Regulation of sPLA₂-IID in Human Decidua: Insights Into the Complexity of the Prostaglandin Pathway in Labor

Reproductive Sciences
2014, Vol. 21(7) 921-929
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DOI: 10.1177/1933719113519176
rs.sagepub.com

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Abstract

Prostaglandins are implicated in the labor process, yet the precise role and regulation of the prostaglandin pathway remains to be elucidated. The first step in the pathway is cleavage of membrane phospholipids by phospholipase A₂ (PLA₂). Previous work demonstrated upregulation of secretory PLA₂ (sPLA₂)-IIA with labor in human myometrium, and recent evidence shows that there are numerous PLA₂ isoforms. The present study investigates the potential of additional sPLA₂ isoforms during pregnancy and labor. Real-time reverse transcriptase–polymerase chain reaction (RT-PCR), Western blotting, and immunohistochemistry were used to determine sPLA₂ expression and localization. Results show the presence of sPLA₂-IID in amnion, chorion, placenta, decidua, and myometrium. Expression of sPLA₂-IID in decidua was significantly decreased in term labor compared to nonlabor patients, whereas no significant labor-associated changes were observed in other gestational tissues. Secretory PLA₂-IID was localized within chorion fibroblasts, placenta trophoblasts, decidual cells, and in myometrial smooth muscle cells. In primary decidual cell cultures, interleukin (IL) 10 (IL-10) increased sPLA₂-IID messenger RNA (mRNA) expression, while IL-1β had no effect on sPLA₂-IID mRNA expression. In conclusion, decreased expression of sPLA₂-IID in the decidua at labor indicates that it is unlikely to contribute to increased prostaglandin production during labor. However, increased expression of sPLA₂-IID, induced by IL-10, suggests that sPLA₂-IID may play an important anti-inflammatory role at the maternal–fetal interface. Nevertheless, precise functions of sPLA₂-IID within the human uterus remain to be determined.

Keywords

decidua, gene expression, gestational tissues, labor, secretory phospholipase A₂-IID

Introduction

Strong evidence supports a pivotal role for prostaglandins (PGs) in uterine contractility and cervical ripening, key processes that characterize human labor, and prostaglandin production is known to be increased with labor onset.^{1–6} In clinical practice, prostaglandin analogs are used to induce labor,^{7,8} and inhibitors of prostaglandin synthesis to delay preterm labor⁹, yet the use of these compounds is not always optimal,^{10–12} largely due to our incomplete understanding of the complex role that prostaglandins play in both maternal and fetal gestational tissues.

Prostaglandin synthesis involves 3 rate-limiting steps¹³ (1) cleavage of arachidonic acid (AA) from membrane phospholipids mediated by phospholipase A₂ (PLA₂) enzymes; (2) conversion of free AA to intermediate prostaglandin molecules, PGG₂ and PGH₂, by the action of cyclooxygenase enzymes, COX-1 and COX-2; and (3) conversion of the intermediate components into distinct prostaglandins by the action of specific PG synthase enzymes. Hence, the increased prostaglandin output

observed with labor may be mediated by increased activity at any or each of the above-mentioned steps. However, it is not clear which step is critical for prostaglandin production nor has it been determined which PLA₂ enzymes are responsible for the increase in PG synthesis at the time of labor.

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Table 1. Gestation and Labor Characteristics of Patients.^a

	Term Nonlabor	Term Labor
Maternal age, years	32.4 ± 1.7 (23-41)	33.3 ± 1.4 (26-41)
Ethnicity, n	11 Caucasian, 1 Asian	9 Caucasian, 3 Asian
Gravidity	2 (1-4)	2 (1-5)
Parity	1 (0-3)	1 (0-3)
Cervical dilation, cm	0.7 ± 0.25 (0-2)	4.625 ± 0.7 ^b (1-9.5)
Hours in labor	0 (0-0)	6.23 ± 0.55 ^b (3-15)
Spontaneous membrane rupture	0	3
Gestational age at delivery, weeks	39 ± 0.3 (38 ⁺ 0.41 ⁺ 4)	38.5 ± 0.42 (37 ⁺ 0.41 ⁺ 2)

Abbreviation: SEM, standard error of the mean.

^a Data are represented as mean ± SEM (range). ^b $P < 0.001$ compared to nonlabor.

From the large PLA₂ “superfamily,” more than 20 distinct members have been identified in human tissues.¹⁴ The PLA₂ isoforms that differ in molecular size, cellular localization, and substrate specificity are broadly classified into 5 categories: secretory phospholipases (sPLA₂), Ca²⁺-dependent cytosolic phospholipases (cPLA₂), Ca²⁺-independent phospholipases (iPLA₂), platelet-activating factor acetylhydrolase (PAF-AH), and lysosomal PLA₂.^{15,16} These PLA₂ groups are further divided into subgroups. For example, the largest group, sPLA₂, includes subgroups I, II, III, V, IX, X, XI, XII, XIII, and XIV. The physiological significance of why multiple PLA₂ isoforms exist is not fully understood. However, with the availability of different PLA₂ enzymes, the potential exists for increased variability or specificity of prostaglandin production within each tissue or cell type. The sPLA₂ group, for example, has been implicated in the generation of diverse kinds of phospholipid-derived mediators.¹⁶ Several members of the sPLA₂ family have been shown to play a role in inflammatory, immune, and apoptotic processes and disease progression,^{17,18,19} hence the interest in developing selective inhibitors for each of these subtypes.^{20,21}

To our knowledge, only 3 of the known PLA₂ enzymes have been identified and partially characterized in the pregnant human uterus, including sPLA₂-IIA, sPLA₂-V, and cPLA₂-IV.^{22,23} These isoforms are differentially expressed and regulated throughout pregnancy and parturition,²⁴⁻²⁹ suggesting different downstream cellular effects. However, these changes do not account for the overall increase in PLA₂ activity observed during pregnancy, suggesting the presence of other PLA₂ enzymes within the pregnant uterus. We performed an initial reverse transcriptase–polymerase chain reaction (RT-PCR) screen and identified the expression of a new secretory PLA₂ enzyme, sPLA₂-group IID (sPLA₂-IID) within the pregnant human uterus.³⁰ Secretory PLA₂-IID was first described in the mouse³¹ and more recently was identified in endothelial cells of the capillary vessels of rheumatoid arthritis patients³² and within mast cells of the human lung³³ and appears to play a role in cytokine-mediated inflammation¹⁹ and immunosuppression.¹⁷ To date, however, there is no information available regarding the sPLA₂-IID expression and regulation in human gestational tissues with labor.

Therefore, the aims of this study were to determine the expression pattern of sPLA₂-IID within human gestational tissues (amnion, chorion, placenta, decidua, and myometrium) at term and to ascertain any labor-associated changes in sPLA₂-IID expression in the gestational tissues. Following the identification of labor-associated changes in the decidua, the next aim of this study was to investigate the regulatory mechanisms of sPLA₂-IID expression. For this aim, we used an in vitro explant model of cultured decidual cells to examine the effects of interleukin (IL) 1β (IL-1β) and IL-10 on sPLA₂-IID expression.

Materials and Methods

Tissue Collection

Human gestational tissues were collected following ethics approval and informed consent (Office of Medical Bioethics, University of Calgary) from women with healthy pregnancies who were undergoing cesarean section (CS) at term. Indications for CS included breech presentation, previous CS, placenta previa, and fetal distress. Samples were collected from healthy term singleton pregnancies, before labor (term nonlabor, TNL; $n = 12$) or after labor (term labor, TL; $n = 12$). Clinical characteristics of labor in the patients included in the study are presented in Table 1. After delivery of the baby, amnion, chorion, and placental samples were isolated. The amnion and chorion samples were obtained from below the incision site of the lower uterine segment at cesarean section delivery. The placental samples were obtained from the middle of the placental disk. In addition, myometrium and decidua biopsies were obtained from within the uterus at the upper margin of the lower uterine segment. Samples were either frozen and stored at -80°C prior to RNA and protein isolation or fixed in 10% neutral-buffered formalin prior to paraffin embedding for immunohistochemistry. Decidua biopsies were also obtained from an additional 4 patients for generating decidua explant cultures. The decidua biopsies were placed immediately into sterile cold Hank Balanced Salt Solution (HBSS; Life Technologies Inc, Burlington, Ontario, Canada) containing an antibiotic–antimycotic solution (Penicillin–Streptomycin–Amphotericin B; Life Technologies Inc) and transported to the laboratory for processing within 30 minutes of delivery.

Decidual Cell Culture

Decidual cells were prepared using modified methods as described previously.^{34,35} In brief, decidua biopsies were dissected from the uterus, cut into 1-mm³ sections, and washed in HBSS supplemented with 100 U/mL penicillin/streptomycin and 2.5 μg/mL amphotericin B. The tissue pieces were incubated in HBSS containing 1 mg/mL collagenase XI and 1 mg/mL collagenase IA for 60 minutes at 37°C. The solution was triturated through a sterile pipette to dissociate decidual cells from the tissue pieces. Digested cells were filtered and centrifuged prior to resuspension in cell culture media supplemented

Table 2. Primers for Conventional and Real-Time RT-PCR.

Gene	Primer Sequence 5'-3'	Product Size	GenBank Accession Number
sPLA ₂ -IID	GTGCAGCATCTACAAGGACT ACACAGCTACTGCCTCAACT	394 bp	NM_012400
sPLA ₂ -IID	CTGCTGCTATGACCACCTGA GTCGCTTCTGGTAGGTGTCC	185 bp	NM_012400
B2M	CTTATGCACGCTTAACCTATCTTAACAA TAGGAGGGCTGGCAACTTAG	127 bp	NM_004048
GAPDH	CCACCCATGGCAAATTCATGGCA TCTAGACGGCAGGTCAGGTCCACC	598 bp	NM_002046

Abbreviations: B2M, β 2-microglobulin; bp, base pair; GAPDH, glyceraldehyde phosphate dehydrogenase; RT-PCR, reverse transcriptase–polymerase chain reaction; sPLA₂, secretory phospholipase.

with 2 mmol/L glutamine, 10% fetal bovine serum, 100 U/mL penicillin/streptomycin and 2.5 μ g/mL amphotericin B. Cells were plated in 12-well culture plates and incubated at 37°C in a humidified atmosphere of 95% air/5% CO₂. Culture medium was changed every 48 hours until the cells reached approximately 80% confluence, at which point the media was changed to serum-free media overnight prior to treatment with IL-1 β (1 ng/mL) or IL-10 (5, 25, or 50 ng/mL) for 18 hours. All experiments were performed at passage 0 or passage 1 and were performed using cultured decidua from 4 women, with each experiment being performed in duplicate. Following treatments, cells were harvested for RNA and protein isolation and the culture supernatant was collected for measurement of IL-8. Cell viability was assessed at the end of selected experiments using the MTT (thiazolyl blue tetrazolium bromide) assay.

RNA Isolation, RT-PCR, and Real-Time RT-PCR

Frozen tissue samples (~30 mg) were homogenized using a Qiagen TissueLyser LT (Qiagen Inc, Toronto, Ontario, Canada). RNA was subsequently isolated using Qiagen RNeasy kits (Qiagen Inc) and 100 ng was used to synthesize complementary DNA (cDNA) with qScript cDNA SuperMix (Quanta BioSciences, Gaithersburg, Maryland). The resultant cDNA was used as a template to perform RT-PCR using gene-specific primers. Conventional PCR was initially used to identify the presence of sPLA₂-IID and the reference gene glyceraldehyde phosphate dehydrogenase (*GAPDH*) using Biolase thermostable enzyme (Bioline Inc, Taunton, Massachusetts) and the Eppendorf EPS Mastercycler (Eppendorf Canada, Mississauga, Ontario, Canada) and PCR was performed for 35 cycles of: denaturing at 94°C, annealing at 60°C, and elongation at 72°C. The PCR amplification products were analyzed by agarose gel electrophoresis and all PCR products were verified by sequencing. Subsequent real-time RT-PCR analysis was performed using an Applied Biosystems 7900HT machine with SYBR Green qPCR SuperMix (Quanta BioSciences) according to manufacturer instructions. Melt curve analysis was performed to verify absence of nonspecific amplification products. Standard curves for each primer pair were generated using serial dilution of pooled cDNA samples as template. Results were calculated according to the methods described by Livak and

Schmittgen^{36,37} and gene expression was normalized to the reference gene β 2-microglobulin (B2M). The PCR primers utilized for conventional and real-time RT-PCR are presented in Table 2.

Immunoblot Analysis

Protein purification from frozen tissue samples (~30 mg) was performed using the Qiagen Mammalian Qproteome protein purification kit and TissueLyser LT (Qiagen Inc) according to manufacturer instructions. Protein samples (8 μ g) were diluted with Laemmli buffer, separated on precast 4% to 12% Bis-Tris gels (Life Technologies Inc) and transferred to Hybond-ECL membranes (GE Healthcare Bio-Sciences, Baie d'Urfe, Quebec, Canada). Membranes were first incubated in blocking solution (1% powdered skim milk in Tris-buffered saline-Tween [TBS-T]) followed by incubation sPLA₂-IID primary antibody (ab47118—Abcam Inc, Toronto, Ontario, Canada—this antibody is specific for sPLA₂-IID) at 1:200 in TBS-T overnight at 4°C or with GAPDH (sc-47724—Santa Cruz Biotechnology Inc, Santa Cruz, California) at 1:50 000 in TBS-T for 20 minutes at room temperature. Subsequently, membranes were washed in TBS-T followed by incubation with a secondary antibody (1:2000 dilution) for 45 minutes at room temperature. For sPLA₂-IID, the secondary antibody was goat anti-rabbit (Santa Cruz Biotechnology Inc) and for GAPDH the secondary antibody was goat anti-mouse (Santa Cruz Biotechnology Inc). The immunoblot image was acquired with a Fujifilm LAS-3000 set for chemiluminescent detection. Optical density of bands was measured and protein expression was assessed as sPLA₂-IID relative to GAPDH expression.

Immunohistochemistry

Tissue sections (5 μ m) were cut onto clear glass slides (ThermoFisher Scientific, Ottawa, Ontario, Canada), dewaxed, rehydrated, and rinsed in phosphate-buffered saline (PBS). The sections were incubated in antigen unmasking solution (10 mmol/L citrate buffer, pH 6.0) for antigen retrieval and in 3% hydrogen peroxide to quench endogenous peroxidase activity. Tissue sections were treated using the ABC staining kit (Santa Cruz Biotechnology Inc) according to the manufacturer instructions. Briefly, sections were incubated for 1 hour in

Figure 1. Representative reverse transcriptase–polymerase chain reaction (RT-PCR) gel showing expression of sPLA₂-IID (394 bp) and the reference gene glyceraldehyde phosphate dehydrogenase (GAPDH; 598 bp) in pooled complementary DNA (cDNA; pooled from $n = 2$ labor and $n = 2$ nonlabor samples). Am indicates amnion; Ch, chorion; Pl, placenta; De, decidua; My, myometrium. M, DNA marker; Bl, PCR blank control.

1.5% blocking serum in PBS in a humidity chamber at room temperature, prior to incubation with primary antibody at 1:200 (sPLA₂-IID; Abcam Inc) overnight at 4°C. Slides were washed in PBS and incubated with biotinylated goat anti-rabbit secondary antibody followed by incubation with Avidin-Biotin enzyme reagent and peroxidase substrate. Finally, sections were counterstained in Harris hematoxylin, dehydrated, and mounted. Images were obtained using a Zeiss Axio Scope.A1 connected to an Axiocam ICc 3 camera and AxioVision 4.7.2 software (Carl Zeiss Canada Ltd, Toronto, Ontario, Canada).

Enzyme-Linked Immunosorbent Assay

A sandwich enzyme-linked immunosorbent assay (ELISA) Kit (R&D Systems, Minneapolis, Minnesota) was used according to manufacturer instructions to quantify IL-8 released into the culture medium in response to treatment. Optical density was measured at 450 nm using a DTX880 Multimode Detector with Multimode Analysis Software (Beckman Coulter Canada Inc, Mississauga, Ontario, Canada).

Statistical Analysis

Data are presented as mean $\pm$ standard error of the mean (SEM). The results were evaluated by unpaired Student *t* test. Statistical analysis and graphic representation was performed with GraphPad PRISM Software (GraphPad Software Inc, San Diego, California). Significance was accepted at a *P* value $< .05$.

Results

The clinical characteristics of patients are depicted in Table 1. There were no significant differences between labor and nonlabor groups for maternal age, gravidity, parity, gestational age at delivery, or ethnicity. In the term labor group, the cervical

dilation and hours of labor were significantly greater than the term nonlabor group.

Expression of sPLA₂-IID in Human Myometrium and Gestational Tissues

Conventional RT-PCR analysis revealed expression of sPLA₂-IID mRNA in human amnion, chorion, placenta, decidua and myometrium at term (Figure 1). Immunohistochemistry was used to examine the cellular localization of sPLA₂-IID in gestational tissues at term. sPLA₂-IID protein expression was identified in amnion (data not shown), chorion, placenta, decidua, and myometrium (Figure 2) with unique staining patterns observed. In chorion, sPLA₂-IID expression was observed within chorion fibroblast and trophoblast cells (Figure 2A) and within the trophoblasts of the placenta (Figure 2B) and in the myometrium, the myometrial smooth muscle bundles showed staining and localization of sPLA₂-IID protein (Figure 2C). Within the decidua, staining was observed in the vacuolated decidual cells and decidual stromal cells (Figure 2D); also shown are higher magnification images of the sPLA₂-IID staining in the decidua (Figure 2E, $\times 20$ and 2F $\times 40$, respectively). No staining was observed in controls, with no primary antibody treatment (Figure 2 insets).

sPLA₂-IID mRNA and Protein Expression With Labor

To determine whether expression of sPLA₂-IID mRNA or protein changed with labor, we employed real-time RT-PCR and Western blot analysis, respectively. Expression of sPLA₂-IID was identified in the maternal gestational tissues, decidua, and myometrium (Figure 3), and the fetal gestational tissues, amnion, chorion, and placenta (Figure 4). In decidua, expression of sPLA₂-IID mRNA and protein was significantly lower in term labor compared to term nonlabor samples ($*P < .05$), suggesting a labor-associated decrease in sPLA₂-IID. No significant labor-associated changes were observed in myometrium (Figure 3), amnion, chorion, or placenta (Figure 4). These results are in contrast to the labor-associated increase observed for sPLA₂-IIA mRNA expression in myometrium,²³ suggesting that the regulatory mechanisms that govern the expression of these 2 isoforms may be unique.

Effect of Pro- and Anti-Inflammatory Mediators on sPLA₂-IID Expression in Decidua Cells

Regulation of sPLA₂-IID gene expression within the decidua was examined in response to treatment with the proinflammatory cytokine, IL-1 β and the anti-inflammatory cytokine, IL-10. Treatment of cultured decidua cells with IL-1 β (1 ng/mL) did not influence the expression of sPLA₂-IID mRNA expression. However, treatment with IL-10 (5, 25, and 50 ng/mL) resulted in a concentration-dependent increase in sPLA₂-IID mRNA expression as compared with nontreated cells (Figure 5A). To determine whether the decidua cells responded to IL-1 β , we tested the effect of IL-1 β and IL-10

Figure 2. Immunohistochemistry localization of sPLA₂-IID protein in maternal and fetal gestational tissues at term. Panels show sPLA₂-IID expression within (A) chorion, (B) placenta, (C) myometrium, and (D) decidua at term nonlabor. Panels (E) and (F) show decidua at $\times 20$ and $\times 40$ magnification, respectively. (The brown color indicates positive staining for sPLA₂-IID, arrows indicate points of interest. Negative controls with no primary antibody are shown in the insets. Scale bars are 100 μ m and 50 μ m).

on IL-8 output from decidua cells. A significant increase in IL-8 release was observed in response to IL-1 β treatment (Figure 5B). In contrast, IL-10 (50 ng/mL) treatment had no effect on IL-8 output and levels of IL-8 output were equivalent to non-treated controls. Cotreatment of cells with IL-1 β and IL-10 had no significant effect on IL-8 release compared to IL-1 β treatment alone, suggesting that IL-10 does not modulate the effects of IL-1 β .

Discussion

In this study, we demonstrate that the sPLA₂-IID isoform is expressed within the pregnant human uterus and that there is differential expression and regulation of sPLA₂-IID within human gestational tissues. Strong evidence suggests that labor

is an inflammatory process.³⁸ Therefore, manipulation of the classically inflammatory prostaglandin pathway has been pursued as a therapeutic target for preterm labor. Nevertheless, current treatment strategies are not always successful, suggesting that our understanding of the role of the PG pathway within the human uterus is incomplete. There are multiple genes involved at each step of the pathway, particularly the initial cleavage of membrane phospholipids by phospholipases. This led us to hypothesize that additional sPLA₂ isoforms may be expressed within human gestational tissues, contributing to the complexity of this pathway during pregnancy and labor.

In the present experiments, we demonstrate for the first time the presence of sPLA₂-IID mRNA and protein in the maternal and fetal term gestational tissues with a distinct pattern of expression observed in the different gestational tissues. Most

Figure 3. Expression of sPLA₂-IID in decidua and myometrium. (A) Real-time reverse transcriptase–polymerase chain reaction (RT-PCR) analysis demonstrating relative expression of sPLA₂-IID messenger RNA (mRNA) in decidua and myometrium comparing term nonlabor (TNL) to term labor (TL) patients (n = 12 each TL and TNL). Data are presented as mean ± standard error of the mean (SEM) of relative expression of sPLA₂-IID / B2 M. (B) Western blot analysis of sPLA₂-IID protein in decidua and myometrium. Inset shows a representative immunoblot with glyceraldehyde phosphate dehydrogenase (GAPDH) as loading control (n = 4 each TL and TNL). Data are presented as mean ± SEM of optical density units (ODUs) of sPLA₂-IID/GAPDH. Statistical analysis performed by unpaired Student *t* test. **P* < .05 compared to decidua TNL.

Figure 4. Expression of sPLA₂-IID in fetal membranes and placenta. (A) Reverse transcriptase–polymerase chain reaction (RT-PCR) analysis demonstrating relative expression of sPLA₂-IID messenger RNA (mRNA) in amnion, chorion, and placenta, comparing term nonlabor (TNL) to term labor (TL) patients (n = 12 each TL and TNL). Data are presented as mean ± standard error of the mean (SEM) of relative expression sPLA₂-IID / B2M. (B) Western blot analysis of sPLA₂-IID protein in amnion, chorion, and placenta. Inset shows a representative immunoblot with glyceraldehyde phosphate dehydrogenase (GAPDH) as loading control (n = 4 each TL and TNL). Data are presented as mean ± SEM of optical density units (ODU) of sPLA₂-IID / GAPDH. Statistical analysis performed by unpaired Student *t* test.

Figure 5. Measurement of sPLA₂-IID messenger RNA (mRNA) expression and interleukin (IL)-8 release from decidual cells in response to IL-1 β and IL-10. Decidual cells were cultured and either nonstimulated (NS) or treated with IL-1 β (1 ng/mL) or IL-10 (5, 25, or 50 ng/mL) and incubated for 18 hours. A, Real-time reverse transcriptase polymerase chain reaction (RT-PCR) was used to quantify sPLA₂-IID mRNA expression, which was normalized to the reference gene β 2-microglobulin (B2M). Data represent the mean $\pm$ standard error of the mean (SEM) of relative expression sPLA₂-IID/B2M. B, IL-8 release into the cell culture supernatant assessed by enzyme-linked immunosorbent assay (ELISA). Data represent the mean $\pm$ SEM for $n = 4$ patients. Statistical analysis performed by unpaired Student t test. * $P < .001$ compared to not significant (NS).

importantly, we show that there is a significant decrease in the expression of sPLA₂-IID mRNA and protein in decidual cells with labor, while changes were not observed in amnion, chorion, placenta, or myometrium. We now show that sPLA₂-IID mRNA expression is decreased in decidual cells in term laboring patients compared to term nonlaboring patients (Figure 3). Previous data showed that sPLA₂-IIA mRNA expression is increased in myometrium with the onset of labor,²³ whereas cPLA₂-IV activity is decreased in amnion with labor onset.²⁴ Such tissue-specific distribution of PLA₂ isoforms may impart a mechanism to permit intricate control of PG synthesis during labor progression. Indeed, the decidual cells would be a desirable location for this level of control because it is located at the interface between maternal and fetal tissues. However, since we observe decreased expression of sPLA₂-IID mRNA in the decidual cells, coupled with an absence of change in other gestational tissues, it is unlikely that sPLA₂-IID contributes to the

increased prostaglandin production implicated in the transition from uterine quiescence to active contractions during labor. The present observation of a downregulation of the IID isoform within decidual cells during labor implies reduced sPLA₂-IID enzymatic activity, suggesting a decrease in PG production via this pathway. Since the changes in mRNA expression were closely followed by a decrease in protein expression (Figure 3A and B), this may lead us to speculate that the fall in sPLA₂-IID in decidual cells with parturition may limit AA release and prostaglandin formation from the cell membranes at the maternal-fetal interface, the significance of which is not clear and warrants further investigation. We can speculate, however, that sPLA₂-IID mRNA regulation in the decidual cells strongly suggests a dynamic cross-talk at the interface between maternal and fetal tissues. Such an assumption is supported by the sensitivity of sPLA₂-IID expression to anti-inflammatory cytokine stimulation (Figure 5). A switch in the expression ratio of pro- to anti-inflammatory mediators in the decidual cells could mediate barrier function to promote or inhibit labor onset, and the maternal immune system may be actively involved in the process of labor onset at term.⁴⁰ This role for the decidual cells would not be surprising, given that in the nonpregnant state, the endometrium acts as a barrier to the external environment where a strong immune component is required.

More recently, an emerging role for sPLA₂-IID in immune function has been proposed.¹⁷ During normal pregnancy, there is an increase in the number of regulatory T cells in the maternal periphery and decidual cells, which are thought to be important for maintaining immune tolerance. A fine balance between the developing fetal and the maternal immune systems is preserved during pregnancy and a disturbance in this balance may lead to miscarriage. Indeed, women who have recurrent spontaneous miscarriage have decreased levels of regulatory T cells, leading to immune imbalance and rejection of the fetus.³⁹ Additionally, it has recently been demonstrated that sPLA₂-IID can act as an effector molecule of regulatory T cells, where it was shown to promote generation of additional regulatory T cells but inhibit T cell proliferation. An enzymatically inactive mutated form of sPLA₂-IID also produced this effect, suggesting that the catalytic domain is not required to elicit this effector function, implying that sPLA₂-IID may have anti-inflammatory and immunosuppressive properties not reliant upon its ability to cleave membrane phospholipids.¹⁷ It is possible that sPLA₂-IID may have a similar effect in the pregnant human uterus, where it could act to suppress the maternal immune system during pregnancy to prevent the rejection of the fetus and decreased sPLA₂-IID expression with labor onset could permit the influx of immune cells necessary to prevent infection during labor.

In summary, we have identified the presence of sPLA₂-IID in human gestational tissues with distinct tissue localization. We show significantly decreased expression in term laboring decidual cells compared to nonlaboring decidual cells. It is therefore probable that sPLA₂-IID is contributing to arachidonic acid production in these tissues during pregnancy but less likely to be implicated in the increased prostaglandin synthesis associated with labor onset. However, higher levels of sPLA₂-IID throughout pregnancy may

contribute to the production of relaxatory PGs, such as PGI₂, implying a proquiescent role of sPLA₂-IID in human pregnancy. In addition, regulation of sPLA₂-IID expression at the maternal–fetal interface may point to an as yet undefined role of the decidua in the maintenance of pregnancy by modulating immune function. There is a need for more specific and effective treatments for preterm labor. Our results highlight the complexity of the prostaglandin pathway in the pregnant human uterus and suggest that further research is needed to better define the roles of the PLA₂ isoforms in pregnancy and labor.

Authors' Note

Authors Andrea A. Mosher and Kelly J. Rainey had an equal contribution to the manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by funding from the Canadian Institutes of Health Research grant # MOP-82765 and Alberta Innovates-Health Solutions (AI-HS), Preterm Birth and Healthy Outcomes Team (PreHOT) grant 200700595. Over the duration of this study AAM was supported by an AI-HS graduate studentship, KJR by an ACHRI-CIHR trainee award and DMS with an AI-HS Research Scholar award.

References

1. Skinner KA, Challis JR. Changes in the synthesis and metabolism of prostaglandins by human fetal membranes and decidua at labor. *Am J Obstet Gynecol.* 1985;151(4):519-523.
2. Wikland M, Lindblom B, Wilhelmsson L, Wijkvist N. Oxytocin, prostaglandins, and contractility of the human uterus at term pregnancy. *Acta Obstet Gynecol Scand.* 1982;61(5):467-472.
3. Wijkvist N, Bryman I, Lindblom B, Norstrom A, Wikland M. The role of prostaglandins for the coordination of myometrial forces during labour. *Acta Physiol Hung.* 1985;65(3):313-322.
4. Keirse MJ, Hicks BR, Mitchell MD, Turnbull AC. Increase of the prostaglandin precursor, arachidonic acid, in amniotic fluid during spontaneous labour. *Br J Obstet Gynaecol.* 1977;84(12):937-940.
5. Keirse MJ. Prostaglandins in preinduction cervical ripening. Meta-analysis of worldwide clinical experience. *J Reprod Med.* 1993;38(1 suppl):89-100.
6. Durn JH, Marshall KM, Farrar D, et al. Lipidomic analysis reveals prostanoid profiles in human term pregnant myometrium. *Prostaglandins Leukot Essent Fatty Acids.* 2010;82(1):21-26.
7. Sanchez Ramos L. Induction of labor. *Obstet Gynecol Clin North Am.* 2005;32(2):181-200.
8. Crane J, Leduc L, Farine D, Hodges S, Reid GJ, Van Aerde J. *Induction of labour at term.* SOGC Clinical practice guideline No.107, August 2001.
9. Klam SL, Leduc L. Management options for preterm labour in Canada. *J Obstet Gynaecol Can.* 2004;26(4):339-345.
10. Ben Haroush A, Yogeve Y, Bar J, Glickman H, Kaplan B, Hod M. Indicated labor induction with vaginal prostaglandin E2 increases the risk of cesarean section even in multiparous women with no previous cesarean section. *J Perinat Med.* 2004;32(1):31-36.
11. Groom KM, Shennan AH, Jones BA, Seed P, Bennett PR. TOCOX—a randomised, double-blind, placebo-controlled trial of rofecoxib (a COX-2-specific prostaglandin inhibitor) for the prevention of preterm delivery in women at high risk. *Br J Obstet Gynaecol.* 2005;112:725-730.
12. Hendricks SK, Smith JR, Moore DE, Brown ZA. Oligohydramnios associated with prostaglandin synthetase inhibitors in preterm labour. *Br J Obstet Gynaecol.* 1990;97(4):312-316.
13. Buczynski MW, Dumlaio DS, Dennis EA. Thematic Review Series: Proteomics. An integrated omics analysis of eicosanoid biology. *J Lipid Res.* 2009;50(6):1015-1038.
14. Schaloske RH, Dennis EA. The phospholipase A2 superfamily and its group numbering system. *Biochim Biophys Acta.* 2006;1761(11):1246-1259.
15. Burke JE, Dennis EA. Phospholipase A2 structure/function, mechanism and signaling. *J Lipid Research.* 2009;50 suppl: S237-S242.
16. Six DA, Dennis EA. The expanding superfamily of phospholipase A(2) enzymes classification and characterization. review. *Biochem Biophys Acta.* 2000;1488(1-2):1-19.
17. von Allmen CE, Schmitz N, Bauer M, et al. Secretory phospholipase A2-IID is an effector molecule of CD4+CD25+ regulatory T cells. *Proc Natl Acad Sci U S A.* 2009;106(28):11673-11678.
18. Boilard E, Lai Y, Larabee K, et al. A novel anti-inflammatory role for secretory phospholipase A2 in immune complex-mediated arthritis. *EMBO Mol Med.* 2010;2(5):172-187.
19. Lindbom J, Ljungman AG, Lindahl M, Tagesson C. Increased gene expression of novel cytosolic and secretory phospholipase A(2) types in human airway epithelial cells induced by tumor necrosis factor-alpha and IFN-gamma. *J Interferon Cytokine Res.* 2002;22(9):947-955.
20. Nevalainen TJ, Haapamäki MM, Grönroos JM. Roles of secretory phospholipases A(2) in inflammatory diseases and trauma. *Biochim Biophys Acta.* 2000;1488(1-2):83-90.
21. Burke JE, Dennis EA. Phospholipase A2 biochemistry. *Cardiovasc Drugs Ther.* 2009;23(1):49-59.
22. Lappas M, Rice GE. Phospholipase A2 isozymes in pregnancy and parturition. *Prostaglandins Leukot Essent Fatty Acids.* 2004;70(2):87-100.
23. Slater DM, Astle S, Bennett PR, Thornton S. Labour is associated with increased expression of type-IIA secretory phospholipase A2 but not type-IV cytosolic phospholipase A2 in human myometrium. *Mol Hum Reprod.* 2004;10(11):799-805.
24. Skannal DG, Brockman DE, Eis AL, Xue S, Siddiqi TA, Myatt L. Changes in activity of cytosolic phospholipase A2 in human amnion at parturition. *Am J Obstet Gynecol.* 1997;177(1):179-184.
25. Skannal DG, Eis AL, Brockman D, Siddiqi TA, Myatt L. Immunohistochemical localization of phospholipase A2 isoforms in human myometrium during pregnancy and parturition. *Am J Obstet Gynecol.* 1997;176(4):878-882.
26. Bennett P, Slater D, Berger L, Moor G. The expression of phospholipase A2 and lipocortins (annexins) I, II and V in human fetal

- membranes and placenta in association with labour. *Prostaglandins*. 1994;48(2):81-90.
27. Freed KA, Moses EK, Brennecke SP, Rice GE. Differential expression of type II, IV and cytosolic PLA2 messenger RNA in human intrauterine tissues at term. *Mol Hum Reprod*. 1997; 3(6):493-499.
28. Rice GE, Brennecke SP, Scott KF, Smith GM, Rajkovic IA, Bishop GJ. Elevated maternal plasma immunoreactive phospholipase A2 in human preterm and term labour. *Eicosanoids*. 1992; 5(1):9-12.
29. Munns MJ, Farrugia W, King RG, Rice GE. Secretory type II PLA2 immunoreactivity and PLA2 enzymatic activity in human gestational tissues before, during and after spontaneous - onset labour at term. *Placenta*. 1999;20(1):21-26.
30. Slater DM, King EM, Thornton S, Vatish M. Differential expression of novel phospholipase A₂ enzymes in human gestational tissue. 54th Annual Meeting of the Society for Gynecologic Investigation. 2007;14:57A-292A.
31. Valentin E, Koduri RS, Scimeca JC, et al. Cloning and recombinant expression of a novel mouse-secreted phospholipase A2. *J Biol Chem*. 1999;274(27):19152-19160.
32. Masuda S, Murakami M, Ishikawa Y, Ishii T, Kudo I. Diverse cellular localizations of secretory phospholipase A2 enzymes in several human tissues. *Biochim Biophys Acta*. 2005;1736(3):200-210.
33. Triggiani M, Giannattasio G, Calabrese C, et al. Lung mast cells are a source of secreted phospholipases A2. *J Allergy Clin Immunol*. 2009;124(3):558-565.
34. Delvin EE, Arabian A, Glorieux FH, Mamer OA. In vitro metabolism of 25-hydroxycholecalciferol by isolated cells from human decidua. *J Clin Endocrinol Metab*. 1985;60(5):880-885.
35. Baston Büst DM, Hess AP, Hirchenhain J, et al. A possible ambivalent role for relaxin in human myometrial and decidual cells in vitro. *Arch Gynecol Obstet*. 2009;280(6):961-969.
36. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*. 2001;25(4):402-408.
37. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc*. 2008;3(6):1101-1108.
38. Golightly E, Jabbour HN, Norman JE. Endocrine immune interactions in human parturition. *Mol Cell Endocrinol*. 2011;335(1): 52-59.
39. Jin LP, Chen QY, Zhang T, Guo PF, Li DJ. The CD4⁺CD25^{bright} regulatory T cells and CTLA-4 expression in peripheral and decidual lymphocytes are down-regulated in human miscarriage. *Clin Immunol*. 2009;133(3):402-410.
40. Nagamatsu T, Schust DJ. The immunomodulatory roles of macrophages at the maternal-fetal interface. *Reprod Sci*. 2010;17(3): 209-218.