology, Life Science Center, University Hospital and Medical Faculty of the Heinrich-Heine University Duesseldorf, Duesseldorf, Germany. [2]Department of OB/GYN & REI, UniKID, University Hospital and Faculty of Medicine, Heinrich Heine University, Duesseldorf, Germany. [3]Department of Obstetrics and Gynecology, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai 200072, China. [4]Institute of Gynecological Minimally Invasive Medicine, Tongji University School of Medicine, Shanghai 200072, China.

Received: 6 June 2023 Accepted: 20 January 2024
Published online: 03 February 2024

References

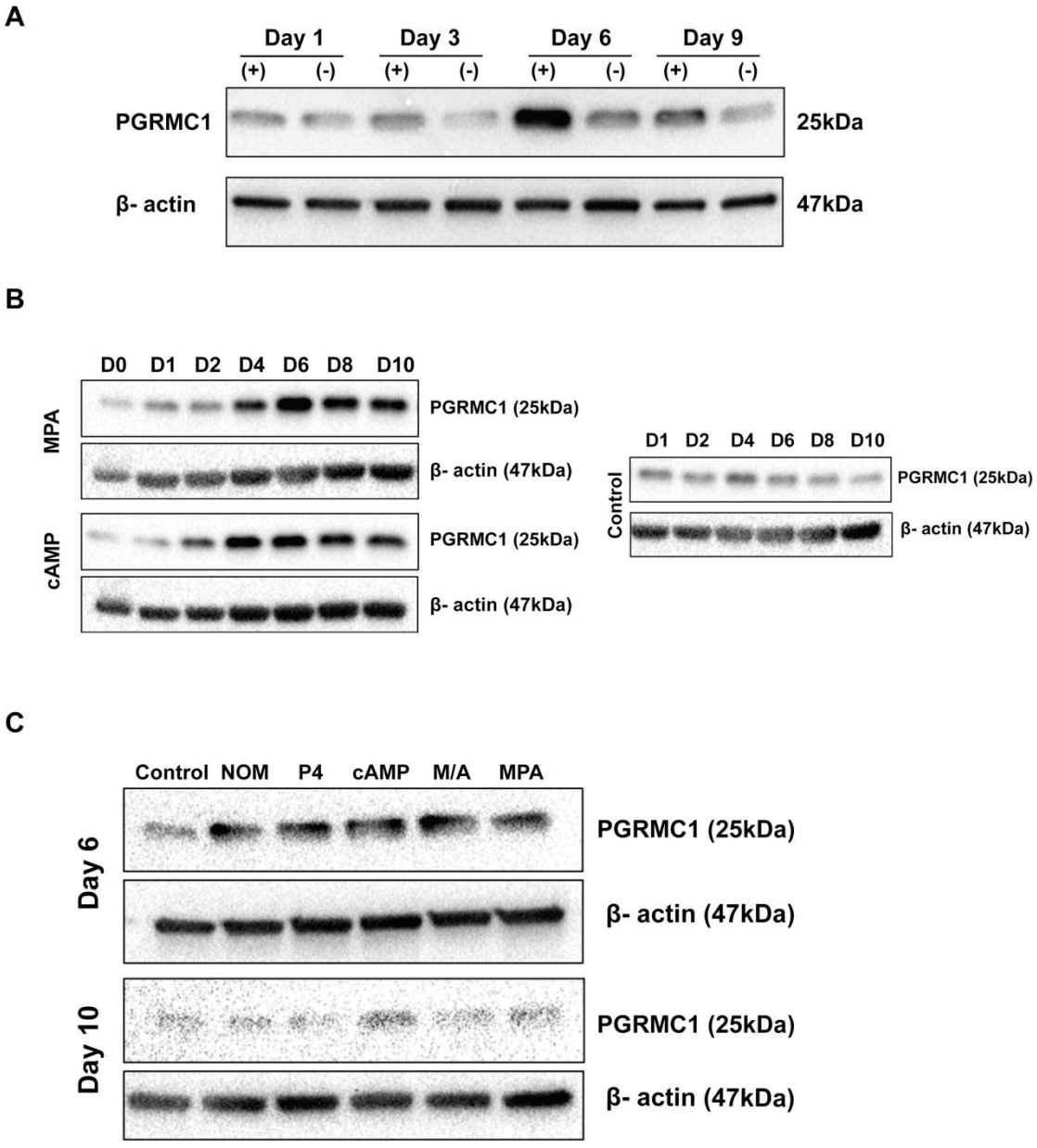
1. Reed BG, Carr BR. The Normal Menstrual Cycle and the Control of Ovulation. [Updated 2018 Aug 5]. In: Feingold KR, Anawalt B, Blackman MR, Boyce A, Chrousos G, Corpas E, de Herder WW, Dhatariya K, Dungan K, Hofland J, Kalra S, Kaltsas G, Kapoor N, Koch C, Kopp P, Korbonits M, Kovacs CS, Kuohung W, Laferrère B, Levy M, McGee EA, McLachlan R, New M, Purnell J, Sahay R, Shah AS, Singer F, Sperling MA, Stratakis CA, Trence DL, Wilson DP, editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000.
2. Jabbour HN, et al. Endocrine regulation of menstruation. Endocr Rev. 2006;27(1):17–46.
3. Mihm M, Gangooly S, Muttukrishna S. The normal menstrual cycle in women. Anim Reprod Sci. 2011;124(3–4):229–36.
4. Gellersen B, Brosens JJ. Cyclic decidualization of the human endometrium in reproductive health and failure. Endocr Rev. 2014;35(6):851–905.
5. Ng SW, et al. Endometrial Decidualization: The Primary Driver of Pregnancy Health. Int J Mol Sci. 2020;21(11):4092.
6. Chen JI, et al. Proteomic characterization of midproliferative and midsecretory human endometrium. J Proteome Res. 2009;8(4):2032–44.
7. Salsano S, et al. Dynamic expression of PGRMC1 and SERBP1 in human endometrium: an implication in the human decidualization process. Fertil Steril. 2017;108(5):832-842 e1.
8. Abdalla M, et al. miRNAs as a novel clinical biomarker and therapeutic targets in polycystic ovary syndrome (PCOS): A review. Life Sci. 2020;259:118174.

9. Bunch K, et al. Expression patterns of progesterone receptor membrane components 1 and 2 in endometria from women with and without endometriosis. Reprod Sci. 2014;21(2):190–7.
10. Feng L, et al. Progesterone receptor membrane component 1 (PGRMC1) expression in fetal membranes among women with preterm premature rupture of the membranes (PPROM). Placenta. 2014;35(5):331–3.
11. Gerhardt K. PGRMC1: A Fertility Stabilizer. Biol Reprod. 2016;95(3):49.
12. Mishra S, Murphy LC, Murphy LJ. The Prohibitins: emerging roles in diverse functions. J Cell Mol Med. 2006;10(2):353–63.
13. Signorile A, et al. Prohibitins: A Critical Role in Mitochondrial Functions and Implication in Diseases. Cells. 2019;8(1):71.
14. Hernando-Rodriguez B, Artal-Sanz M. Mitochondrial Quality Control Mechanisms and the PHB (Prohibitin) Complex. Cells. 2018;7(12):238.
15. He B, et al. A repressive role for prohibitin in estrogen signaling. Mol Endocrinol. 2008;22(2):344–60.
16. Zhang LF, et al. PHB regulates meiotic recombination via JAK2-mediated histone modifications in spermatogenesis. Nucleic Acids Res. 2020;48(9):4780–96.
17. Wang T, Zhang M, Jiang Z, Seli E. Mitochondrial dysfunction and ovarian aging. Am J Reprod Immunol. 2017;77(5). https://doi.org/10.1111/aji.12651. Epub 2017 Feb 13. PMID: 28194828.
18. Qi X, et al. Knockdown of prohibitin expression promotes glucose metabolism in eutopic endometrial stromal cells from women with endometriosis. Reprod Biomed Online. 2014;29(6):761–70.
19. He B, et al. Estrogen-regulated prohibitin is required for mouse uterine development and adult function. Endocrinology. 2011;152(3):1047–56.
20. Bai Y, et al. PGRMC1 Promotes Progestin-Dependent Proliferation of Breast Cancer Cells by Binding Prohibitins Resulting in Activation of ERalpha Signaling. Cancers (Basel). 2021;13(22):5635.
21. Talbi S, et al. Molecular phenotyping of human endometrium distinguishes menstrual cycle phases and underlying biological processes in normo-ovulatory women. Endocrinology. 2006;147(3):1097–121.
22. Burney RO, et al. Gene expression analysis of endometrium reveals progesterone resistance and candidate susceptibility genes in women with endometriosis. Endocrinology. 2007;148(8):3814–26.
23. Vestergaard AL, et al. Transcriptional expression of type-I interferon response genes and stability of housekeeping genes in the human endometrium and endometriosis. Mol Hum Reprod. 2011;17(4):243–54.
24. Nayyar A, et al. Menstrual Effluent Provides a Novel Diagnostic Window on the Pathogenesis of Endometriosis. Front Reprod Health. 2020;2:3.
25. Thieffry C, et al. Spatiotemporal expression pattern of Progesterone Receptor Component (PGRMC) 1 in endometrium from patients with or without endometriosis or adenomyosis. J Steroid Biochem Mol Biol. 2022;223:106153.
26. Salsano S, et al. Novel nonclassic progesterone receptor PGRMC1 pulldown-precipitated proteins reveal a key role during human decidualization. Fertil Steril. 2020;113(5):1050-1066 e7.
27. Peluso JJ, Pru JK. Progesterone Receptor Membrane Component (PGRMC)1 and PGRMC2 and Their Roles in Ovarian and Endometrial Cancer. Cancers (Basel). 2021;13(23):5953.
28. Tsuru A, et al. PGRMC1 Regulates Cellular Senescence via Modulating FOXO1 Expression in Decidualizing Endometrial Stromal Cells. Biomolecules. 2022;12(8):1046.
29. Thieffry C, et al. AG-205 Upregulates Enzymes Involved in Cholesterol Biosynthesis and Steroidogenesis in Human Endometrial Cells Independently of PGRMC1 and Related MAPR Proteins. Biomolecules. 2021;11(10):1472.
30. Salsano S, et al. Deciphering the Role of PGRMC1 During Human Decidualization Using an In Vitro Approach. J Clin Endocrinol Metab. 2021;106(8):2313–27.
31. Wang-Eckhardt L, Eckhardt M. A progesterone receptor membrane component 1 antagonist induces large vesicles independent of progesterone receptor membrane component 1 expression. Biol Chem. 2020;401(9):1093–9.
32. Teakel SL, et al. Protein complexes including PGRMC1 and actin-associated proteins are disrupted by AG-205. Biochem Biophys Res Commun. 2020;524(1):64–9.
33. Ahmed IS, et al. Progesterone receptor membrane component 1 (Pgrmc1): a heme-1 domain protein that promotes tumorigenesis and is inhibited by a small molecule. J Pharmacol Exp Ther. 2010;333(2):564–73.
34. Cahill MA. Progesterone receptor membrane component 1: an integrative review. J Steroid Biochem Mol Biol. 2007;105(1–5):16–36.
35. Velazquez Hernandez DM, Vazquez-Martinez ER, Camacho-Arroyo I. The role of progesterone receptor membrane component (PGRMC) in the endometrium. Steroids. 2022;184:109040.
36. Cahill MA, et al. The emerging role of progesterone receptor membrane component 1 (PGRMC1) in cancer biology. Biochim Biophys Acta. 2016;1866(2):339–49.
37. McCallum ML, et al. Conditional Ablation of Progesterone Receptor Membrane Component 1 Results in Subfertility in the Female and Development of Endometrial Cysts. Endocrinology. 2016;157(9):3309–19.
38. Ahmed IS, et al. Pgrmc1 (progesterone receptor membrane component 1) associates with epidermal growth factor receptor and regulates erlotinib sensitivity. J Biol Chem. 2010;285(32):24775–82.
39. Wang R, Sheehan PM, Brennecke SP. Changes in myometrial expression of progesterone receptor membrane components 1 and 2 are associated with human parturition at term. Reprod Fertil Dev. 2016;28(5):618–27.
40. Chen YJ, et al. PGRMC1 acts as a size-selective cargo receptor to drive ER-phagic clearance of mutant prohormones. Nat Commun. 2021;12(1):5991.
41. Merkwirth C, Langer T. Prohibitin function within mitochondria: essential roles for cell proliferation and cristae morphogenesis. Biochim Biophys Acta. 2009;1793(1):27–32.

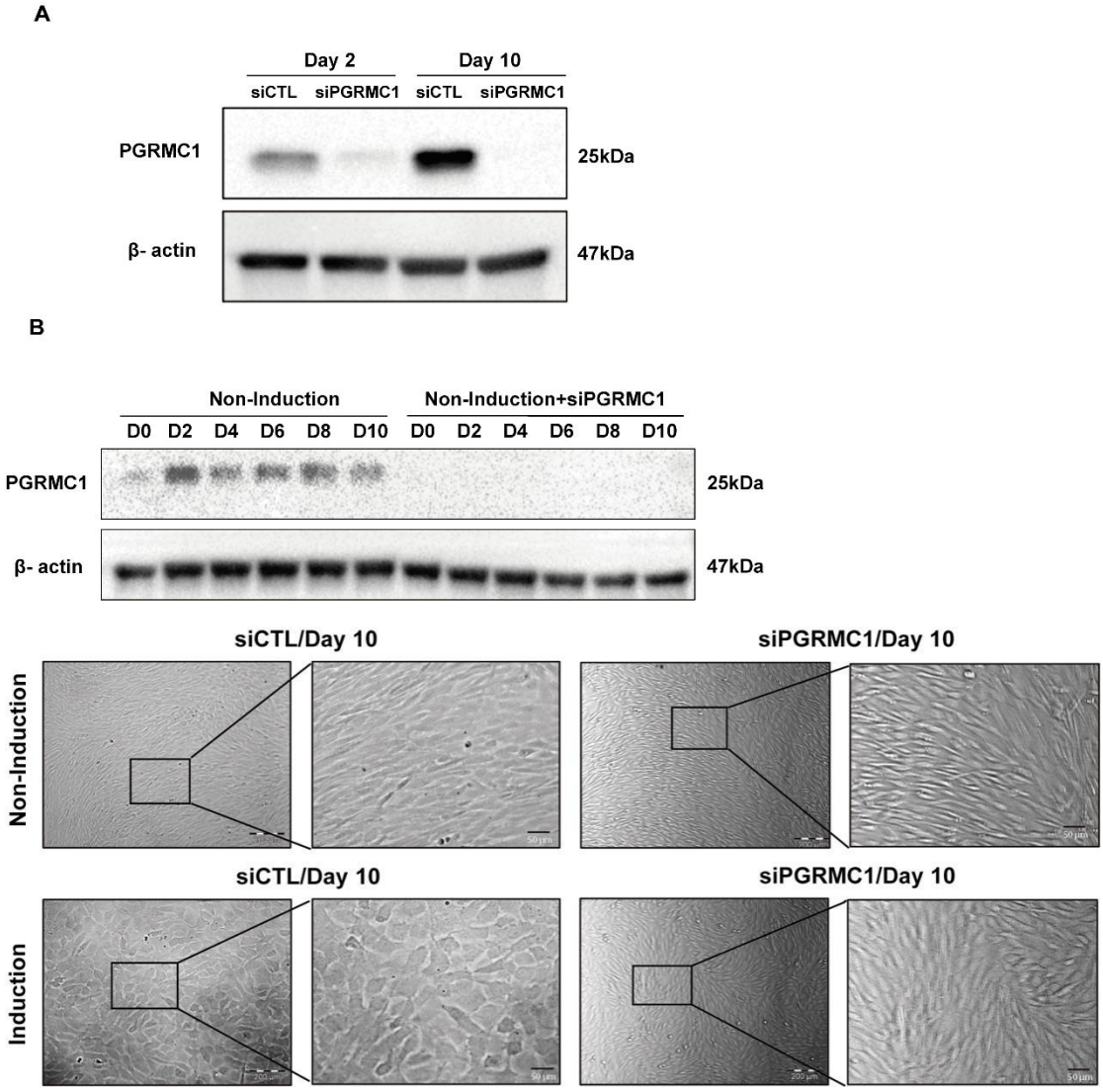
Publisher's Note

4. Supplementary Figures:

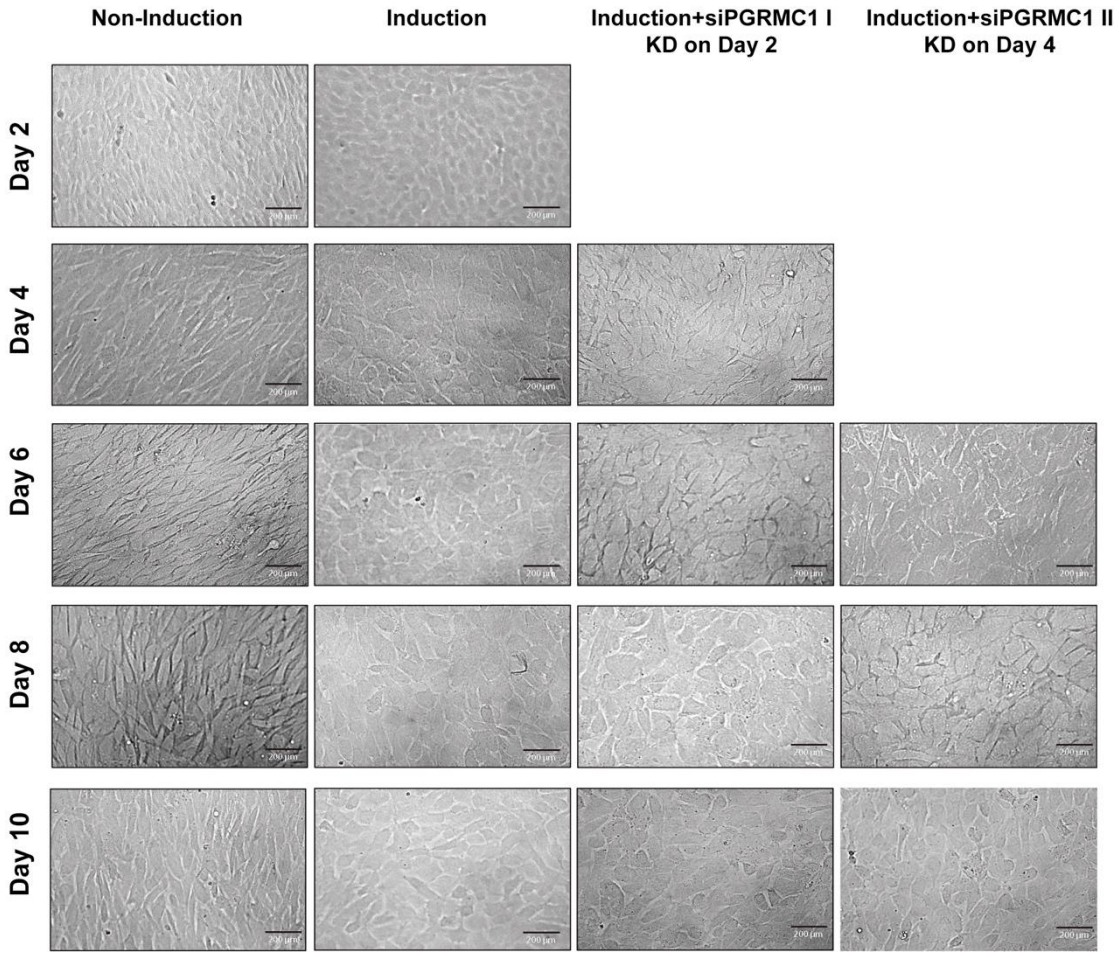
Supplementary Figure 1



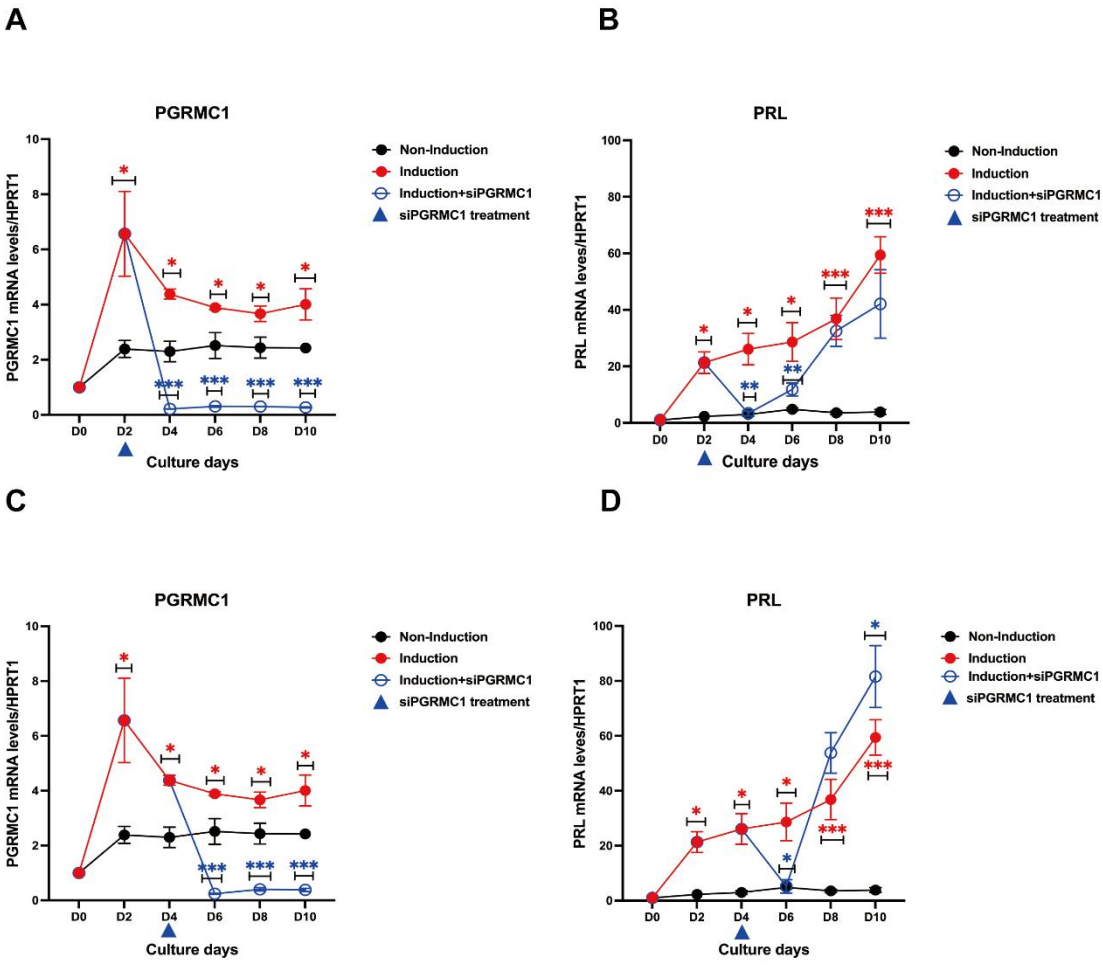
Supplementary Figure 2



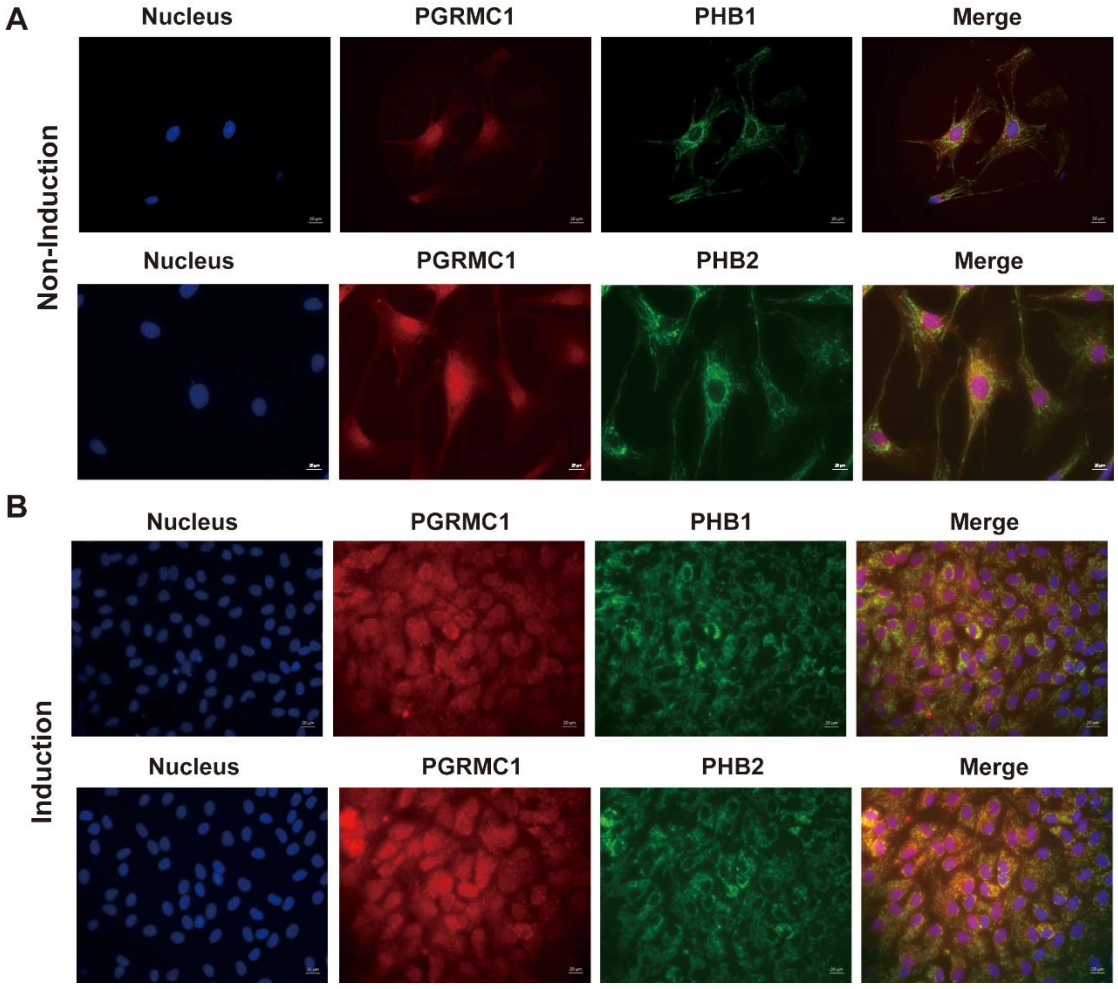
Supplementary Figure 3



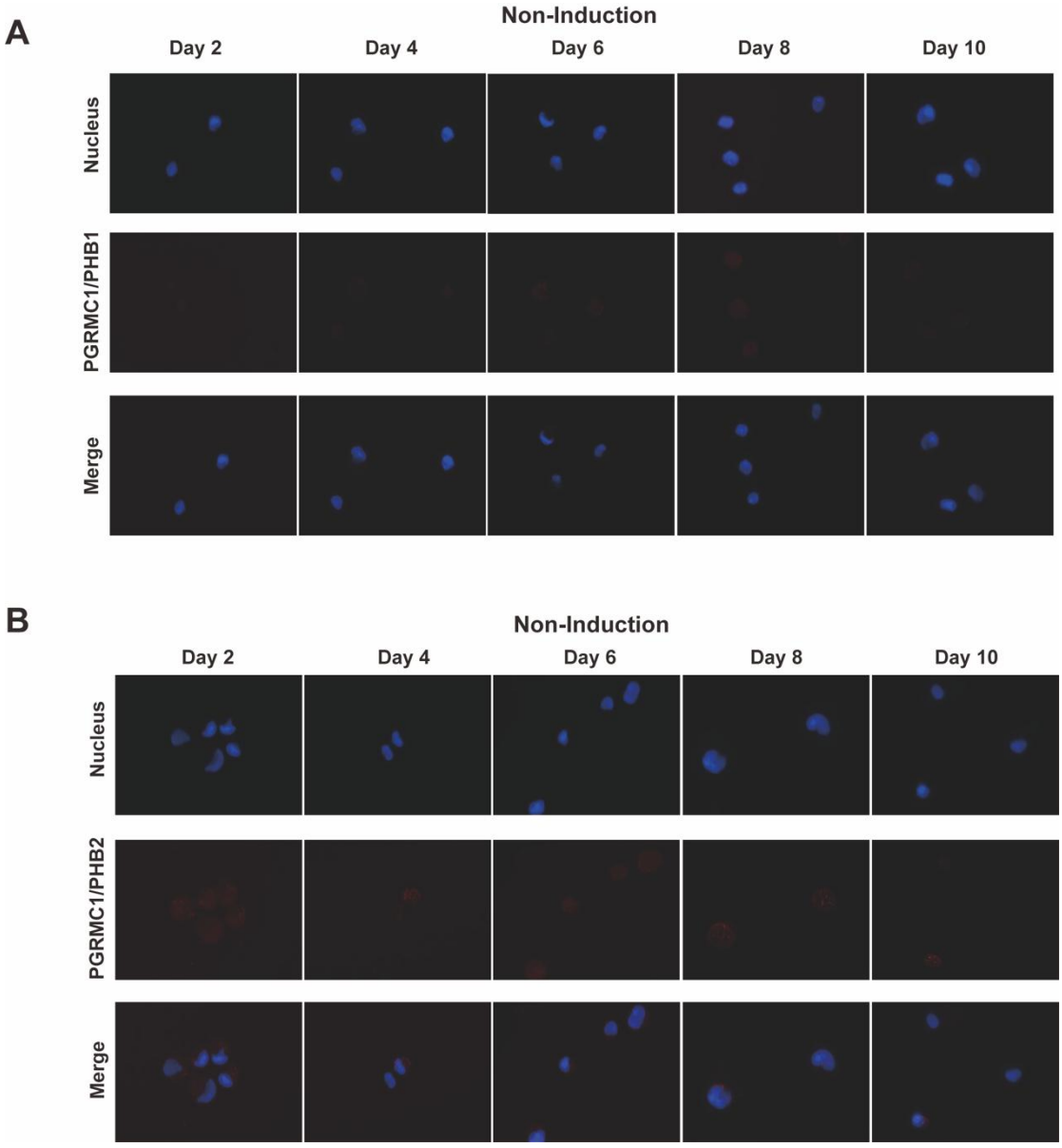
Supplementary Figure 4



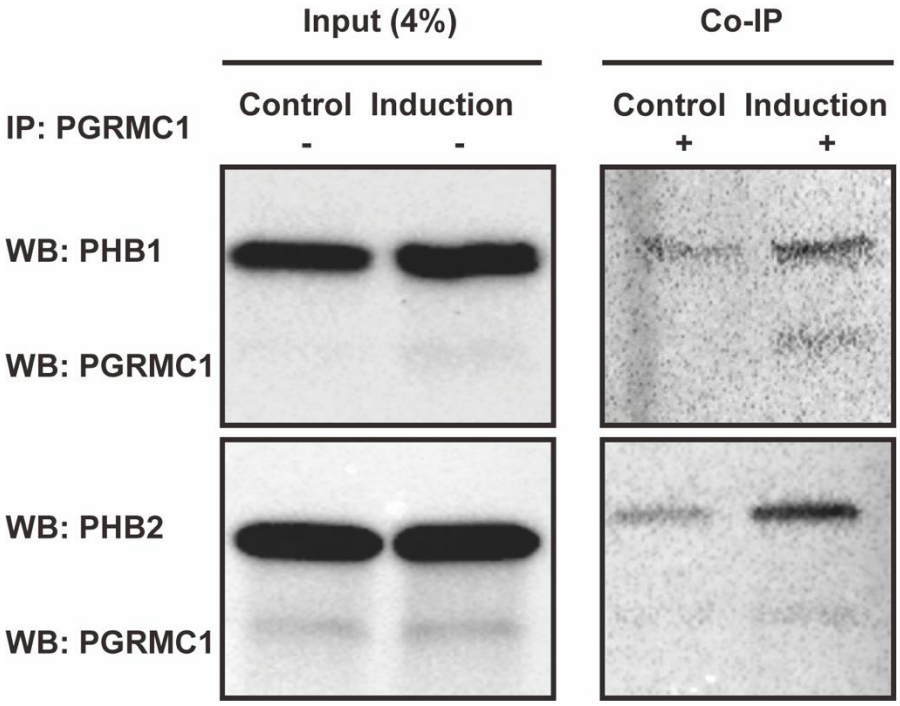
Supplementary Figure 5



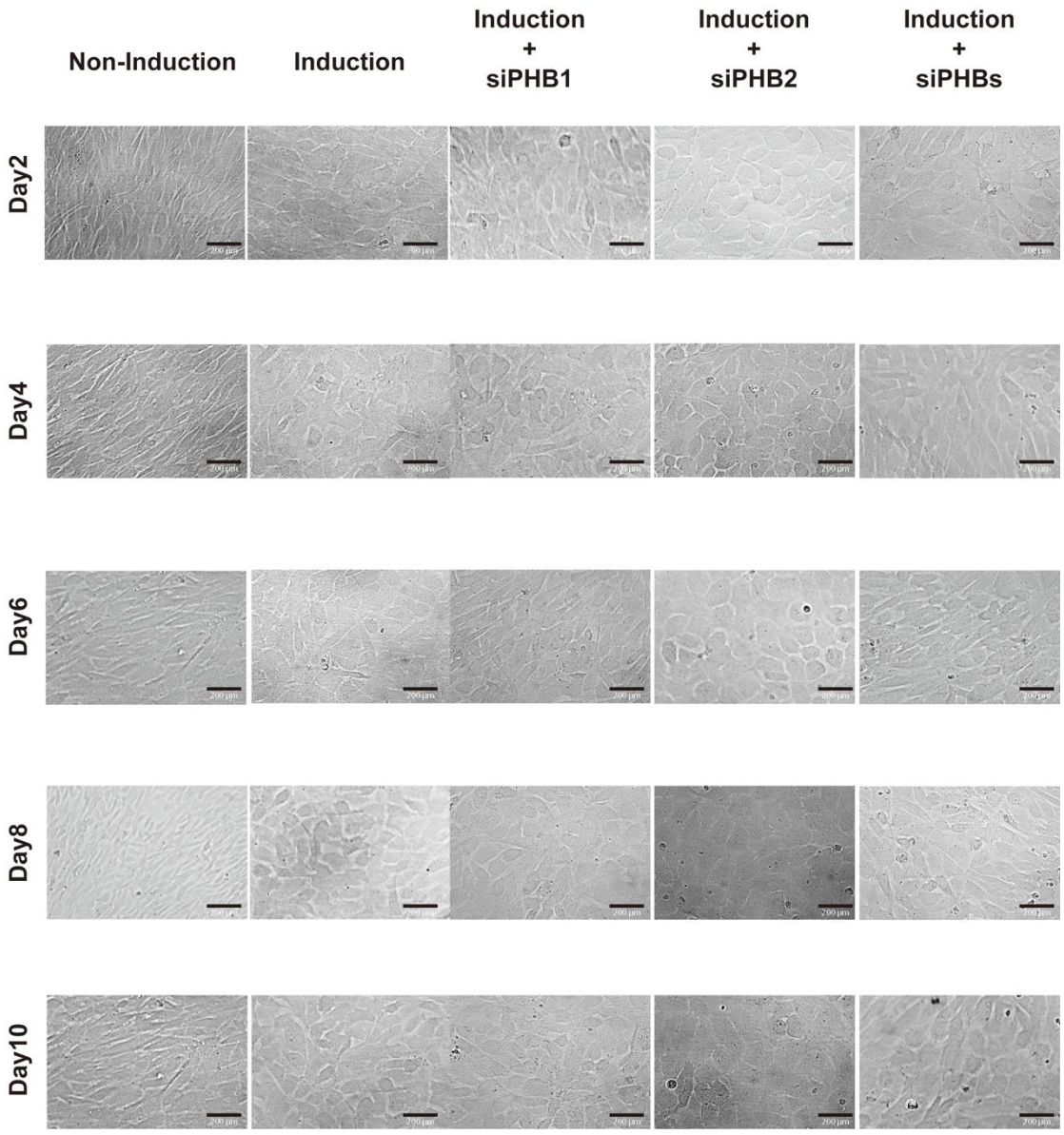
Supplementary Figure 6



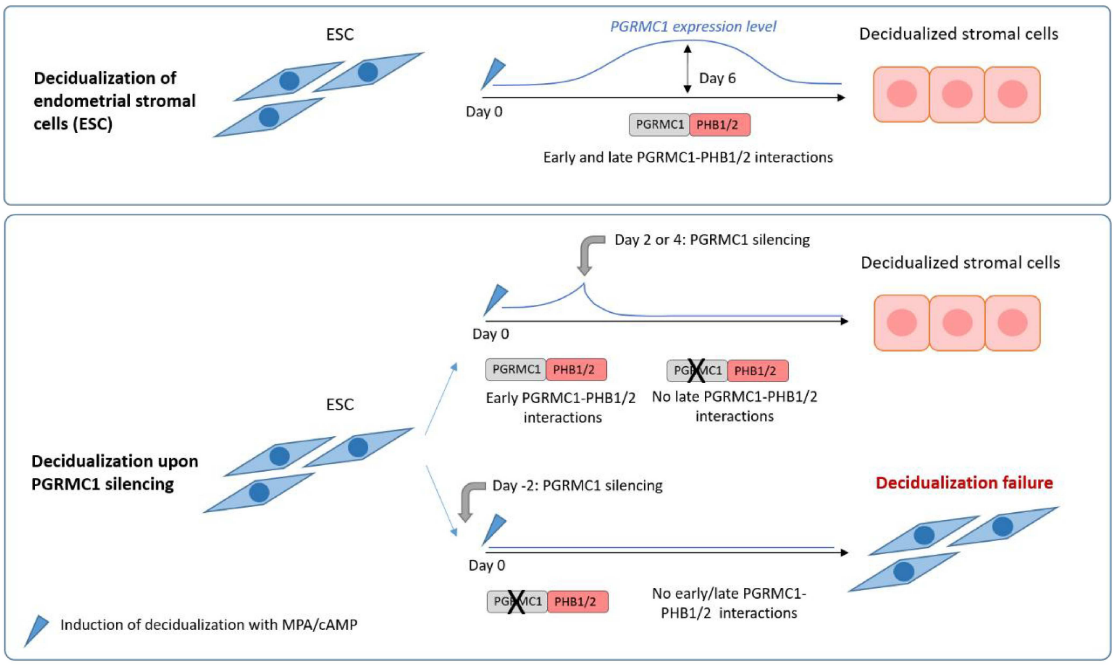
Supplementary Figure 7



Supplementary Figure 8



Supplementary Figure 9



5. General Discussion

PGRMC1 is expressed across various cellular systems and contexts, playing a critical role in a wide range of cellular functions(*77, 80*). It is predominantly located in the liver, kidney, brain, breast, lung and pancreas(*154, 155*). Beyond its involvement in membrane trafficking, cytochrome P450-mediated steroidogenesis, lipid synthesis, stress-response, and tumor progression(*77, 86*), PGRMC1 is implicated in carcinogenesis particularly in breast cancer, and is a potential target for cancer therapy(*80*). Its expression is associated with larger tumor size, metastasis, poor overall survival and tumor-free survival rate(*156, 157*). Accumulating evidence indicates that PGRMC1 acts as a fertility stabilizer, closely linked to non-genomic progesterone responses in the female reproductive tract(*81, 90, 97, 158*). Dysregulation of PGRMC1 is observed in multiple reproductive disorders, including endometriosis, chronic placental inflammation (CPI), and repeated implantation failure (RIF)(*158*). Women with reproductive disorders often have multiple risk factors contributing to negative obstetric outcomes. Inflammation is implicated as one of the major events of female infertility-related diseases. A hyperinflammatory or chronic inflammatory state can disrupt the immune-endocrine interaction within the endometrium(*159*), leading to conditions such as CPI, endometriosis, and shifted window of receptivity in RIF(*160-162*). PGRMC1 plays an essential role as a mediator of the anti-inflammatory effects of progesterone, to attenuate the activities of tumor necrosis factor-α (TNF-α)-induced matrix metalloproteinase 9 (MMP9)(*103, 163, 164*). The inflammatory response of fetal membranes associated with decreased PGRMC1, highlights the importance of preserving PGRMC1 for pregnancy maintenance. Furthermore, PGRMC1's role in the placenta's stress response indicates that oxidative stress induction and inflammation reduce its expression. Progesterone action is crucial to decreasing inflammation in the endometrium, normally triggering an endometrial response by inhibiting estrogen-dependent proliferation of epithelial cells and transforming stromal cells into decidual cells. Defective progesterone signaling results in a proinflammatory phenotype, while chronic inflammation can

induce progesterone resistance(*165, 166*). This insensitivity, associated with changes in progesterone receptor isoforms(*167-169*) and disruption in progesterone signaling downstream(*170, 171*), has been linked to infertility-related disorders such as endometriosis and RIF. Given that decidualization is crucial for reproduction and disordered decidualization can also lead to advanced pregnancy complications(*48*), the low implantation rates in assistive reproduction technology (ART), many couples still experiencing infertility. The implantation rates in ART remain low, even with high-quality embryos, underscoring the importance of addressing impaired decidualization as a major cause of female infertility(*172*). With PGRMC1's dysregulation found in serval infertility-related diseases, the role of PGRMC1 in decidualization merits further investigation, despite some studies have already shed light on its function (*105, 112, 113, 122*).

Our main finding offers a detailed and dynamic expression profile of PGRMC1 during decidualization, unveiling that its expression follows a rise-to-decline pattern post-progestin stimulation, essential for proper decidualization (see Supplementary figure 10). Concurrently, the interaction between prohibitin proteins (PHB1/PHB2) with PGRMC1 in decidualization was observed, indicating their collective role in this process. Our *in-silico* analysis revealed that, throughout a typical menstrual cycle, the dynamic changes of the PGRMC1 protein levels consist of two peaks: one in the secretory phase and another one in the proliferative phase, as previously documented(*127, 173*). In our decidualization induction model using T-HESCs cells, we noted a similar rise-to-decline pattern in both protein and mRNA levels of PGRMC1. During the first 6 days of induction, mirroring the menstrual cycle's proliferative phase (analogous to the ovary's follicular phase), PGRMC1 expression increases. This phase is marked by rising levels of 17-ß-estradiol (E2), which thickens the endometrial lining through stromal and glands proliferation, and spiral artery elongation(*9, 174*). The up-regulation of PGRMC1 suggests it may be regulated by E2 concentration or a similar mechanism. Given that PGRMC1 overexpression leads to higher E2 secretion in breast cancer cells(*99*), it is plausible that E2 production in

human endometrial stromal cells relies on PGRMC1 activation. Following a peak on induction day 6, PGRMC1 expression starts to decline until the endometrial stromal cells complete decidualization. This process occurs in the secretory phase of the menstrual cycle, where endometrial stromal cells morphologically and biochemically prepared under the control of progesterone for successful blastocyst implantation, placenta development, and modulation of the immune response(*175*).

The endometrial transformation is initiated by E2 through estrogen receptors alpha and beta (ERα and ERβ, respectively). E2 significantly influences the expression of the progesterone receptor (PR), enabling the endometrium's response to progesterone during the secretory phase(*176*). The activation of G protein-coupled membrane receptors by key endogenous factors such progesterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), human chorionic gonadotropin (hCG), relaxin and prostaglandins (PGs) reportedly lead to an increase in intracellular cyclic adenosine monophosphate (cAMP) levels(*177*). The rise in cAMP triggers the activation of protein kinase A (PKA) and exchange proteins activated by cAMP, regulating the expression of genes associated with decidualization(*178*). Several *in vitro* studies employing different protocols to induce decidualization in primary ESCs(*179, 180*) or other immortalized ESCs(*181, 182*), including a combination of progestin (progesterone or medroxyprogesterone acetate [MPA]), E2 and cAMP analogs, their mixture or of MPA with cAMP, have demonstrated the induction of decidualization markers at different periods. Our initial observations in the T-HESC cell line and another immortalized ESC cell line (St-T1) revealed a PGRMC1 rise-to-decline pattern induced by MPA + cAMP treatment. A similar pattern of PGRMC1 expression could also be induced using MPA and cAMP alone (see Supplementary figure 1). However, we did not detect the decidualization markers expression level under different induction schemes. Further studies are necessary to explore the decidualization efficacy of those different schemes, which is crucial for comprehending the molecular physiology of the endometrium.

Adequate PGRMC1 levels are necessary to initiate decidualization upon progesterone/cAMP stimulation. PGRMC1 levels in ESCs are significantly lower during the secretory phase than in the proliferative phase, suggesting a stage-specific regulation of PGRMC1 throughout the menstrual cycle. Knocking down PGRMC1 before induction inhibits decidualization, highlighting its crucial role as a 'switch' at this stage. However, PGRMC1 appears less necessary after the initiation of decidualization, as knocking it down either does not affect or even facilitates the process. Reducing PGRMC1 levels after progestin treatment does not hamper decidualization, indicating its critical role during the increasing phase and induction. This aligns with the observations that PGRMC1 expression decreases in the endometrial stroma during the late secretory phase and may even promote decidualization(*105, 113*). Although, PGRMC1 activation by progesterone may facilitate the switch from cellular proliferation to decidualization initiation through various biological processes, the underlying mechanisms by which PGRMC1 levels is downregulated in the secretory phase remain unexplored. Tsuru et al. reported an upregulation of miR-98 in parallel with PGRMC1 downregulation in db-cAMP/P4-induced decidual cells, indicating miR-98's involve-ment in PGRMC1 regulation during decidualization. In the rat uterus, miR-98 downregulation promotes ESCs proliferation and inhibits apoptosis by targeting the anti-apoptotic protein Bcl-xl(*183*). Patients experiencing early spontaneous abortion exhibit lower miR-98 expression in decidual tissues compared to women with normal pregnancies(*184*). Further studies are required to determine the relationship between miR-98 mediated PGRMC1 regulation and decidualization.

PGRMC1 is known to occupy multiple subcellular locations, from endoplasmic reticulum, cytoplasm, plasma membrane, nucleus, and mitochondria(*82*). Its localization is regulated by processes such as phosphorylation, ubiquitination, and sumoylation(*77, 80*). In granulosa cells (GCs), the cellular localization of PGRMC1 can shift under different physiological conditions. In rat follicles, PGRMC1 expression increases in luteal cells and its diffusely localized throughout the cytoplasm and

nucleus following hCG-induction(*185*). Therefore, we sought to analyze PGRMC1's subcellular localization during the decidualization process. Interestingly, we noticed an induction-associated peri-nuclear phenomenon, characterized by more intense signals in the peri-nuclear region at 10 days of induction (Figure 4A). However, when analyzing the subcellular protein amount of PGRMC1 via western blot, we found PGRMC1 was barely detected in the nucleus before and after induction (Figure 4B). Our results are not fully consistent with Salsano *et al*., who reported that PGRMC1's subcellular protein amount could be detected in both non-decidualized and decidualized stromal cells, with higher amounts observed in decidualized stromal cells(*105*). The nuclear localization of PGRMC1 in decidualized stromal cells, as noted in macaque(*186*) and during early gestation in mice(*101*), suggests a potential role for PGRMC1 in regulating the expression of genes distinct from those targeted by the classic nuclear PR during decidualization. Luciano et al. found PGRMC1 colocalized with active phosphorylated aurora kinase B on chromosomes and centromeres during oocyte maturation, an indication that nucleus-localized PGRMC1 in decidualized stromal cells could be associated with cell cycle progression(*97*). Our study does not rule out the possibility of PGRMC1 translocating towards the nuclear during decidualization; however, PGRMC1 was not detected in the nucleus in our system, regardless of decidualization status. This conclusion is partially in agreement with Thieffry et al., who indicated that PGRMC1 was essentially present in the cytoplasm and was barely or not detected in the nucleus in T-HESC and HEC-1A (Human Endometrial Cancer One A) cell lines(*125*).

PGRMC1 has been reported to be associated with proteins involved in protein biosynthesis, intracellular transport, and mitochondrial activity, all of which promote decidualization(*112*). The specifics of how PGRMC1 interacts with these proteins to regulate decidualization is little known. Following induction, we found that PGRMC1 binds to prohibitins (PHBs) at the periphery of the nucleus, indicating it might serve as a scaffold protein for decidualization. Knocking down PHBs partially impaired decidualization, similar to PGRMC1 knockdown, suggesting PGRM1-PHBs

interactions may affect decidualization as a complex. In breast cancer cells, we previously demonstrated overexpression of PGRMC1 leads to increased synthesis of E2 and the upregulation of ER, with elevated levels of E2 and ER responsible for increased proliferation of E2-dependent tumor cells. PGRMC1 is binding PHB1 and PHB2, thereby facilitating the release of ER for transcriptional activity in the nucleus(*99*). We speculate that PGRMC1's binding to PHBs may inhibit cellular proliferation and facilitate differentiation, acting as a proliferation-differentiation switch, a concept which requires further investigation.

AG205, purportedly an inhibitor of PGRMC1, has had its specificity recently questioned. Previous studies showed reduced cell viability with AG205 treatment, through the effective concentration of AG205 varies across in different cell types. In MDA-MB-231 breast cancer cells, cell viability was reduced by 40% and 60% under 20μM and 40μM of AG205 treatment, respectively; in lung cancer-derived stem cells, cell viability was reduced by approximately 25%, 40%, and 50% after 24 h at concentration of 25μM, 50μM, and 100μM of AG205 treatment, respectively(*156*). Prior to analysis, we optimized the concentration of AG205 and incubation time, assessing its potential effects on cell viability and PGRMC1 expression. Based on our MTT assay, we selected 15μM of AG205 for further use. We noticed that AG205 did not affect PGRMC1-PHBs interaction, nor did it impact decidualization in our study. Our data align with another study demonstrating that 15μM of AG205 has no effect on the expression of PGRMC1, and did not promote increased nuclear localization of PGRMC1(*125*). In ESCs, even a higher concentration of AG205 (50μM) did not affect decidualization(*122*).

Thus, our study postulates that the progesterone/progestin-induced rise-to-decline expression pattern of PGRMC1 is critical for initiating decidualization. Once decidualization begins, PGRMC1 is no longer necessary to sustain the process. PGRMC1's expression is specifically important at the onset of decidualization, and disruption if this expression could impair decidualization.

6. Limitations of this work

As limitations, proximity ligation assay (PLA) results only indicate a close proximity and potential physical interaction between PGRMC1 and PHBs. A study involving a larger cohort from primary patient's endometrium tissues and mouse model would likely yield more available and significant results.

7. References

1. B. Zhang, W. Li, J. Cao, Y. Zhou, X. Yuan, Prohibitin 2: A key regulator of cell function. *Life Sci* **338**, 122371 (2024).
2. H. C. Fitzgerald, D. J. Schust, T. E. Spencer, In vitro models of the human endometrium: evolution and application for women's health. *Biol Reprod* **104**, 282-293 (2021).
3. S. Kyurkchiev, A. Shterev, R. Dimitrov, Assessment of presence and characteristics of multipotent stromal cells in human endometrium and decidua. *Reprod Biomed Online* **20**, 305-313 (2010).
4. L. Garcia-Alonso *et al.*, Mapping the temporal and spatial dynamics of the human endometrium in vivo and in vitro. *Nat Genet* **53**, 1698-1711 (2021).
5. R. D. Martin, The evolution of human reproduction: a primatological perspective. *Am J Phys Anthropol* **Suppl 45**, 59-84 (2007).
6. X. Wang, S. M. Hawkins, Using advanced spatial and single-cell transcriptomics to characterize the human endometrium. *Nat Genet* **53**, 1628-1630 (2021).
7. P. Diaz-Gimeno *et al.*, A genomic diagnostic tool for human endometrial receptivity based on the transcriptomic signature. *Fertil Steril* **95**, 50-60, 60 e51-15 (2011).
8. W. Wang *et al.*, Single-cell transcriptomic atlas of the human endometrium during the menstrual cycle. *Nat Med* **26**, 1644-1653 (2020).
9. M. Mihm, S. Gangooly, S. Muttukrishna, The normal menstrual cycle in women. *Anim Reprod Sci* **124**, 229-236 (2011).
10. J. Le, N. Thomas, C. Gurvich, Cognition, The Menstrual Cycle, and Premenstrual Disorders: A Review. *Brain Sci* **10**, (2020).
11. H. N. Jabbour, R. W. Kelly, H. M. Fraser, H. O. Critchley, Endocrine regulation of menstruation. *Endocr Rev* **27**, 17-46 (2006).
12. B. G. D. Figueiredo, M. T. C. Rezende, N. A. D. Santos, M. J. O. Andrade, Mapping changes in women's visual functions during the menstrual cycle: narrative review. *Sao Paulo Med J* **139**, 662-674 (2021).
13. K. Itriyeva, The normal menstrual cycle. *Curr Probl Pediatr Adolesc Health Care* **52**, 101183 (2022).
14. G. A. Johnson *et al.*, Osteopontin expression in uterine stroma indicates a decidualization-like differentiation during ovine pregnancy. *Biol Reprod* **68**, 1951-1958 (2003).
15. S. W. Ng *et al.*, Endometrial Decidualization: The Primary Driver of Pregnancy Health. *Int J Mol Sci* **21**, (2020).
16. L. K. Harris, M. Benagiano, M. M. D'Elios, I. Brosens, G. Benagiano, Placental bed research: II. Functional and immunological investigations of the placental bed. *Am J Obstet Gynecol* **221**, 457-469 (2019).
17. G. S. Vince, P. M. Starkey, M. C. Jackson, I. L. Sargent, C. W. Redman, Flow cytometric characterisation of cell populations in human pregnancy decidua

and isolation of decidual macrophages. *J Immunol Methods* **132**, 181-189 (1990).
18. F. Yang, Q. Zheng, L. Jin, Dynamic Function and Composition Changes of Immune Cells During Normal and Pathological Pregnancy at the Maternal-Fetal Interface. *Front Immunol* **10**, 2317 (2019).
19. M. Massimiani *et al.*, Molecular Signaling Regulating Endometrium-Blastocyst Crosstalk. *Int J Mol Sci* **21**, (2019).
20. M. Haller, Y. Yin, L. Ma, Development and utilization of human decidualization reporter cell line uncovers new modulators of female fertility. *Proc Natl Acad Sci U S A* **116**, 19541-19551 (2019).
21. B. Gellersen, J. J. Brosens, Cyclic decidualization of the human endometrium in reproductive health and failure. *Endocr Rev* **35**, 851-905 (2014).
22. F. J. Cornillie, J. M. Lauweryns, I. A. Brosens, Normal human endometrium. An ultrastructural survey. *Gynecol Obstet Invest* **20**, 113-129 (1985).
23. A. M. Lawn, E. W. Wilson, C. A. Finn, The ultrastructure of human decidual and predecidual cells. *J Reprod Fertil* **26**, 85-90 (1971).
24. T. Kajihara *et al.*, Androgens modulate the morphological characteristics of human endometrial stromal cells decidualized in vitro. *Reprod Sci* **21**, 372-380 (2014).
25. M. A. Ochoa-Bernal, A. T. Fazleabas, Physiologic Events of Embryo Implantation and Decidualization in Human and Non-Human Primates. *Int J Mol Sci* **21**, (2020).
26. G. P. Wagner, E. M. Erkenbrack, A. C. Love, Stress-Induced Evolutionary Innovation: A Mechanism for the Origin of Cell Types. *Bioessays* **41**, e1800188 (2019).
27. L. C. Giudice, Microarray expression profiling reveals candidate genes for human uterine receptivity. *Am J Pharmacogenomics* **4**, 299-312 (2004).
28. J. A. Horcajadas *et al.*, Determinants of endometrial receptivity. *Ann N Y Acad Sci* **1034**, 166-175 (2004).
29. P. A. Essah, K. I. Cheang, J. E. Nestler, The pathophysiology of miscarriage in women with polycystic ovary syndrome. Review and proposed hypothesis of mechanisms involved. *Hormones (Athens)* **3**, 221-227 (2004).
30. C. H. Graham *et al.*, Establishment and characterization of first trimester human trophoblast cells with extended lifespan. *Exp Cell Res* **206**, 204-211 (1993).
31. G. Krikun *et al.*, A novel immortalized human endometrial stromal cell line with normal progestational response. *Endocrinology* **145**, 2291-2296 (2004).
32. A. Samalecos *et al.*, Characterization of a novel telomerase-immortalized human endometrial stromal cell line, St-T1b. *Reprod Biol Endocrinol* **7**, 76 (2009).
33. J. J. Brosens *et al.*, Human endometrial fibroblasts immortalized by simian virus 40 large T antigen differentiate in response to a decidualization stimulus. *Endocrinology* **137**, 2225-2231 (1996).

34. J. C. Irwin, D. Kirk, R. J. King, M. M. Quigley, R. B. Gwatkin, Hormonal regulation of human endometrial stromal cells in culture: an in vitro model for decidualization. *Fertil Steril* **52**, 761-768 (1989).
35. N. J. Hannan, P. Paiva, E. Dimitriadis, L. A. Salamonsen, Models for study of human embryo implantation: choice of cell lines? *Biol Reprod* **82**, 235-245 (2010).
36. J. J. Brosens, N. Hayashi, J. O. White, Progesterone receptor regulates decidual prolactin expression in differentiating human endometrial stromal cells. *Endocrinology* **140**, 4809-4820 (1999).
37. B. Gellersen, J. Brosens, Cyclic AMP and progesterone receptor cross-talk in human endometrium: a decidualizing affair. *J Endocrinol* **178**, 357-372 (2003).
38. C. Y. Ramathal, I. C. Bagchi, R. N. Taylor, M. K. Bagchi, Endometrial decidualization: of mice and men. *Semin Reprod Med* **28**, 17-26 (2010).
39. R. Telgmann, B. Gellersen, Marker genes of decidualization: activation of the decidual prolactin gene. *Hum Reprod Update* **4**, 472-479 (1998).
40. B. Gellersen, I. A. Brosens, J. J. Brosens, Decidualization of the human endometrium: mechanisms, functions, and clinical perspectives. *Semin Reprod Med* **25**, 445-453 (2007).
41. T. J. Lindsay, K. R. Vitrikas, Evaluation and treatment of infertility. *Am Fam Physician* **91**, 308-314 (2015).
42. M. Vander Borght, C. Wyns, Fertility and infertility: Definition and epidemiology. *Clin Biochem* **62**, 2-10 (2018).
43. W. Ombelet, I. Cooke, S. Dyer, G. Serour, P. Devroey, Infertility and the provision of infertility medical services in developing countries. *Hum Reprod Update* **14**, 605-621 (2008).
44. F. Zegers-Hochschild *et al.*, The International Glossary on Infertility and Fertility Care, 2017. *Hum Reprod* **32**, 1786-1801 (2017).
45. A. L. Greil, K. Slauson-Blevins, J. McQuillan, The experience of infertility: a review of recent literature. *Sociol Health Illn* **32**, 140-162 (2010).
46. M. P. Dougherty *et al.*, Unexplained Female Infertility Associated with Genetic Disease Variants. *N Engl J Med* **388**, 1055-1056 (2023).
47. J. Cha, X. Sun, S. K. Dey, Mechanisms of implantation: strategies for successful pregnancy. *Nat Med* **18**, 1754-1767 (2012).
48. C. Ticconi, N. Di Simone, L. Campagnolo, A. Fazleabas, Clinical consequences of defective decidualization. *Tissue Cell* **72**, 101586 (2021).
49. G. Dallenbach-Hellweg, The endometrium of infertility. A review. *Pathol Res Pract* **178**, 527-537 (1984).
50. T. Liu, B. He, X. Xu, Repairing and Regenerating Injured Endometrium Methods. *Reprod Sci* **30**, 1724-1736 (2023).
51. L. Jiang *et al.*, Infertility psychological distress in women undergoing assisted reproductive treatment: A grounded theory study. *J Clin Nurs*, (2024).
52. H. Okada, T. Tsuzuki, H. Murata, Decidualization of the human endometrium. *Reprod Med Biol* **17**, 220-227 (2018).

53. M. P. Snijders *et al.*, Immunocytochemical analysis of oestrogen receptors and progesterone receptors in the human uterus throughout the menstrual cycle and after the menopause. *J Reprod Fertil* **94**, 363-371 (1992).
54. M. Mori, A. Bogdan, T. Balassa, T. Csabai, J. Szekeres-Bartho, The decidua-the maternal bed embracing the embryo-maintains the pregnancy. *Semin Immunopathol* **38**, 635-649 (2016).
55. X. Wang, S. P. Wu, F. J. DeMayo, Hormone dependent uterine epithelial-stromal communication for pregnancy support. *Placenta* **60 Suppl 1**, S20-S26 (2017).
56. S. Sharma, G. Godbole, D. Modi, Decidual Control of Trophoblast Invasion. *Am J Reprod Immunol* **75**, 341-350 (2016).
57. E. D. Albrecht *et al.*, Effect of estrogen on angiogenesis in co-cultures of human endometrial cells and microvascular endothelial cells. *Hum Reprod* **18**, 2039-2047 (2003).
58. C. Dunk *et al.*, Failure of Decidualization and Maternal Immune Tolerance Underlies Uterovascular Resistance in Intra Uterine Growth Restriction. *Front Endocrinol (Lausanne)* **10**, 160 (2019).
59. S. Dambaeva *et al.*, Decidualization score identifies an endometrial dysregulation in samples from women with recurrent pregnancy losses and unexplained infertility. *F S Rep* **2**, 95-103 (2021).
60. D. Wu *et al.*, Chronic endometritis modifies decidualization in human endometrial stromal cells. *Reprod Biol Endocrinol* **15**, 16 (2017).
61. T. Garrido-Gomez *et al.*, Defective decidualization during and after severe preeclampsia reveals a possible maternal contribution to the etiology. *Proc Natl Acad Sci U S A* **114**, E8468-E8477 (2017).
62. T. Garrido-Gomez, N. Castillo-Marco, T. Cordero, C. Simon, Decidualization resistance in the origin of preeclampsia. *Am J Obstet Gynecol* **226**, S886-S894 (2022).
63. X. Cai *et al.*, Endometrial stromal PRMT5 plays a crucial role in decidualization by regulating NF-kappaB signaling in endometriosis. *Cell Death Discov* **8**, 408 (2022).
64. R. Navarro, L. Poder, D. Sun, P. Jha, Endometriosis in pregnancy. *Abdom Radiol (NY)* **45**, 1741-1753 (2020).
65. H. Kobayashi, Endometrial Inflammation and Impaired Spontaneous Decidualization: Insights into the Pathogenesis of Adenomyosis. *Int J Environ Res Public Health* **20**, (2023).
66. Y. Peng, Z. Jin, H. Liu, C. Xu, Impaired decidualization of human endometrial stromal cells from women with adenomyosisdagger. *Biol Reprod* **104**, 1034-1044 (2021).
67. M. Khatun *et al.*, Decidualized endometrial stromal cells present with altered androgen response in PCOS. *Sci Rep* **11**, 16287 (2021).
68. J. H. You, J. Lee, J. L. Roh, PGRMC1-dependent lipophagy promotes ferroptosis in paclitaxel-tolerant persister cancer cells. *J Exp Clin Cancer Res* **40**, 350 (2021).

69. E. Falkenstein *et al.*, Localization of a putative progesterone membrane binding protein in porcine hepatocytes. *Cell Mol Biol (Noisy-le-grand)* **44**, 571-578 (1998).
70. D. Gerdes, M. Wehling, B. Leube, E. Falkenstein, Cloning and tissue expression of two putative steroid membrane receptors. *Biol Chem* **379**, 907-911 (1998).
71. C. Meyer, R. Schmid, P. C. Scriba, M. Wehling, Purification and partial sequencing of high-affinity progesterone-binding site(s) from porcine liver membranes. *Eur J Biochem* **239**, 726-731 (1996).
72. E. Falkenstein, C. Meyer, C. Eisen, P. C. Scriba, M. Wehling, Full-length cDNA sequence of a progesterone membrane-binding protein from porcine vascular smooth muscle cells. *Biochem Biophys Res Commun* **229**, 86-89 (1996).
73. F. S. Raza, H. Takemori, H. Tojo, M. Okamoto, G. P. Vinson, Identification of the rat adrenal zona fasciculata/reticularis specific protein, inner zone antigen (IZAg), as the putative membrane progesterone receptor. *Eur J Biochem* **268**, 2141-2147 (2001).
74. L. Min *et al.*, Characterization of the adrenal-specific antigen IZA (inner zone antigen) and its role in the steroidogenesis. *Mol Cell Endocrinol* **215**, 143-148 (2004).
75. R. A. Hand, N. Jia, M. Bard, R. J. Craven, Saccharomyces cerevisiae Dap1p, a novel DNA damage response protein related to the mammalian membrane-associated progesterone receptor. *Eukaryot Cell* **2**, 306-317 (2003).
76. A. L. Hughes *et al.*, Dap1/PGRMC1 binds and regulates cytochrome P450 enzymes. *Cell Metab* **5**, 143-149 (2007).
77. M. A. Cahill, Progesterone receptor membrane component 1: an integrative review. *J Steroid Biochem Mol Biol* **105**, 16-36 (2007).
78. R. M. Losel, D. Besong, J. J. Peluso, M. Wehling, Progesterone receptor membrane component 1--many tasks for a versatile protein. *Steroids* **73**, 929-934 (2008).
79. M. Suchanek, A. Radzikowska, C. Thiele, Photo-leucine and photo-methionine allow identification of protein-protein interactions in living cells. *Nat Methods* **2**, 261-267 (2005).
80. M. A. Cahill *et al.*, The emerging role of progesterone receptor membrane component 1 (PGRMC1) in cancer biology. *Biochim Biophys Acta* **1866**, 339-349 (2016).
81. L. Zhang *et al.*, Expression of progesterone receptor membrane component 1 and its partner serpine 1 mRNA binding protein in uterine and placental tissues of the mouse and human. *Mol Cell Endocrinol* **287**, 81-89 (2008).
82. M. R. McGuire, P. J. Espenshade, PGRMC1: An enigmatic heme-binding protein. *Pharmacol Ther* **241**, 108326 (2023).

83. W. Mifsud, A. Bateman, Membrane-bound progesterone receptors contain a cytochrome b5-like ligand-binding domain. *Genome Biol* **3**, RESEARCH0068 (2002).
84. M. A. Cahill, J. A. Jazayeri, Z. Kovacevic, D. R. Richardson, PGRMC1 regulation by phosphorylation: potential new insights in controlling biological activity. *Oncotarget* **7**, 50822-50827 (2016).
85. B. M. Thejer *et al.*, PGRMC1 phosphorylation affects cell shape, motility, glycolysis, mitochondrial form and function, and tumor growth. *BMC Mol Cell Biol* **21**, 24 (2020).
86. M. A. Cahill, H. Neubauer, PGRMC Proteins Are Coming of Age: A Special Issue on the Role of PGRMC1 and PGRMC2 in Metabolism and Cancer Biology. *Cancers (Basel)* **13**, (2021).
87. H. Neubauer *et al.*, Breast cancer proteomics reveals correlation between estrogen receptor status and differential phosphorylation of PGRMC1. *Breast Cancer Res* **10**, R85 (2008).
88. E. Runko, Z. Kaprielian, Caenorhabditis elegans VEM-1, a novel membrane protein, regulates the guidance of ventral nerve cord-associated axons. *J Neurosci* **24**, 9015-9026 (2004).
89. Y. Kabe *et al.*, Haem-dependent dimerization of PGRMC1/Sigma-2 receptor facilitates cancer proliferation and chemoresistance. *Nat Commun* **7**, 11030 (2016).
90. J. K. Pru, N. C. Clark, PGRMC1 and PGRMC2 in uterine physiology and disease. *Front Neurosci* **7**, 168 (2013).
91. T. Cao *et al.*, Clozapine Induced Disturbances in Hepatic Glucose Metabolism: The Potential Role of PGRMC1 Signaling. *Front Endocrinol (Lausanne)* **12**, 727371 (2021).
92. L. Terzaghi *et al.*, PGRMC1 localization and putative function in the nucleolus of bovine granulosa cells and oocytes. *Reproduction* **155**, 273-282 (2018).
93. H. Neubauer *et al.*, Membrane-initiated effects of progesterone on proliferation and activation of VEGF in breast cancer cells. *Climacteric* **12**, 230-239 (2009).
94. P. Thomas, Y. Pang, J. Dong, Enhancement of cell surface expression and receptor functions of membrane progestin receptor alpha (mPRalpha) by progesterone receptor membrane component 1 (PGRMC1): evidence for a role of PGRMC1 as an adaptor protein for steroid receptors. *Endocrinology* **155**, 1107-1119 (2014).
95. P. J. Thul *et al.*, A subcellular map of the human proteome. *Science* **356**, (2017).
96. J. J. Peluso, J. DeCerbo, V. Lodde, Evidence for a genomic mechanism of action for progesterone receptor membrane component-1. *Steroids* **77**, 1007-1012 (2012).
97. A. M. Luciano, V. Lodde, F. Franciosi, F. Ceciliani, J. J. Peluso, Progesterone receptor membrane component 1 expression and putative function in bovine oocyte maturation, fertilization, and early embryonic development. *Reproduction* **140**, 663-672 (2010).

98. V. Lodde, J. J. Peluso, A novel role for progesterone and progesterone receptor membrane component 1 in regulating spindle microtubule stability during rat and human ovarian cell mitosis. *Biol Reprod* **84**, 715-722 (2011).
99. Y. Bai *et al.*, PGRMC1 Promotes Progestin-Dependent Proliferation of Breast Cancer Cells by Binding Prohibitins Resulting in Activation of ERalpha Signaling. *Cancers (Basel)* **13**, (2021).
100. S. R. Lee *et al.*, Loss of PGRMC1 Delays the Progression of Hepatocellular Carcinoma via Suppression of Pro-Inflammatory Immune Responses. *Cancers (Basel)* **13**, (2021).
101. M. L. McCallum *et al.*, Conditional Ablation of Progesterone Receptor Membrane Component 1 Results in Subfertility in the Female and Development of Endometrial Cysts. *Endocrinology* **157**, 3309-3319 (2016).
102. J. J. Peluso, X. Liu, A. Gawkowska, E. Johnston-MacAnanny, Progesterone activates a progesterone receptor membrane component 1-dependent mechanism that promotes human granulosa/luteal cell survival but not progesterone secretion. *J Clin Endocrinol Metab* **94**, 2644-2649 (2009).
103. J. J. Peluso, J. Romak, X. Liu, Progesterone receptor membrane component-1 (PGRMC1) is the mediator of progesterone's antiapoptotic action in spontaneously immortalized granulosa cells as revealed by PGRMC1 small interfering ribonucleic acid treatment and functional analysis of PGRMC1 mutations. *Endocrinology* **149**, 534-543 (2008).
104. J. J. Peluso, Progesterone receptor membrane component 1 and its role in ovarian follicle growth. *Front Neurosci* **7**, 99 (2013).
105. S. Salsano *et al.*, Dynamic expression of PGRMC1 and SERBP1 in human endometrium: an implication in the human decidualization process. *Fertil Steril* **108**, 832-842 e831 (2017).
106. Y. A. Lyzikova, D. A. Zinovkin, M. Z. I. Pranjol, Increase in FoxP3, CD56 immune cells and decrease in glands PGRMC1 expression in the endometrium are associated with recurrent miscarriages. *Eur J Obstet Gynecol Reprod Biol* **245**, 121-126 (2020).
107. L. C. Kao *et al.*, Global gene profiling in human endometrium during the window of implantation. *Endocrinology* **143**, 2119-2138 (2002).
108. S. Talbi *et al.*, Molecular phenotyping of human endometrium distinguishes menstrual cycle phases and underlying biological processes in normo-ovulatory women. *Endocrinology* **147**, 1097-1121 (2006).
109. R. O. Burney *et al.*, Gene expression analysis of endometrium reveals progesterone resistance and candidate susceptibility genes in women with endometriosis. *Endocrinology* **148**, 3814-3826 (2007).
110. C. I. Ace, W. C. Okulicz, Microarray profiling of progesterone-regulated endometrial genes during the rhesus monkey secretory phase. *Reprod Biol Endocrinol* **2**, 54 (2004).
111. J. I. Chen *et al.*, Proteomic characterization of midproliferative and midsecretory human endometrium. *J Proteome Res* **8**, 2032-2044 (2009).

112. S. Salsano *et al.*, Novel nonclassic progesterone receptor PGRMC1 pulldown-precipitated proteins reveal a key role during human decidualization. *Fertil Steril* **113**, 1050-1066 e1057 (2020).
113. A. Tsuru *et al.*, PGRMC1 Regulates Cellular Senescence via Modulating FOXO1 Expression in Decidualizing Endometrial Stromal Cells. *Biomolecules* **12**, (2022).
114. N. Yoshitani *et al.*, A structure-based strategy for discovery of small ligands binding to functionally unknown proteins: combination of in silico screening and surface plasmon resonance measurements. *Proteomics* **5**, 1472-1480 (2005).
115. S. U. Mir, I. S. Ahmed, S. Arnold, R. J. Craven, Elevated progesterone receptor membrane component 1/sigma-2 receptor levels in lung tumors and plasma from lung cancer patients. *Int J Cancer* **131**, E1-9 (2012).
116. L. Wang-Eckhardt, I. Becker, M. Eckhardt, The PGRMC1 Antagonist AG-205 Inhibits Synthesis of Galactosylceramide and Sulfatide. *Cells* **10**, (2021).
117. I. S. Ahmed, H. J. Rohe, K. E. Twist, R. J. Craven, Pgrmc1 (progesterone receptor membrane component 1) associates with epidermal growth factor receptor and regulates erlotinib sensitivity. *J Biol Chem* **285**, 24775-24782 (2010).
118. R. B. Piel, 3rd *et al.*, A Novel Role for Progesterone Receptor Membrane Component 1 (PGRMC1): A Partner and Regulator of Ferrochelatase. *Biochemistry* **55**, 5204-5217 (2016).
119. I. S. Ahmed, H. J. Rohe, K. E. Twist, M. N. Mattingly, R. J. Craven, Progesterone receptor membrane component 1 (Pgrmc1): a heme-1 domain protein that promotes tumorigenesis and is inhibited by a small molecule. *J Pharmacol Exp Ther* **333**, 564-573 (2010).
120. C. Sueldo, X. Liu, J. J. Peluso, Progestin and AdipoQ Receptor 7, Progesterone Membrane Receptor Component 1 (PGRMC1), and PGRMC2 and Their Role in Regulating Progesterone's Ability to Suppress Human Granulosa/Luteal Cells from Entering into the Cell Cycle. *Biol Reprod* **93**, 63 (2015).
121. J. J. Peluso, D. Griffin, X. Liu, M. Horne, Progesterone receptor membrane component-1 (PGRMC1) and PGRMC-2 interact to suppress entry into the cell cycle in spontaneously immortalized rat granulosa cells. *Biol Reprod* **91**, 104 (2014).
122. S. Salsano, R. Gonzalez-Martin, A. Quinonero, S. Perez-Deben, F. Dominguez, Deciphering the Role of PGRMC1 During Human Decidualization Using an In Vitro Approach. *J Clin Endocrinol Metab* **106**, 2313-2327 (2021).
123. L. Wang-Eckhardt, M. Eckhardt, A progesterone receptor membrane component 1 antagonist induces large vesicles independent of progesterone receptor membrane component 1 expression. *Biol Chem* **401**, 1093-1099 (2020).

124. Y. Kabe *et al.*, Glycyrrhizin Derivatives Suppress Cancer Chemoresistance by Inhibiting Progesterone Receptor Membrane Component 1. *Cancers (Basel)* **13**, (2021).
125. C. Thieffry *et al.*, AG-205 Upregulates Enzymes Involved in Cholesterol Biosynthesis and Steroidogenesis in Human Endometrial Cells Independently of PGRMC1 and Related MAPR Proteins. *Biomolecules* **11**, (2021).
126. L. Feng *et al.*, Progesterone receptor membrane component 1 (PGRMC1) expression in fetal membranes among women with preterm premature rupture of the membranes (PPROM). *Placenta* **35**, 331-333 (2014).
127. C. Thieffry *et al.*, Spatiotemporal expression pattern of Progesterone Receptor Component (PGRMC) 1 in endometrium from patients with or without endometriosis or adenomyosis. *J Steroid Biochem Mol Biol* **223**, 106153 (2022).
128. T. Garrido-Gomez *et al.*, Deciphering the proteomic signature of human endometrial receptivity. *Hum Reprod* **29**, 1957-1967 (2014).
129. S. Mishra, L. C. Murphy, L. J. Murphy, The Prohibitins: emerging roles in diverse functions. *J Cell Mol Med* **10**, 353-363 (2006).
130. J. K. McClung *et al.*, Isolation of a cDNA that hybrid selects antiproliferative mRNA from rat liver. *Biochem Biophys Res Commun* **164**, 1316-1322 (1989).
131. T. Sato *et al.*, The human prohibitin (PHB) gene family and its somatic mutations in human tumors. *Genomics* **17**, 762-764 (1993).
132. T. Sato *et al.*, The human prohibitin gene located on chromosome 17q21 is mutated in sporadic breast cancer. *Cancer Res* **52**, 1643-1646 (1992).
133. S. Mishra, L. C. Murphy, B. L. Nyomba, L. J. Murphy, Prohibitin: a potential target for new therapeutics. *Trends Mol Med* **11**, 192-197 (2005).
134. F. Thuaud, N. Ribeiro, C. G. Nebigil, L. Desaubry, Prohibitin ligands in cell death and survival: mode of action and therapeutic potential. *Chem Biol* **20**, 316-331 (2013).
135. T. Tatsuta, T. Langer, Quality control of mitochondria: protection against neurodegeneration and ageing. *EMBO J* **27**, 306-314 (2008).
136. L. G. Nijtmans *et al.*, Prohibitins act as a membrane-bound chaperone for the stabilization of mitochondrial proteins. *EMBO J* **19**, 2444-2451 (2000).
137. K. Kasashima, E. Ohta, Y. Kagawa, H. Endo, Mitochondrial functions and estrogen receptor-dependent nuclear translocation of pleiotropic human prohibitin 2. *J Biol Chem* **281**, 36401-36410 (2006).
138. V. Kurtev *et al.*, Transcriptional regulation by the repressor of estrogen receptor activity via recruitment of histone deacetylases. *J Biol Chem* **279**, 24834-24843 (2004).
139. K. J. Teittinen *et al.*, Nucleolar proteins with altered expression in leukemic cell lines. *Leuk Res* **36**, 232-236 (2012).
140. Y. Shen *et al.*, Prohibitin-2 negatively regulates AKT2 expression to promote prostate cancer cell migration. *Int J Mol Med* **41**, 1147-1155 (2018).

141. A. Bavelloni *et al.*, Prohibitin 2 represents a novel nuclear AKT substrate during all-trans retinoic acid-induced differentiation of acute promyelocytic leukemia cells. *FASEB J* **28**, 2009-2019 (2014).
142. I. C. Morrow *et al.*, Flotillin-1/reggie-2 traffics to surface raft domains via a novel golgi-independent pathway. Identification of a novel membrane targeting domain and a role for palmitoylation. *J Biol Chem* **277**, 48834-48841 (2002).
143. Y. T. Peng, P. Chen, R. Y. Ouyang, L. Song, Multifaceted role of prohibitin in cell survival and apoptosis. *Apoptosis* **20**, 1135-1149 (2015).
144. Y. Zhang, Y. Wang, Y. Xiang, W. Lee, Y. Zhang, Prohibitins are involved in protease-activated receptor 1-mediated platelet aggregation. *J Thromb Haemost* **10**, 411-418 (2012).
145. M. Uhlen *et al.*, A pathology atlas of the human cancer transcriptome. *Science* **357**, (2017).
146. A. Bavelloni, M. Piazzi, M. Raffini, I. Faenza, W. L. Blalock, Prohibitin 2: At a communications crossroads. *IUBMB Life* **67**, 239-254 (2015).
147. J. W. Kim *et al.*, Activation of an estrogen/estrogen receptor signaling by BIG3 through its inhibitory effect on nuclear transport of PHB2/REA in breast cancer. *Cancer Sci* **100**, 1468-1478 (2009).
148. T. Yoshimaru *et al.*, Targeting BIG3-PHB2 interaction to overcome tamoxifen resistance in breast cancer cells. *Nat Commun* **4**, 2443 (2013).
149. B. He *et al.*, A repressive role for prohibitin in estrogen signaling. *Mol Endocrinol* **22**, 344-360 (2008).
150. J. Mao *et al.*, Elevated prohibitin 1 expression mitigates glucose metabolism defects in granulosa cells of infertile patients with endometriosis. *Mol Hum Reprod* **28**, (2022).
151. X. Qi *et al.*, Knockdown of prohibitin expression promotes glucose metabolism in eutopic endometrial stromal cells from women with endometriosis. *Reprod Biomed Online* **29**, 761-770 (2014).
152. S. Park *et al.*, Uterine development and fertility are dependent on gene dosage of the nuclear receptor coregulator REA. *Endocrinology* **153**, 3982-3994 (2012).
153. Y. Zhao, S. Park, M. K. Bagchi, R. N. Taylor, B. S. Katzenellenbogen, The coregulator, repressor of estrogen receptor activity (REA), is a crucial regulator of the timing and magnitude of uterine decidualization. *Endocrinology* **154**, 1349-1360 (2013).
154. C. S. Ryu, K. Klein, U. M. Zanger, Membrane Associated Progesterone Receptors: Promiscuous Proteins with Pleiotropic Functions - Focus on Interactions with Cytochromes P450. *Front Pharmacol* **8**, 159 (2017).
155. I. Kimura *et al.*, Functions of MAPR (membrane-associated progesterone receptor) family members as heme/steroid-binding proteins. *Curr Protein Pept Sci* **13**, 687-696 (2012).
156. K. K. Hampton, R. Stewart, D. Napier, P. P. Claudio, R. J. Craven, PGRMC1 Elevation in Multiple Cancers and Essential Role in Stem Cell Survival. *Adv Lung Cancer (Irvine)* **4**, 37-51 (2015).

157. X. Ruan *et al.*, Increased expression of progesterone receptor membrane component 1 is associated with aggressive phenotype and poor prognosis in ER-positive and negative breast cancer. *Menopause* **24**, 203-209 (2017).
158. K. Gerhardt, PGRMC1: A Fertility Stabilizer. *Biol Reprod* **95**, 49 (2016).
159. Y. Duan, Y. Zhou, Y. Peng, X. Shi, C. Peng, Inflammatory Markers in Women with Infertility: A Cross-Sectional Study. *Int J Gen Med* **16**, 1113-1121 (2023).
160. B. Smolarz, K. Szyllo, H. Romanowicz, Endometriosis: Epidemiology, Classification, Pathogenesis, Treatment and Genetics (Review of Literature). *Int J Mol Sci* **22**, (2021).
161. R. L. Goldenberg, J. F. Culhane, J. D. Iams, R. Romero, Epidemiology and causes of preterm birth. *Lancet* **371**, 75-84 (2008).
162. F. Amjadi *et al.*, The uterine immunological changes may be responsible for repeated implantation failure. *J Reprod Immunol* **138**, 103080 (2020).
163. T. K. Allen, L. Feng, C. A. Grotegut, A. P. Murtha, Progesterone receptor membrane component 1 as the mediator of the inhibitory effect of progestins on cytokine-induced matrix metalloproteinase 9 activity in vitro. *Reprod Sci* **21**, 260-268 (2014).
164. N. Schwartz, X. Xue, M. A. Elovitz, O. Dowling, C. N. Metz, Progesterone suppresses the fetal inflammatory response ex vivo. *Am J Obstet Gynecol* **201**, 211 e211-219 (2009).
165. B. G. Patel, M. Rudnicki, J. Yu, Y. Shu, R. N. Taylor, Progesterone resistance in endometriosis: origins, consequences and interventions. *Acta Obstet Gynecol Scand* **96**, 623-632 (2017).
166. W. B. Nothnick, MicroRNAs and Progesterone Receptor Signaling in Endometriosis Pathophysiology. *Cells* **11**, (2022).
167. R. O. Burney, L. C. Giudice, Pathogenesis and pathophysiology of endometriosis. *Fertil Steril* **98**, 511-519 (2012).
168. E. Attar, S. E. Bulun, Aromatase and other steroidogenic genes in endometriosis: translational aspects. *Hum Reprod Update* **12**, 49-56 (2006).
169. S. E. Bulun *et al.*, Role of estrogen receptor-beta in endometriosis. *Semin Reprod Med* **30**, 39-45 (2012).
170. J. Y. Yoo *et al.*, KRAS Activation and over-expression of SIRT1/BCL6 Contributes to the Pathogenesis of Endometriosis and Progesterone Resistance. *Sci Rep* **7**, 6765 (2017).
171. S. E. Bulun *et al.*, Estrogen receptor-beta, estrogen receptor-alpha, and progesterone resistance in endometriosis. *Semin Reprod Med* **28**, 36-43 (2010).
172. K. Nikolakopoulou, M. Y. Turco, Investigation of infertility using endometrial organoids. *Reproduction* **161**, R113-R127 (2021).
173. K. Bunch *et al.*, Expression patterns of progesterone receptor membrane components 1 and 2 in endometria from women with and without endometriosis. *Reprod Sci* **21**, 190-197 (2014).
174. D. K. Thiyagarajan, H. Basit, R. Jeanmonod, in *StatPearls*. (Treasure Island (FL) relationships with ineligible companies. Disclosure: Hajira Basit declares

no relevant financial relationships with ineligible companies. Disclosure: Rebecca Jeanmonod declares no relevant financial relationships with ineligible companies., 2023).
175. D. M. Velazquez Hernandez, E. R. Vazquez-Martinez, I. Camacho-Arroyo, The role of progesterone receptor membrane component (PGRMC) in the endometrium. *Steroids* **184**, 109040 (2022).
176. M. Wetendorf, F. J. DeMayo, The progesterone receptor regulates implantation, decidualization, and glandular development via a complex paracrine signaling network. *Mol Cell Endocrinol* **357**, 108-118 (2012).
177. K. Kasahara *et al.*, The role of human chorionic gonadotropin on decidualization of endometrial stromal cells in vitro. *J Clin Endocrinol Metab* **86**, 1281-1286 (2001).
178. J. de Rooij *et al.*, Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. *Nature* **396**, 474-477 (1998).
179. H. S. Kaya *et al.*, Roles of progesterone receptor A and B isoforms during human endometrial decidualization. *Mol Endocrinol* **29**, 882-895 (2015).
180. E. C. Mazur *et al.*, Progesterone receptor transcriptome and cistrome in decidualized human endometrial stromal cells. *Endocrinology* **156**, 2239-2253 (2015).
181. V. K. Maurya *et al.*, Decidualization of human endometrial stromal cells requires steroid receptor coactivator-3. *Front Reprod Health* **4**, 1033581 (2022).
182. K. T. Rytkonen *et al.*, Decidualization of Human Endometrial Stromal Fibroblasts is a Multiphasic Process Involving Distinct Transcriptional Programs. *Reprod Sci* **26**, 323-336 (2019).
183. H. F. Xia, X. H. Jin, Z. F. Cao, T. Shi, X. Ma, MiR-98 is involved in rat embryo implantation by targeting Bcl-xl. *FEBS Lett* **588**, 574-583 (2014).
184. Y. Wang *et al.*, MicroRNAome in decidua: a new approach to assess the maintenance of pregnancy. *Fertil Steril* **103**, 980-989 e986 (2015).
185. J. J. Peluso, A. Pappalardo, R. Losel, M. Wehling, Progesterone membrane receptor component 1 expression in the immature rat ovary and its role in mediating progesterone's antiapoptotic action. *Endocrinology* **147**, 3133-3140 (2006).
186. C. S. Keator, K. Mah, O. D. Slayden, Alterations in progesterone receptor membrane component 2 (PGRMC2) in the endometrium of macaques afflicted with advanced endometriosis. *Mol Hum Reprod* **18**, 308-319 (2012).

8. Appendix

8.1 Declaration of author contributions

Title: Progesterone-induced Progesterone Receptor Membrane Component 1 Rise-to-Decline Changes are Essential for Decidualization

Authors: Hailun Liu[1], André Franken[1], Alexandra P. Bielfeld[2], Tanja Fehm[1], Dieter Niederacher[1], Zhongping Cheng[3, 4], Hans Neubauer[1,*,†] and Nadia Stamm[1,*, †]

Affiliations:

1. Department of Obstetrics and Gynecology, University Hospital and Medical Faculty of the Heinrich-Heine University Duesseldorf, Life Science Center, Duesseldorf, Germany

2. UniKiD-Department of OB/GYN & REI, University Hospital and Faculty of Medicine, Heinrich Heine University Duesseldorf, Germany

3. Department of Obstetrics and Gynecology, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai 200072, China

4. Institute of Gynecological Minimally Invasive Medicine, Tongji University School of Medicine, Shanghai 200072, China

Correspondence:

* Corresponding Authors:

Hans Neubauer; Department of Obstetrics and Gynecology, University Hospital and Medical Faculty of the Heinrich-Heine University Duesseldorf, Life Science Center, Duesseldorf, Germany

Universitätsklinikum Düsseldorf Life Science Center, EG, Raum 0.028, Merowingerplatz 1A, 40225 Düsseldorf; +49 211 81-06026; Hans.Neubauer@med.uni-duesseldorf.de

Nadia Stamm; Department of Obstetrics and Gynecology, University Hospital and Medical Faculty of the Heinrich-Heine University Duesseldorf, Life Science Center, Duesseldorf, Germany

Universitätsklinikum Düsseldorf Life Science Center, EG, Raum 0.032, Merowingerplatz 1A, 40225 Düsseldorf; +49 211 81-06030; Nadia.Stamm@med.uni-duesseldorf.de

Authors' contributions:

Conceptualization, H.L., H.N.; Investigation, H.L., N.S.; Methodology, H.L., A.P.B. and N.S.; Supervision, T.F., D.N., Z.C., N.S., H.N.; Project administration, T.F., H.N.; Writing-original draft, H.L.; Writing-review and editing, A P.B., A.F., N.S. and H.N. All authors have read and agreed to the published version of the manuscript.

8.2 List of figures

Supplementary figure 3. PGRMC1-downregulation before decidualization induction impairs morphological remodeling of T-HESCs.

Supplementary figure 4. PGRMC1-downregulation after decidualization induction does not impair morphological remodeling of T-HESCs.

Supplementary figure 5. PGRMC1-downregulation after progestin induction does not impair decidualization in the St-T1 cell line.

Supplementary figure 6. PGRMC1 and PHB1/PHB2 co-localize in T-HESCs.

Supplementary figure 7. PGRMC1 does not interact with PHBs without induction.

Supplementary figure 8. PGRMC1 co-precipitate with PHB1/PHB2 upon decidualization induction.

Supplementary figure 9. Morphological changes during decidualization upon PHBs downregulation.

8.3 Acknowledgements

At the end of my unforgettable journey as an M.D candidate, I extend my deepest gratitude to my supervisor, Prof. Dr. rer. nat. Hans Neubauer, who has accompanied me throughout this project. His vast knowledge, experience, and continuous patience have been a source of motivation and support during my research. I also wish to express my sincere appreciation to my co-supervisor, Prof. Dr. med. Alexandra Bielfeld, for her clinical advice and for providing the cell lines essential for my research. Additionally, I am grateful to all members of our obstetrics and gynecology lab, including Prof. Dr. rer. nat. Dieter Niederacher, Nadia Stamm, Dr. rer. nat. André Franken, Liwen Yang, and Mahdi Rivandi, for their invaluable suggestions and technical support during every labmeeting and presentation. Special thanks go to Nadia Stamm, whose extensive help and guidance were indispensable in advancing my project and experiment design.

Reflecting on my valuable memories with friends in Düsseldorf, Germany, I have been blessed to be surrounded by nice and warm-hearted friends, including Jiabao Lu, Yingxue Bai, Chen Chen, Xinkun Cheng, Shanshan Deng, and Menglin Sun. We have so many joyful times and created lasting memories. We have encouraged and supported to each other. We are friends and family.

Last but not the least, I am profoundly grateful to my parents and my husband. Without my parents' support, I cannot overcome all the difficulties in study and life! And my husband, Qinhui Rao, we have been separated in the U.S. and Germany since 2019, his never-ending patience, support and love have only made me stronger!

Thank you all, with my heart!