Pregnancy Hypertension 15 (2019) 64–71

Contents lists available at ScienceDirect

Pregnancy Hypertension

journal homepage: www.elsevier.com/locate/preghy

Decidual cells from women with preeclampsia exhibit inadequate decidualization and reduced sFlt1 suppression☆

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ARTICLE INFO

Keywords:
Preeclampsia
Placenta
Decidua
Stromal cell
Decidualization
sFlt1

ABSTRACT

Uterine stromal cell decidualization of maternal tissue is essential for implantation of and local adaptation to the fetal allograft, as well as growth and maintenance of the placenta in healthy pregnancies. Maternal defects in decidualization have been suggested as a possible driver of preeclampsia. Preeclampsia (PE) pregnancies demonstrate shallow implantation, inadequate spiral artery remodeling, and elevated levels of the anti-angiogenic protein, sFlt1. To test whether stromal cells (DSCs) isolated from PE placentas exhibit inadequate re-decidualization and increased expression of sFlt1, DSCs from normotensive (NT-DSCs) and PE (PE-DSCs) placentas were treated for 8 days (D8) with cAMP to induce decidualization and levels of decidualization markers (PRL, IGFBP1, VEGF) and sFlt1 were measured at day 0 (D0), D8, and after reversal of treatment. NT-DSCs achieved statistically significant elevations in PRL and IFGBP1 expression (25.72 [5.78–50.04], $p = 0.0008$ and 92.09 [1.79–543.10], $p = 0.005$). PE-DSCs increased PRL and IFGBP1 expression to 6.15 [2.30–10.73] ($p = 0.18$) and 8.67 [1.64–376.10] ($p = 0.04$). NT-DSCs reduced sFlt1 expression at D8 to 0.25 [0.17–0.49] ($p = 0.0021$) compared to 0.31 [0.25–0.82] ($p = 0.087$) in PE-DSCs. These results show that, when induced to decidualize, PE-DSCs fail to increase expression of decidualization markers to levels achieved by NT-DSCs. sFlt1 expression is higher in PE-DSCs during decidualization, suggesting inadequate suppression during the crucial implantation period. These defects at the maternal fetal interface may lead to the failed spiral artery modification, decreased placental invasion of the uterus, and elevated circulating sFlt1 levels seen in PE pathology.

1. Introduction

Preeclampsia (PE) is a hypertensive disorder of pregnancy that affects 8 million pregnancies annually worldwide and is responsible for an estimated 15% of premature births in the US [1]. PE occurs after 20 weeks of gestation with the onset of hypertension and widespread vascular dysfunction. If untreated, severe disease leads to fatal end-organ damage in the mother, including stroke, kidney failure, liver rupture, pulmonary edema, and frank eclampsia [2]. The only known treatment of the condition once it has developed is the delivery of both the placenta and the fetus, resulting in higher rates of premature births and infant growth restriction [1]. PE has a lasting effect on both mother and child: it is associated with a 2-fold increased risk of future cardiovascular disease (CVD) and a 5- to 12-fold increased risk of end-stage renal disease in mothers, and an increased risk of CVD in offspring [3,4].

Though the etiology of PE is unknown, there are several proposed mechanisms, including immune response to the fetal allograft, endothelial dysfunction, and placental hypoxia [5]. Until recently most models focused on the placenta as the primary causal agent in PE development [6]. New research has begun to implicate dysfunctional interactions between placenta and uterus at the maternal-fetal interface

Abbreviations: PE, preeclampsia; NT, normotensive; DSC, decidual stromal cell; MFI, maternal-fetal interface; sFlt1, soluble fms-like tyrosine kinase-1; VEGF, vascular endothelial growth factor; IGFBP1, insulin-like growth factor binding protein-1; PRL, prolactin

☆ This work was presented as a poster at the 65th SRI meeting, San Diego CA, March 7–10, 2018.

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https://doi.org/10.1016/j.preghy.2018.11.003
Received 27 September 2018; Received in revised form 15 November 2018; Accepted 16 November 2018
Available online 17 November 2018

(MFI). PE placentas demonstrate inadequate spiral artery remodeling and shallow implantation into the uterine wall; these characteristics have directed investigations into the angiogenic environment and the behavior of both migratory and receptive cells at the MFI [7].

The degree of placental invasion and implantation into the uterine wall is consistently reduced in PE placentas. This process is dependent on carefully-orchestrated interactions between invading extravillous trophoblasts and vascular cells at the MFI [8]. Endometrial stromal cells (ESCs) of menstruating species cyclically undergo decidualization, during which they change shape and function to secrete factors promoting placental growth and embryo implantation, including insulin-like growth factor binding protein 1 (IGFBP1) and prolactin (PRL) [9–11]. If implantation occurs successfully, decidualization must be sustained by factors produced by the incoming embryo; however, the initial penetration and growth of the conceptus during and in the early stages of implantation are dependent on proper reception and maintenance by the transient decidual layer [12]. As such, studies have begun to investigate defects in the decidua of women with past or current PE pregnancies [7]. Garrido-Gomez et al., have shown that endometrial stromal cells derived from non-pregnant women with a prior PE pregnancy failed to fully decidualize *in vitro* and exhibit a global depression in transcription when compared to controls, suggesting that failed decidualization may contribute to the development of PE [13].

Dysregulated production of the anti-angiogenic soluble VEGF receptor 1 (sFlt1) is thought to contribute to the disorganized and incomplete remodeling of spiral arteries observed in PE placentas [14,15]. sFlt1 is found at much higher circulating levels in women with active PE and inactivates angiogenic factors such as vascular endothelial growth factor (VEGF) and placental growth factor (PIGF) [14]. It is produced in a controlled manner by healthy placentas to support the creation of arterial networks to supply the conceptus, as well as by maternal peripheral blood mononuclear cells and ESCs [15,16]. Our lab recently demonstrated that sFlt1 production and decidualization are negatively correlated, suggesting that adequate suppression of this anti-angiogenic protein at the MFI is crucial for successful implantation of the conceptus [17].

Together, the above findings indicate that defects in the decidualization process may initiate a disruption in uterine-placental interaction, leading to poor development of the placental spiral arteries, hypoxia and interruption of nutrient delivery to the conceptus, local immune disruption, and systemic maternal derangements as seen in the clinical PE phenotype. Given this, it is essential to better characterize the behavior of the maternal decidua early in pregnancy and its relationship with vascular remodeling potential. In this *in vitro* study, we hypothesized that decidual stromal cells obtained from PE placentas (PE-DSCs) would demonstrate altered decidualization potential and increased levels of sFlt1 expression compared to DSCs from normotensive pregnancies (NT-DSCs).

2. Materials and methods

2.1. Human tissue sample collection

Preeclamptic (n = 6) and normotensive (n = 7) pregnant women were enrolled in this study through the Emory University Hospital–Midtown (Atlanta, GA) Gynecology-Obstetric clinic. This protocol was approved by the Emory Institutional Review Board and signed informed consent was obtained from all subjects under IRB00078902. PE was defined per ACOG Hypertension Guidelines [18]. We included non-smoking English-speaking women above 18 years old and excluded women with a preexisting chronic condition complicating pregnancy (diabetes, pre-gestational or chronic hypertension, congenital heart disease, autoimmune disorder, diagnosed past or present malignancy), acute complication such as non-pregnancy-related infection, known or suspected fetal anomaly, or known/suspected complication of pregnancy other than PE. Patient characteristics are summarized and shown in Table 1.

Table 1
Clinical characteristics of study population.

Variable	Normal Pregnancy (n = 7)	Preeclampsia (n = 6)
Maternal age (years)	35.7 ± 5.3	29.8 ± 4.3
Race	Caucasian = 4 Black = 3 Asian = 0	Caucasian = 1 Black = 4 Asian = 1
Maternal BMI (kg/m2)	30.6 ± 5.1	35.0 ± 3.3
Systolic blood pressure (mmHg)	118 ± 8	155 ± 18 **
Diastolic blood pressure (mmHg)	70 ± 5	92 ± 4 **
Gestational age at delivery (weeks)	39.8 ± 0.6	34.0 ± 4.0 ***
Placental weight (g)	589 ± 77	376 ± 167 *
Neonatal weight (g)	3401 ± 210	1989 ± 1001 *
Proteinuria	N/A	5/7
Cesarean section	4/7	4/6

Clinical characteristics of enrolled subjects. Quantitative characteristics were compared with Mann-Whitney *U* test. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

2.2. Decidual stromal cell (DSC) isolation and expansion

The decidual layer of each placenta was dissected and used in stromal cell preparation within 30 min of delivery. Per published protocols, tissue was enzymatically digested using 0.1% collagenase and filtered using 100–40 μm sieves [17]. Filtrates were subcultured at least twice to enrich the stromal cell population, and resultant primary DSCs were trypsinized and frozen in liquid nitrogen (10% DMSO & 90% FBS). Cells were thawed and grown in DMEM/Ham's F-12 supplemented with L-glutamine containing 10% fetal bovine serum, 1.0 nM sodium pyruvate, 1% nonessential amino acid, 1% penicillin-streptomycin, and 1% amphotericin B (Hyclone). Cells were passaged at 90% confluency and treatment was initiated at passage 4. Equivalent stromal cell lineage was confirmed with vimentin staining using a previously published protocol [19].

2.3. In vitro re-decidualization and reversal

In this study we use the term "re-decidualization" to describe *in vitro* re-stimulation of stromal cells which were obtained from the placental decidua. These cells have previously undergone monthly cyclical conversions from spindle-shaped stromal cells to cobblestone-shaped secretory decidual cells [9,20]. After isolation from the placental tissue and multiple cell culture passages in the absence of stimuli such as cAMP, they are de-decidualized both in form and function, but their history of prior decidualization distinguishes them from cells obtained from non-secretory endometrium [19]. For re-decidualization and reversal experiments, we used both NT-DSCs (n = 7) and PE-DSCs (n = 6). Initially cells were seeded in 10 cm cell culture plates, allowed to reach 90% confluence and subjected to re-decidualization by 0.5 mM dibutyryl cAMP (hereafter referred to as cAMP) in decidualization medium (phenol red-free DMEM/high glucose modified (Hyclone) with 5% charcoal-stripped fetal bovine serum, 1% penicillin, streptomycin, and 1% amphotericin B [19]). Cyclic AMP was used in isolation without addition of estradiol (E2) or progesterone (P4) as per previously published studies, which showed no appreciable difference in decidualization with this approach [17]. Appropriate medium was changed every 4 days and decidualization medium without cAMP served as control. At the end of 8 days, supernatant was harvested, cells were snap frozen in liquid nitrogen and stored in −80 °C for further analysis. To measure effect on decidualization reversal, another subset of DSCs was simultaneously decidualized for 8 days, followed by the

Table 2
Primer sequences used for mRNA expression quantification.

Primer	Direction	Sequence
hRPL17	Forward	5′-TGAACAAAGCACCTAAGATGCGCC-3′
	Reverse	5′-TGGGCAACCTCCTCTTCTGGTTTA-3′
Flt1	Forward	5′-GTTTAAAAGGCACCCAGCAC-3′
	Reverse	5′-TGTTTGCCATTTCTTCCACA-3′
PRL	Forward	5′-CACCCCCGAAGACAAGGA-3′
	Reverse	5′-CCAGGATCGCAATATGCTGAC-3′
IGFBP1	Forward	5′-TACCTGCCAAACTGCAACAAGA-3′
	Reverse	5′-CCATGGATGTCTCACACTGTCTG-3′
VEGF	Forward	5′-TGCCTGGAAGATTCAGGAGCCT-3′
	Reverse	5′-GAGCAGGAAGAGGATGAGG-3′

supplementation with regular growth medium (free of cAMP) for another 8 days (RD8). The supernatant was harvested and cells were snap frozen in liquid nitrogen and stored in −80 °C for further analysis.

2.4. Quantitative PCR analysis for decidualization markers and sFtl1

Total RNA was isolated from all cells at passage 4 once 80% confluent, prior to exposure to decidualization; on day 8 of decidualization treatment; and on day 8 of reversal using TRIzol (Invitrogen, Carlsbad, CA) per the manufacturer's protocol. cDNA was synthesized from the isolated RNA using Superscript III Reverse Transcriptase (Invitrogen). SYBR green based quantitative PCR (qPCR) analysis was used to analyze the expression of sFlt1, PRL, IGFBP1, and VEGF using SYBR green master (primer sequences shown in Table 2) [17]. Target gene transcript levels of each sample were normalized to human ribosomal protein L17 (hRPL17) housekeeping gene and the fold change was calculated using the $2^{-\Delta\Delta C(T)}$ method [21]. Each sample was run in duplicate for each gene (including control) and the average of both results reported. If duplicates differed from each other by ≥1, results were considered spurious and the plate was repeated.

2.5. ELISA estimation of sFlt1

Human VEGF R1/Flt1 Quantikine ELISA kits (R&D Systems, Minneapolis, MN) were used to estimate sFlt1 levels of NT-DSCs (n = 6) and PE-DSCs (n = 5) in culture medium at day 8 (D8) and reversal day 8 (RD8) according to manufacturer's protocol as described in prior publications [17]. In a previous manuscript, we have shown that mRNA

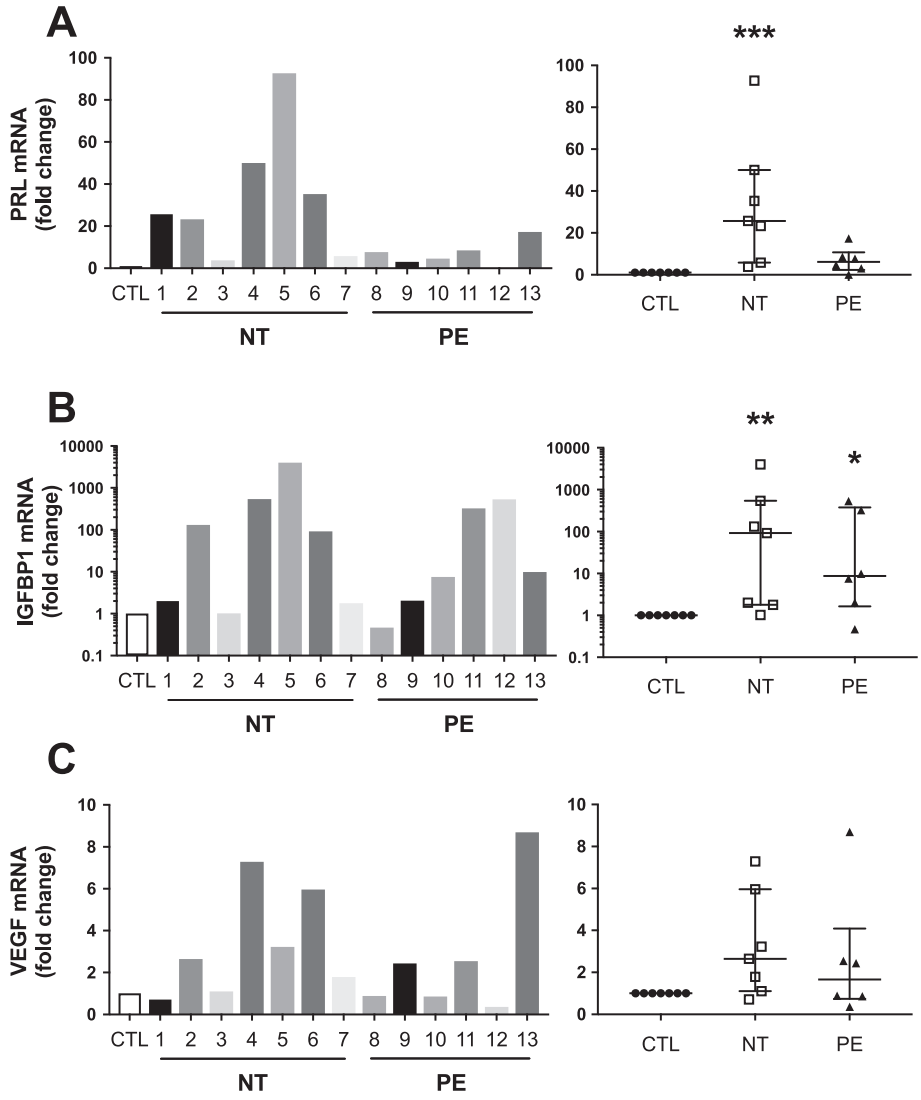

Fig. 1. Effect of cAMP treatment on markers of decidualization in NT-DSCs and PE-DSCs. Human DSCs were treated for 8 days with 0.5 mM cAMP. All treated cells had their own untreated controls. Fold change in RNA expression of (A) *PRL*, (B) *IGFBP1*, (C) *VEGF* at treatment day 8 (D8) are shown both individually and grouped with median and interquartile range. $n = 7$ NT-DSCs and 6 PE-DSCs. Fold changes at D8 were compared to controls. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ using the Kruskall-Wallis test.

levels of PRL, IGFBP1, and VEGF follow protein levels as measured by ELISA [17]. We therefore elected to measure and report only RNA levels of these markers for this exploratory investigation.

2.6. Statistical analysis

Normally distributed data such as patient characteristics are presented as mean ± standard deviation or as percent proportion of total group. Nonparametric data is reported as median [25th quartile – 75th quartile]. Clinical parameters of PE and NP patients were compared with non-parametric Mann Whitney *U* test. Gene expression profiles and ELISA protein levels were analyzed by Kruskall-Wallis test. All treated samples were standardized to their own controls. p values < 0.05 were considered statistically significant. Graphpad PRISM software version 7.0 (GraphPad Software Inc., San Diego, CA) was used for analysis.

3. Results

3.1. Clinical characteristics of study cohort

For this study, placental tissues for stromal cell isolation were collected from a total of 13 women (7 NT, 6 PE). Their clinical characteristics are shown in Table 1. Maternal age and maternal BMI were comparable between mothers with PE versus NT. The average gestational age at delivery of NT pregnancies is 39.8 ± 0.6 weeks, compared to 34.0 ± 4.0 weeks in PE pregnancies ($p = 0.0006$). NT pregnancies had average systolic and diastolic blood pressures of 118 ± 8 and 70 ± 5 respectively, while PE pregnancies averaged 155 ± 18 and 92 ± 4 (both p = 0.0012). Neonatal weight was significantly lower in PE pregnancies compared to NT pregnancies ($p = 0.014$), as was placental weight (p = 0.048), though it is unclear if this is a result of earlier delivery, PE pathology, or some combination thereof.

3.2. Decidualization of placental DSCs by cAMP

Degree of DSC decidualization was assessed at day 8 (D8) of stimulation by comparing fold change in decidualization markers PRL, VEGF, and IGFBP1 (Fig. 1A–C). Individually, response to re-decidualization stimuli in DSCs was highly variable even within each group (Fig. 1). Collectively, at D8 of cAMP treatment, NT-DSCs showed a significant increase in PRL mRNA levels, with a median increase of 25.72 [5.78–50.04] fold compared to untreated controls (1.0) ($p = 0.0008$). In PE-DSCs, PRL mRNA rose to a median of 6.15 [2.30–10.73] ($p = 0.18$). Similarly, NT-DSCs showed increased IGFBP1 mRNA levels to a median of 92.09 [1.79–543.10] ($p = 0.0054$) fold compared to control (1.0). PE-DSC IGFBP1 mRNA rose a median 8.67-fold [1.64–376.10] ($p = 0.04$). VEGF mRNA levels in NT-DSCs at D8 rose to 2.65 [1.10–5.96] ($p = 0.12$) versus 1.66 [0.74–4.09] (p > 0.9999) in PE-DSCs compared to respective controls (1.0).

3.3. Soluble Flt1 expression in DSCs during decidualization

Previously we had demonstrated a downregulation of sFlt1 with decidualization treatment in ESCs derived from normal cycling non-pregnant women [17]. Here we compared patterns of sFlt1 in PE-DSCs throughout re-decidualization to that of NT-DSCs.

Consistent with past findings in ESCs remote of pregnancies, measured sFlt1 mRNA median fold change levels in NT-DSCs at day 8 of cAMP treatment are reduced to one quarter of D0 levels (0.25 [0.17–0.49], $p = 0.0021$) (Fig. 2A), whereas PE-DSCs did not achieve a statistically significant change from baseline (0.31 [0.25–0.82], $p = 0.087$).

ELISA was used to measure levels of secreted sFlt1 protein on D8. Raw levels (pg/ml) were then normalized to levels produced by untreated controls (standardized to 1.0) to obtain proportionate change (Fig. 2B). At D8 of treatment, NT-DSCs reduced sFlt1 protein levels to a median 0.10 [0.07–0.30] ($p = 0.0017$), a decrease of 0.90 or 90%. In comparison, PE-DSCs sFlt1 production was reduced to 0.19 [0.13–0.43] ($p = 0.029$) fold, a decrease of 0.81 or 81%, also shown in Fig. 2B.

3.4. Effect of removal of cAMP on decidualization markers and sFlt1 expression

Levels of decidualization markers during decidualization reversal were assessed at RD8 (Fig. 3A–C). Reported values were produced by normalizing fold change levels in treated cells to levels produced by untreated controls (standardized to 1.0), as was done in calculation of D8 levels. Upon reversal of decidualization, NT-DSC median fold change in expression of PRL was 1.05 [0.48–1.54], IGFBP-1 was 0.80 [0.48–1.21], and VEGF was 0.95 [0.53–1.21]. In PE-DSCs, the fold change levels of PRL, IGFBP-1, and VEGF were 0.63 [0.31–1.13], 0.85 [0.66–1.95], and 1.18 [0.74–1.44], respectively. None of these were statistically significant changes from control values.

By RD8, sFtl1 mRNA transcripts levels in NT-DSCs had risen to a 0.51 [0.34–0.86] fold ($p = 0.033$) change from baseline, while PE-DSC expression had returned to 0.72 [0.61–1.36].

ELISA levels at RD8 were also measured and compared. When standardized to controls, measured sFlt1 levels were 0.78 [0.63–1.09] ($p = 0.01$) in NT-DSCs and 0.77 [0.58–1.69] ($p = 0.19$) in PE-DSCs.

3.5. Day 0 expression of sFlt1 and decidualization markers in DSCs

To examine DSC production of decidualization markers PRL, IGFBP1, and VEGF after passaging and before treatment, all cell lines were passaged three times in normal cell medium. RNA expression levels were then measured and compared in all untreated DSCs (Fig. 4A and B). NT- and PE-DSCs expressed grossly higher levels of decidualization markers than previously observed in ESCs, as expected of cells originating from the maternal decidua [17]. We find no significant difference in expression levels of sFlt1, IGFBP1, VEGF, or PRL between the two groups (Fig. 4C and D). There was considerable individual variation in all baseline gene expression values in both PE and NT-DSCs.

4. Discussion

In this manuscript, we report two novel findings related to molecular events at the MFI in the development of PE. First, our results indicate that DSCs obtained from PE placentas are unable to adequately respond to decidualization stimuli *in vitro*, consistent with recent results in ESCs published by Garrido-Gomez et al. In addition, we show that when stimulated to re-decidualize these cells only partially downregulate sFlt1, the pathogenic molecule associated with PE. This contrasts with ESCs obtained from non-pregnant women and NT-DSCs, both of which significantly downregulate sFlt1 production when decidualized [13,17]. To our knowledge, this is the first report addressing the relationship between insufficient decidualization and sFlt1 production in stromal cells obtained from the decidua of PE placentas. Our findings add to the growing body of evidence implicating the maternal decidua as an early driver of the PE phenotype. We suggest that a defect in the ability of maternal stromal cells to properly decidualize is responsible for a chain of downstream effects which together lead to aberrant overexpression of sFlt1.

The notion that failed decidualization is a contributor to negative pregnancy outcomes such as PE and fetal growth restriction (FGR) has been considered for some time. In 1986, the Brosens group published evidence of inadequate physiological changes in preeclamptic maternal uterine tissue in response to trophoblastic invasion [22]. Soon after, Craven et al., showed that structural changes necessary for implantation occurred in the maternal decidua prior to cellular contact with extravillous cytotrophoblasts [23]. Taylor et al., related lower

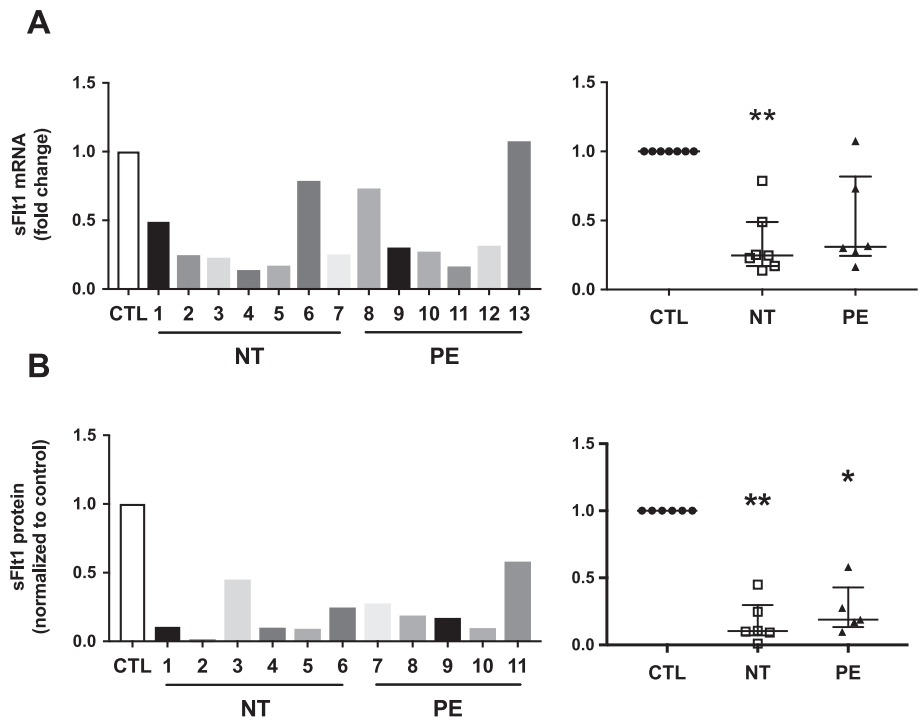

Fig. 2. Effect of decidualization treatment on sFlt1 protein and mRNA expression levels. (A) represents fold changes in mRNA expression and (B) secreted protein quantification of sFlt1 protein levels using ELISA. Individual levels within each cell line and grouped median and interquartile range levels are shown. Significant changes at D8 are relative to controls. Analysis was conducted using the Kruskall-Wallis test to compare each group. $^{*}p < 0.05$, $^{**}p < 0.01$.

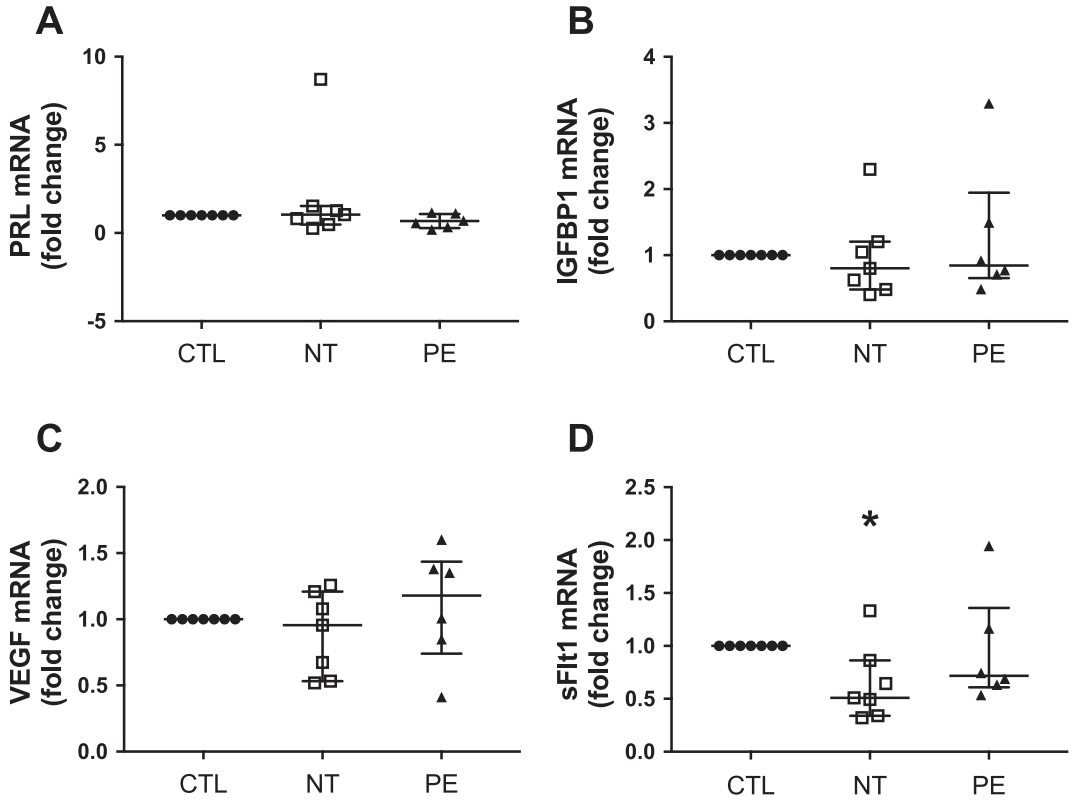

Fig. 3. Effect of decidualization treatment reversal on decidualization marker (A–C) and sFlt1 (D) expression levels. Significant changes at RD8 are relative to control values. Analysis was conducted using the Kruskall-Wallis test to compare each group. $^{*}p < 0.05$.

circulating levels of IGFBP1 to reduced trophoblastic invasion of the decidua in to-be-preeclamptic pregnancies in 1996 [24]. In a 2002 review it was suggested that partial or disrupted decidualization could initiate a series of events leading to inadequate implantation [24,25]. These and other contributions to the concept of defective decidualization have recently been summarized in a review by Conrad et al. [26].

More recently, Garrido-Gomez et al., published the first experimental evidence showing that ESCs obtained from non-pregnant women with a history of PE have defects in their ability to decidualize [13]. They suggested that women who are destined to develop PE have inherent errors in their response to hormonal decidualization signals. Our findings support this hypothesis. In addition to demonstrating

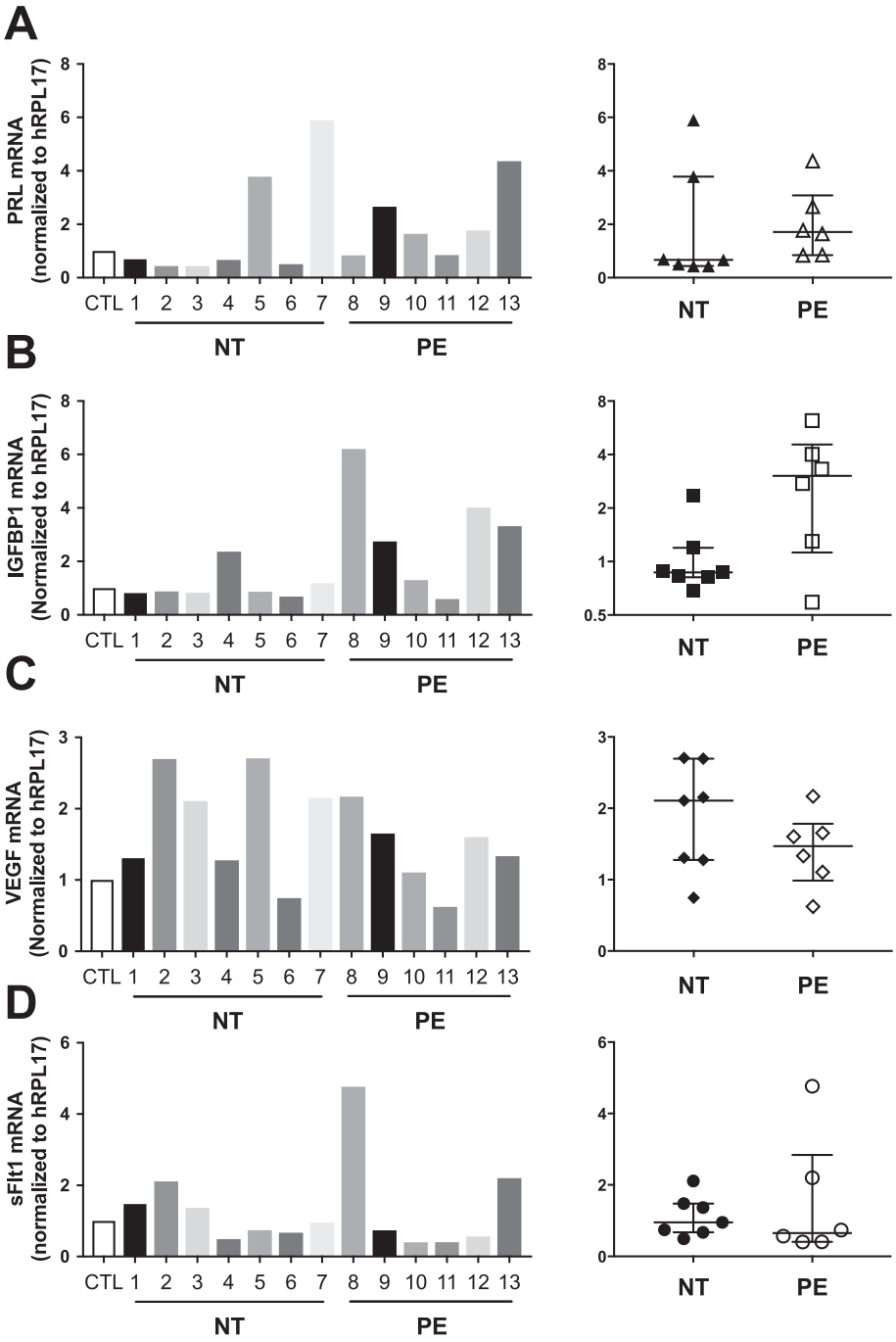

Fig. 4. Baseline decidualization marker (A-C) and sFlt1 (D) expression levels of PE-DSC and NT-DSCs prior to treatment, shown across individual cell lines and as grouped data with overlying median with interquartile range. Expression levels were compared using Kruskall-Wallis test.

deficient decidualization, we also show that IGFBP1 expression by PE-DSCs was 5 times lower than in NT-DSCs – a magnitude of difference which may lead to insufficient trophoblastic invasion [27,28]. This may be an artifact of multiple past dysfunctional decidualization cycles, in which abnormal responses to local decidualization stimuli over many months results in de-synchronous regulation of decidualization hormones. Our repeated passaging of the cells prior to analysis was intended to capture baseline behavior of the cells, removed from local *in vivo* stimuli. Even when passaged in culture conditions three times, PE-DSCs appear to retain a defective decidualization phenotype [17].

One model for PE development proposes that pathologically elevated sFlt1 levels seen in PE may be the result of cross-talk between placental and maternal cells. In 2007, Lockwood et al., described increased expression of sFlt1 by DSCs obtained from first-trimester pregnancies in response to thrombin [29]. Placental hypoxia has been shown to result in an over-secretion of sFlt1 into maternal circulation [30]. A recent study from our lab found that placental villous explants caused co-cultured PE PBMCs to overproduce sFlt1 [31]. These findings suggest cell-placental interactions play a key role in modulating sFlt1 production. We show here that, while NT-DSCs respond to decidualization stimulation by consistently downregulating sFtl1 production, PE-DSCs do not. Taken together, these findings may indicate that inadequate or partial decidualization of maternal DSCs leads to their overexpression of sFlt1, thereby interfering with angiogenic development in the local environment and causing hypoxic stress in the placenta. Discoordination of VEGF and sFlt1 production at this stage may

lead to further dysfunction in remodeling of spiral arteries and oxygen delivery to the uteroplacental interface [32]. The placenta may then in turn produce higher levels of sFtl1 which is secreted into maternal circulation, contributing to global vascular dysfunction as seen in PE.

Ultimately our exploratory study is limited by our use of term tissues only, which are essential to understand behavior of diseased tissue but cannot be used to definitively characterize early causal events. We believe building upon these results by describing behavior of early gestational tissue will be essential to characterizing early pathology at the MFI. While our passaging and enrichment protocol was designed to describe cellular behavior remote of pregnancy and thereby mitigate impact of gestational age on decidualization potential, it is important to note that the gestational age at time of delivery was significantly earlier in the PE mothers in this study. Future studies should also consider the impact that fetal sex, fetal outcome, differential gestational age of sample tissue, and other patient characteristics have on the behavior of maternal cells in preeclamptic pregnancies.

Our results show that decidualization and sFlt1 production are linked and concurrently dysregulated in preeclampsia, supporting a model for disease development that is driven by early dysfunction at the MFI. These findings deserve continued investigation, as better understanding of decidual cell behavior in PE may contribute to the discovery of a clinically useful biomarker for risk quantification, early diagnosis, and treatment to restore of a healthy phenotype.

Acknowledgments

We thank Hongyan Qu for her technical assistance.

Contributors

M.B.S.: I declare that I participated in collection of samples, experimental assays, RNA and protein data collection, data analysis, and production of figures and manuscript. I have seen and approved the final version. I have no conflicts of interest.

V.D.: I declare that I participated in collection of samples, experimental assays, RNA and protein data collection, data analysis, and production of figures and manuscript. I have seen and approved the final version. I have no conflicts of interest.

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B.R.: I declare that I participated in collection of samples and production of figures and manuscript. I have seen and approved the final version. I have no conflicts of interest.

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N.S: I declare that I participated in production of figures and manuscript. I have seen and approved the final version. I have no conflicts of interest.

A.R.: I declare that I participated in collection of samples, experimental assays, RNA and protein data collection, data analysis, and production of figures and manuscript. I have seen and approved the final version. I have no conflicts of interest.

Funding sources

This work was supported by the Abraham J & Phyllis Katz Foundation. M.S. is supported in part by the National Center for Advancing Translational Sciences of the National Institutes of Health, USA (UL1TR002378).

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