Progesterone Receptor Membrane Component 1 as the Mediator of the Inhibitory Effect of Progestins on Cytokine-Induced Matrix Metalloproteinase 9 Activity In Vitro

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Abstract

Progesterone (P4) and the progestin, 17 α -hydroxyprogesterone caproate, are clinically used to prevent preterm births (PTBs); however, their mechanism of action remains unclear. Cytokine-induced matrix metalloproteinase 9 (MMP-9) activity plays a key role in preterm premature rupture of the membranes and PTB. We demonstrated that the primary chorion cells and the HTR8/SVneo cells (cytotrophoblast cell line) do not express the classical progesterone receptor (PGR) but instead a novel progesterone receptor, progesterone receptor membrane component 1 (PGRMC1), whose role remains unclear. Using HTR8/SVneo cells in culture, we further demonstrated that 6 hours pretreatment with medroxyprogesterone acetate (MPA) and dexamethasone (Dex) but not P4 or 17 α -hydroxyprogesterone hexanoate significantly attenuated tumor necrosis factor α -induced MMP-9 activity after a 24-hour incubation period. The inhibitory effect of MPA, but not Dex, was attenuated when PGRMC1 expression was successfully reduced by PGRMC1 small interfering RNA. Our findings highlight a possible novel role of PGRMC1 in mediating the effects of MPA and in modulating cytokine-induced MMP-9 activity in cytotrophoblast cells in vitro.

Keywords

progesterone, preterm delivery, progesterone receptor, inflammation

Introduction

Preterm premature rupture of membranes (PPROM) complicates up to 25% of the preterm deliveries (PTDs).¹ The mechanisms leading to early fetal membrane weakening and rupture are poorly understood. Inflammatory cytokines such as tumor necrosis factor α (TNF- α) are thought to play a key role in this pathogenesis by inducing premature apoptosis and increasing extracellular matrix degradation.²⁻⁴ TNF- α induces matrix metalloproteinase 9 (MMP-9) activity, a zinc-dependent endopeptidase enzyme involved in extracellular matrix remodeling. Significantly elevated levels of MMP-9 expression and activity have been identified in the fetal membranes of women with preterm labor (PTL) and PPRM.^{5,6} Cell cultures originating from fetal membranes have identified placental trophoblast cells, amnion epithelial cells, and chorion trophoblast cells as the primary sites for the production of MMP-9.⁶

Although limited therapeutic options exist for the prevention of PTD and PPRM, recent clinical evidence highlights the potential therapeutic benefit of administering progesterone and its analogs, specifically 17-hydroxyprogesterone caproate and vaginal progesterone for the prevention of spontaneous

PTD in high-risk patients.^{7,8} Progesterone and medroxyprogesterone acetate (MPA) inhibit basal and TNF- α -induced apoptosis in term fetal membranes, protect chorion and decidual cells from calcium-induced cell death, and attenuate cytokine-induced MMP expression and activity in decidual cells.⁹⁻¹¹ Preliminary data from our laboratory demonstrated that pretreatment with MPA reduced TNF- α -induced MMP-9 activity in a cytotrophoblast cell line (HTR8/SVneo). However, the mechanisms underlying these therapeutic effects remain elusive. The classic nuclear progesterone receptor (PGR), which is thought to be responsible for most of the progesterone's key biological effects, is differentially expressed in the layers of

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fetal membranes, limiting its protective functions.¹² Previous work has demonstrated a relative lack of expression of the PR-A and PR-B subtypes of PGR in the chorion and amnion when compared with the decidua.^{12,13} Despite this, progesterone still retains biological actions in ovarian cancer cells not expressing PGR and mutant mice devoid of PGR.^{14,15} These findings strongly suggest that at least some of progesterone's observed effects may be mediated through another novel mechanism independent of PGR.

One such mechanism may involve the novel membrane-associated progesterone receptor, progesterone receptor membrane component 1 (PGRMC1, also called heme-1 domain protein or HPR6.6)¹⁶ PGRMC1 binds progesterone with high affinity, is localized in the endoplasmic reticulum, and is responsible for the antiapoptotic effect seen with progesterone treatment in serum-starved granulosa and luteal cells.¹⁷ PGRMC1 also completely attenuates the apoptotic action of paclitaxel and cisplatin on PGR-negative ovarian cancer cells.¹⁵ However, the expression of PGRMC1 (and PGR) in HTR8/SVneo cells, primary chorion cells, and fetal membrane explants has not been previously described. Furthermore, progesterone's effect on cytokine-mediated MMP-9 activity and the role of PGRMC1 in mediating this effect has not been explored previously. Our hypothesis is that progesterone and its analogs attenuate cytokine-induced MMP-9 activity in cytotrophoblast cells, and this effect is mediated through the nonnuclear progesterone receptor PGRMC1. Our objectives were first to demonstrate that PGRMC1 is expressed in the HTR8/SVneo cells, primary chorion cells, and chorion layer of fetal membranes, second to determine whether progesterone reduced TNF- α -induced MMP-9 activity in the HTR8/SVneo cell line, and third to determine whether this effect is mediated through PGRMC1 by demonstrating a loss of this protective effect when PGRMC1 expression is reduced by PGRMC1-specific small interfering RNA (siRNA) in the HTR8/SVneo cell line. To determine whether this was a nonspecific steroid effect, we also investigated the effect of the corticosteroid dexamethasone (Dex) on TNF- α -induced MMP-9 activity and determined whether this effect was also mediated through PGRMC1.

Materials and Methods

Reagents

Progesterone (4-pregnene 3,20-dione; lyophilized powder), medroxyprogesterone 17-acetate (lyophilized powder), 17 α -hydroxyprogesterone hexanoate (17P; lyophilized powder), and Dex (Sigma-Aldrich, St Louis, Missouri) were reconstituted in ethanol and stored at -20°C.

Cell Culture

The experiments were conducted using the HTR8/SVneo cell line (a gift from Dr C.H. Graham, Queen's University, Kingston, Ontario, Canada) established from explant culture of first-trimester human placenta and immortalized by the simian virus

40 large T antigen.¹⁸ The cells were cultured in RPMI 1640 (Mediatech, Manassas, Virginia) supplemented with 5% fetal bovine serum (FBS), penicillin, streptomycin, and amphotericin B and maintained in a humidified incubator at 5% CO₂ and at 37°C. The cells were trypsinized with 0.05% trypsin-EDTA (Gibco; Life Technologies, Grand Island, New York). Primary chorion cells were harvested from the fetal membranes of women having a nonlaboring planned cesarean delivery at term without rupture of membranes. Harvested tissue was transported into Dulbecco modified Eagle medium (DMEM)/F12 media (Invitrogen; Life technologies) with 10% FBS, penicillin, streptomycin, and amphotericin B. The amnion layer was manually removed, and the chorion and decidua were separated using blunt dissection. Primary chorion cells were harvested from the chorion layer using a previously described methodology and maintained in serum-supplemented DMEM/F12 under similar culture conditions as described previously.¹¹ Harvested cell lysates were used for PGRMC1 and PGR quantification.

Immunohistochemistry Staining

Sections of fetal membranes collected as described previously were fixed in paraffin, and the slides were prepared. Tissue sections were deparaffinized with xylene followed by graded rehydration in ethanol (100%, 95%, 80%, and 70%) and distilled water. Subsequently, the sections were subjected to heat-induced epitope retrieval by heating in antigen unmarking solution (Vector Laboratories, Inc, Burlingame, California) preheated to more than 90°C for 20 minutes (two 10-minute periods with reheating between), followed by a 20-minute cool-down period at room temperature. The slides were stained using UltraVision LP Detection System HRP Polymer & DAB Plus Chromogen kit following manufacturer's instruction (Thermo Fisher Scientific Inc, Fremont, California). This UltraVision detection system detects a specific mouse immunoglobulin (Ig) G (IgG) or rabbit IgG antibody bound to an antigen in tissue sections. The specific antibody is located by a universal secondary antibody formulation conjugated to an enzyme-labeled polymer that recognizes mouse and rabbit Igs. The polymer complex is then visualized with DAB substrate. The PGRMC1 antibody from rabbit (catalog no. HPA002877; Sigma) was used at 1:200 dilution in phosphate-buffered saline with 1% bovine serum albumin and 5% goat serum. For each batch of staining, 1 slide was incubated with polyclonal rabbit IgG antibody (catalog no. AB27472; Abcam, Cambridge, Massachusetts) at a dilution of 1:200 as a negative control. The slides were then counterstained with hematoxylin and eosin stain, and images were photographed using the Zeiss Axio Observer (Carl Zeiss Microscopy, Thornwood, New York).

Experimental Conditions

To determine the effects of progesterone on cytokine-induced MMP-9 activity, HTR8/SVneo cells were plated in 12-well plates and grown to confluence. The cells were pretreated with ethanol (0.01%), P4, 17P, MPA, or Dex at 10⁻⁶ mol/L in

serum-containing media for 6 hours. The cultures were then switched to serum-free media containing vehicle, P4, 17P, MPA, or Dex and incubated with and without 10 ng/mL of human recombinant TNF- α (RnD Systems, Minneapolis, Minnesota) for an additional 24 hours. At the end of the incubation period, cell culture media for substrate zymography was harvested and centrifuged with the supernatant immediately frozen at -80°C until analyzed. Cell protein lysates were harvested and subsequently used for protein concentration determination using the Bradford assay.

Matrix Metalloproteinase 9 Substrate Zymography

Aliquots of cell culture media were mixed in a 1:1 ratio with Novex (Invitrogen; Life Technologies) and Tris-glycine sodium dodecyl sulfate (SDS) sample buffer (Invitrogen; Life Technologies). Sample of 20 μL was loaded on a Novex 10% Zymogram (gelatin) gel and electrophoresed at 125 V for 90 minutes in Novex Tris-Glycine SDS running buffer, along with a protein standard for molecular weight estimation (Novex Sharp Pre-stained Protein Standard). To renature the enzymes, the gel was incubated in Novex renaturing buffer for 30 minutes followed by Novex developing buffer for an additional 30 minutes. The gel was then incubated in fresh zymogram-developing buffer for 16 to 18 hours at 37°C . The gel was stained with a Coomassie G250 stain (SimplyBlue Safestain, Invitrogen; Life Technologies) for 1 hour and then destained with deionized water for 2 hours at room temperature. We quantified total MMP-9 activity by analyzing band densities at 88 and 92 kDa using computer-based image densitometry (Image J; National Institute of Health, Bethesda, Maryland). Zymography was performed on cell culture media harvested from 5 separate experiments, each was performed in duplicates.

Progesterone Receptor Membrane Component 1 siRNA Transfection and Inhibition

HTR8/SVneo cells were plated in 6-well plates and grown to 30% to 40% confluency. The cells were then transfected with scramble (catalog no. AM4611, Ambion; Life Technologies) or predesigned PGRMC1 siRNA (catalog no. s21310, Ambion; Life Technologies) using Lipofectamine RNAiMAX (Invitrogen) to yield a final concentration of 10 nmol/L. The siRNA transfection was performed as outlined in protocols provided by Invitrogen. The cells were pretreated with MPA or Dex at a dose of 10^{-6} mol/L followed by incubation with or without TNF- α at the concentration of 10 ng/mL for 24 hours as described previously, 72 hours after transfection. (Preliminary data from our laboratory had identified MPA as the most effective progestin at attenuating cytokine-induced MMP-9 activity.) The MMP-9 activity was quantified in harvested cell media using zymography as described previously. The cell lysates were harvested and processed for Western blotting to determine knockdown of PGRMC1 expression using standard protocols.

Western Blot Analysis

Western blot analysis was performed on harvested cell lysates. Protein concentrations were measured using the Bradford assay. Total protein of 25 μg were loaded onto the 10% SDS polyacrylamide gels, separated, and then transferred onto a polyvinylidene difluoride membrane. The membranes were incubated with rabbit anti-human glyceraldehyde 3-phosphate dehydrogenase antibody (1:20,000; Cell Signaling Technology, Danvers, Massachusetts), rabbit anti-human PGRMC1 antibody (1:2000), and monoclonal mouse anti-human PGR (clone PgR 1294, 1:1000; Dako, Carpinteria, California) in blocking buffer (5% milk in Tris-buffered saline and Tween 20 buffer) over night at 4°C . Following incubation with the appropriate secondary antibody (1:2000, horseradish peroxidase-linked anti-rabbit or anti-mouse; Cell Signaling Technology) for 1 hour at room temperature, the membranes were incubated with SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific, Rockford, Illinois) and exposed on film. Cell lysates from T47D cell line were used as positive controls.

Statistical Analysis

For MMP-9 zymography, the MMP-9 activity was normalized to mean cell protein concentration for each experimental condition. The relative MMP-9 activity was calculated by first subtracting the measurements from each unstimulated control from the corresponding TNF- α stimulated group. The resulting MMP-9 densitometry for the TNF- α stimulated groups were then divided by the vehicle (control)-stimulated group. The relative MMP-9 activity of each TNF- α -treated progesterone/progestin or Dex group was compared with the control TNF- α -stimulated group using 1-way analysis of variance with the post hoc Dunnett's test or the unpaired t test. The relative MMP-9 activity for the control TNF- α -stimulated group and the MPA TNF- α stimulated for the siRNA treatments were compared using the paired and unpaired t tests where appropriate. Experiments were repeated on 3 or 5 occasions. Data were summarized as mean $\pm$ standard error of the mean. $P < .05$ was considered significant. Data were analyzed using Graphpad Prism (version 5.0d; GraphPad Software, Inc, La Jolla, California).

Results

HTR8/SVneo and Primary Chorion Cells Lack PGR and Express PGRMC1

Western blotting demonstrated PGRMC1 expression in both the HTR8/SVneo cells and the primary chorion cells but not the PGR (Figure 1A). Similarly, PGRMC1 was highly expressed in the chorion layer of fetal membranes (Figure 1B). Slides stained with the polyclonal rabbit IgG antibody were used as a negative control (Figure 1C). Both the receptors were expressed in T47D cells (positive control). These findings validate the use of the HTR8/SVneo cell line for the subsequent experiments.

Figure 1. A, Western blot demonstrating that PGRMC1 but not PGR (specifically the PR-A and PR-B subtypes) is expressed in primary chorion cells (CHs) and HTR8/Svneo cells (HTR8). Both receptors are expressed in the T47D breast cancer cell line (positive control). B, Immunohistochemistry stain ($\times 10$) demonstrating that PGRMC1 is highly expressed in the chorion layer of fetal membranes. C, Negative control slide ($\times 10$). Slides were counterstained with hematoxylin and

Progesterone's Effect on TNF- α -Induced MMP-9 Activity

TNF- α increased MMP-9 activity relative to unstimulated vehicle control by 42% ($P < .001$; Figure 2). Pretreatment with MPA significantly reduced TNF- α -induced MMP-9 activity when compared with the stimulated controls (41% reduction vs TNF- α stimulated control, $P < .05$; Figure 2). Pretreatment with Dex also significantly attenuated TNF- α -induced MMP-9 activity (71% reduction vs TNF- α stimulated control, $P < .05$; Figure 3). However, pretreatment with P4 and 17P did not attenuate TNF- α -induced MMP-9 activity.

Progesterone Receptor Membrane Component 1 siRNA Effect on Cytokine-Induced MMP-9 Zymography

Western blot analysis confirmed that PGRMC1 expression was significantly reduced (at least $>50\%$) when treated with PGRMC1 siRNA compared to controls (Figure 4).

In cells pretreated with PGRMC1 siRNA, the inhibitory effect of MPA pretreatment on TNF- α -induced MMP-9 activity (Figure 4) was diminished when compared to cells treated with the scramble siRNA group. Pretreatment with MPA reduced TNF- α -induced MMP-9 activity by 45% when compared to the stimulated controls ($P < .008$). However, in the PGRMC1 siRNA group this effect was lost with MPA pretreatment reducing MMP-9 activity to a lesser degree when compared with stimulated controls (15%, $P = .100$). In contrast, Dex was effective in reducing TNF- α -induced MMP-9 activity in both the scramble and the PGRMC1 siRNA group when compared with stimulated controls (92% reduction in MMP-9 activity in the scramble group, $P = .003$ vs 83% reduction in the PGRMC1 siRNA group, $P = .005$; Figure 5).

Discussion

Progesterone's role in the prevention of PTD remains an area of ongoing debate. Using a cell line that expressed PGRMC1 and lacked PGR, we demonstrated that (1) MPA and Dex significantly attenuate TNF- α -induced MMP-9 activity but not P4 and 17P, in vitro and (2) the inhibitory effect of MPA, but not Dex, occurs partly through a novel mechanism involving PGRMC1. Furthermore, our findings provide some initial evidence that PGRMC1 possibly plays a role in regulating MMP-9 activity in cytotrophoblast cells.

Both PPROM and PTL are associated with elevated levels of cytokines in both intra-amniotic fluid and fetal membranes.⁴ Cytokine treatment decreases the strength and force needed to rupture fetal membranes. This effect may be in part due to increased MMP-9 activity and extensive collagen remodeling.¹⁹ TNF- α induces increased MMP-9 expression and activity from cultured primary human chorion trophoblast cells

Figure 1. (continued) eosin staining (A, amnion; C, chorion; and D, decidua). Experiments were replicated on tissue samples from 3 patients. PGR indicates nuclear progesterone receptor; PGRMC1, progesterone receptor membrane component 1.

Figure 2. A, A representative zymogram (top) showing the increase in MMP-9 activity following TNF- α stimulation in an unstimulated (vehicle) control in HTR8/SVneo cells. TNF- α increased relative MMP-9 activity when compared with unstimulated controls by 42% ($^{^^}P < .001$ vs control). The effect of progesterone pretreatment on MMP-9 activity in HTR8/SVneo cells. B, A representative zymogram demonstrating the effects of progesterone and progestin pretreatment on TNF- α induced MMP-9 activity. In addition to the unstimulated (vehicle) control, each progesterone and progestin-treatment group is represented with both the unstimulated and the TNF- α stimulated MMP-9 activity. The relative MMP-9 activity represents activity in excess of baseline for each experimental group. The unstimulated control only is represented graphically. C, MPA pretreatment significantly reduced relative MMP-9 activity, while P4 and 17P were ineffective ($^{*}P < .05$ vs TNF- α). Experiments were replicated on 5 separate occasions. Data are mean $\pm$ standard error of the mean. MMP-9 indicates matrix metalloproteinase 9; MPA, medroxyprogesterone acetate; TNF- α , tumor necrosis factor α .

Figure 3. A, A representative zymogram showing the effect of dexamethasone pretreatment on TNF- α -induced MMP-9 activity. B, Dexamethasone pretreatment significantly reduced relative MMP-9 activity when compared with the TNF- α -stimulated group (* $P < .05$ vs TNF- α). The unstimulated control only is represented graphically. Experiments were replicated on 3 separate occasions. Data are mean $\pm$ standard error of the mean. MMP-9 indicates matrix metalloproteinase 9; TNF- α , tumor necrosis factor α .

through TNF receptor 1 signaling via I κ B–nuclear factor kappa B (NF κ B) and mitogen-activated protein kinase (MAPK) signaling pathways.²⁰ Kumar et al demonstrated that cytokine-conditioned choriodecidua leads to remodeling and weakening of the amnion and identified the choriodecidua layers as key intermediaries in the fetal membrane remodeling process.²¹ Our efforts have been focused on the role of the chorion layer in PPROM based on the previous work from our laboratory identifying the chorion layer as a site for inflammation-induced accelerated cell death and destruction in patients with PPROM.² However, due to the difficulty in performing siRNA transfection in primary chorion trophoblast cells, the

experiments were carried out using a cytotrophoblast cell line that shares several phenotypic characteristics with these cells.²² Strategies aimed at attenuating MMP-9 expression and activity may prevent subsequent fetal membrane weakening and PTD.²³ Progestins, specifically MPA, have been shown to attenuate TNF- α and interleukin 1 β -induced MMP-1 and 3 activity in decidua cells via p38 MAPK signaling.¹⁰ We hypothesize that the observed reduction in cytokine-induced MMP-9 activity by MPA pretreatment in cytotrophoblast cells could prevent extracellular matrix remodeling both at local and at distant sites and aid in preserving fetal membrane strength and integrity in the face of increased inflammation via a PGRMC1-dependent mechanism.

Previous studies examining PGR expression in human term gestational tissues have produced mixed and conflicting results. These contradictory findings are likely due to the differences in the specificity of the various PGR antibodies used in different studies. Mesiano group using the PGR-specific antibody PgR1294 (Dako Corp) confirmed the presence of PGR in decidual but not amnion and chorion cells.¹² We also used this specific antibody PgR1294 to characterize the PGR in cytotrophoblast cell line HTR8/SVneo and primary chorion cells. The lack of expression of PGR coupled with the expression of PGRMC1 in the HTR8/SVneo cells and primary cultured chorion cells is not surprising. An inverse relationship appears to exist between PGR and PGRMC1 expression, with greater expression of PGRMC1 observed in PGR knockout mice when compared with their wild-type controls.¹⁵ As a result, defining the role of PGRMC1 in cytotrophoblast cells therefore assumes greater clinical significance. We present initial evidence that PGRMC1 may play a protective role in progestin-mediated attenuation of cytokine-induced MMP-9 activity and PTD. This is based on the observations that depletion of PGRMC1 by siRNA specifically targeting PGRMC1 results in a reduced ability of MPA to attenuate TNF- α -induced MMP-9 activity in the HTR8/SVneo cell line. PGRMC1 may mediate its effects by nongenomic mechanisms initiated at the cell membrane or cytoplasm and genomic mechanisms initiated by PGRMC1 dimers located in the nucleus. The PGRMC1 dimers located in the nucleus have been shown to influence the expression of genes and transcription factors involved in regulating apoptosis cell cycling.^{15,24} Both the mechanisms may partially explain the attenuation in TNF- α -induced MMP-9 expression observed with MPA pretreatment. However, further work is needed to clearly elucidate the pathways by which PGRMC1 modulates MMP-9 activity, particularly in primary chorion cells.

Interestingly, in our model, P4 and 17P did not attenuate the TNF- α -induced MMP-9 activity despite evidence that PGRMC1 binds P4 and harbors both high ($k_d = 11$ nmol/L) and low (286 nmol/L) affinity P4 binding sites in spontaneously immortalized granulosa cells overexpressing the receptor.¹⁶ The reasons for these differences are not immediately apparent. A major limitation of our study is that we did not determine whether the P4 and 17P preparations used were biologically active in the HTR8/SVneo cell line. The P4 is also

Figure 4. A, A Western blot demonstrating significant reduction in PGRMC1 expression in HTR8/SVneo cells with PGRMC1-specific siRNA treatment. B, A representative zymogram demonstrating that knocking down PGRMC1 expression reduces the efficacy of MPA pretreatment to inhibit TNF- α -induced MMP-9 activity. Both the unstimulated (vehicle) control and the MPA groups are shown in the zymogram (C). MPA pretreatment significantly reduced TNF- α -induced relative MMP-9 activity in the scramble siRNA group by 45% ($P < .008$ vs TNF- α) but not in the PGRMC1 siRNA groups. The relative MMP-9 activity represents activity in excess of baseline for each experimental group. The unstimulated (vehicle) control only is represented graphically for both siRNA treatments. Experiments were replicated on 5 separate occasions. Data are mean $\pm$ standard error of the mean. MMP-9 indicates matrix metalloproteinase 9; MPA, medroxyprogesterone acetate; PGRMC1, progesterone receptor membrane component 1; siRNA, small interfering RNA; TNF α , tumor necrosis factor α .

known to undergo rapid and extensive metabolism in vitro, in cell cultures with 70% metabolized in 8 hours and 90% in 24 hours, reducing its bioavailability and effectiveness at the end of 24 hours of incubation with TNF- α .²⁵ There is also some initial evidence that 17P may mediate its effect through PGR with PGR polymorphism altering the clinical response to 17P when used for the prevention of preterm birth.²⁶ The lack of PGR expression in the HTR8/SVneo cell line could therefore account for the inability of 17P to attenuate TNF- α -induced MMP-9 activity in our model. The MPA in contrast is known to possess anti-inflammatory properties.^{10,27} This anti-

inflammatory effect may be partly attributed to its intrinsic glucocorticoid activity and its higher relative binding affinity to the glucocorticoid receptor when compared with other progestins.^{28,29} In fact, we were able to demonstrate profound inhibition of TNF- α -induced MMP-9 activity with Dex. The Dex's anti-inflammatory effects may be mediated by suppressing NF κ B activation, which may or may not be glucocorticoid receptor mediated.³⁰ However, in contrast with MPA, these observed effects appear not to be PGRMC1 mediated. Further evidence of MPA mediating its effects through PGRMC1 is implied by the strong proliferative response elicited by MPA in the presence of

Figure 5. A, A representative zymogram demonstrating that knocking down PGRMC1 expression in the HTR8/SVneo cells did not significantly reduce the efficacy of dexamethasone pretreatment to reduce TNF- α -induced MMP-9 activity. B, Dexamethasone pretreatment significantly reduced TNF- α -induced relative MMP-9 activity in both the scramble ($P = .003$ vs TNF- α) and the PGRMC1 siRNA groups ($P = .005$ vs TNF- α). The relative MMP-9 activity represents activity in excess of baseline for each experimental group. Experiments were replicated on 3 separate occasions. Data are mean $\pm$ standard error of the mean. MMP-9 indicates matrix metalloproteinase 9; PGRMC1, progesterone receptor membrane component 1; siRNA, small interfering RNA; TNF α , tumor necrosis factor α .

estradiol in wild-type MCF-7 (a breast cancer cell line) cells over-expressing PGRMC1, while P4 had no effect.^{31,32} Although our findings provide additional functional evidence that MPA may mediate some of its effects through PGRMC1, the nature of this interaction and the downstream consequences remain unknown. Furthermore, MPA is not a progestin clinically used for preventing PTD, so although these findings maybe important, they could represent a pharmacological in vitro effect. There is currently little data available on the binding properties of synthetic progestins with PGRMC1. However, the biologic effects of P4 and progestins may depend on the specific pharmacology of the agents and vary in different target tissues. It is widely accepted that progestins of the same class may differ in their respective steroid receptor interactions through either activating receptors by recruiting coactivators or preventing activation by corepressors leading to different downstream effects.²⁸ In this regard, it is possible that while P4 is a ligand for PGRMC1, its effects may not modulate

MMP-9 activity in the cytotrophoblast cell line. Similarly, MPA may be an important ligand for mediating this effect in response to inflammation.

In conclusion, PGRMC1 appears to play a role in vitro in mediating the protective effects of MPA on cytokine-induced MMP-9 activity in cytotrophoblast cells and may play an, as yet, unclear role in preventing inflammation-induced fetal membrane rupture. In addition, PGRMC1 may represent a new molecular target in the prevention of PTD and PPROM. Further studies are needed to clearly determine whether these pharmacological effects occur in vivo.

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