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Steroid sulfatase is increased in the placentas and whole blood of women with early-onset preeclampsia

Amy M. Gratton [a], Louie Ye [a], Fiona C. Brownfoot [a], Natalie J. Hannan [a], Clare Whitehead [a], Ping Cannon [a], Minh Deo [a], Peter J. Fuller [b], Stephen Tong [a], Tu'uhevaha J. Kaitu'u-Lino [a, *]

[a] Translational Obstetrics Group, The Department of Obstetrics and Gynaecology, Mercy Hospital for Women, University of Melbourne, Heidelberg, Victoria, Australia
[b] The Hudson Institute of Medical Research, Monash Health, Clayton, Victoria, Australia

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ABSTRACT

Introduction: Preeclampsia is a serious complication of pregnancy affecting 5% of pregnancies. Our team identified 137 genes highly expressed in placenta relative to other human tissues. Here, we have explored a role for steroid sulfatase (STS) in preeclampsia by characterising STS expression and the functional effects of STS on primary placental trophoblasts.

Methods: Characterisation of STS was performed on preterm preeclamptic and gestation-matched normotensive preterm controls who delivered at <34 weeks gestation. We characterised placental and maternal whole blood STS mRNA and placental protein expression via qRT-PCR, immunohistochemistry and Western Blot. To assess whether STS is involved in sFlt1 secretion and syncytialisation, we administered siRNA to silence STS in primary trophoblasts before measuring sFlt1 and hCG secretion and E-Cadherin expression.

Results: A custom array containing 45 placental specific genes identified 10 genes significantly altered in the placentas of preeclamptic patients relative to normotensive gestation-matched controls. Of these genes, qRT-PCR and western blot on a larger cohort confirmed that the expression of STS was significantly elevated in preeclamptic placentas ($n = 44$) relative to gestation matched controls ($n = 26$). Given placental RNA leaks in to the maternal circulation, we also assessed STS mRNA expression in the whole blood of patients with preeclampsia and found it was significantly increased relative to normotensive controls. siRNA knockdown of STS in primary trophoblast resulted in a modest but significant reduction in sFlt1 secretion, but had no affect on hCG secretion or E-Cadherin protein expression.

Discussion: STS is increased in preeclamptic placentas and maternal whole blood. Our data suggests that STS may affect sFlt1 secretion by regulating sFlt1-i13 transcription, and not via alterations in syncytialisation.

1. Introduction

Preeclampsia is a serious hypertensive disorder complicating approximately 5% of pregnancies [1–3]. It is the single leading cause of maternal mortality [4], responsible for over 70,000 global maternal deaths every year [1]. The cost of this disease is compounded by limited effective preventative measures and a lack of adequate screening.

Preeclampsia has traditionally been considered a two-stage disease [5]. The first asymptomatic stage comprises abnormal placental implantation and failure of spiral artery remodeling. Resulting dysregulated placental perfusion and ischemia causes release of anti-angiogenic factors such as soluble fms-like Tyrosine Kinase 1 (sFlt1). sFlt1 disrupts the maternal endothelium by binding circulating angiogenic factors, resulting in the symptomatic second stage of the disease [6–8]. Clinically, preeclampsia manifests as a multi-system disorder affecting maternal vessels (causing hypertension and endothelial dysfunction), kidneys, liver, the haematological system, brain (causing seizures, or eclampsia) and the fetus (growth restriction).

The two-stage model underpins much of our understanding of the disease and it is clear that the placenta is central to the pathogenesis [9]. Using in silico analysis, we identified 137 genes that were highly expressed in the placenta relative to other non-

* Corresponding author. Department of Obstetrics and Gynaecology, Mercy Hospital for Women, 163 Studley Rd, Heidelberg 3084, Victoria, Australia.
E-mail address: t.klino@unimelb.edu.au (T.J. Kaitu'u-Lino).

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malignant human tissues [10]. We reasoned that these Placental Specific Genes (PSGs) may be differentially altered in preeclamptic placenta and involved in the pathogenesis of the disease. Steroid Sulfatase (STS) is one of the 137 identified PSGs. In silico analysis suggests it is 34.4-fold more highly expressed in placenta compared to other human tissues [11].

STS is one of a larger family of 12 mammalian sulfatases that are encoded by the STS gene. STS has been shown to be responsible for the hydrolysis of abundant conjugated steroids converting them to active steroids with estrogenic properties [12]. While studies have identified a role for STS in breast cancer [13–15], its role in placentally-derived conditions such as preeclampsia has not been explored.

This study explores a role for STS in preeclampsia. We hypothesized that STS may be differentially expressed in preeclamptic placenta and play a functional role in the pathogenesis of the disease. We assessed this by characterising STS expression at the mRNA and protein level in preeclamptic placenta. We silenced STS in primary trophoblasts to examine the functional effect on sFlt1 secretion and syncytialisation. We also investigated whether STS mRNA is detectable in maternal whole blood.

2. Materials and methods

2.1. Tissue collection

Women presenting to the Mercy Hospital for Women provided written and informed consent for placental tissue collection. Placentas were obtained from preterm pregnancies and those complicated by preterm preeclampsia. Preterm preeclampsia was diagnosed in accordance with American College of Obstetricians and Gynaecologists (ACOG) guidelines [16]. Samples were acquired from cases of preterm preeclampsia, defined as requiring delivery <34 weeks gestation. Control placentas were from women presenting with preterm rupture of membranes or spontaneous preterm labour without evidence of infection, hypertensive disease or maternal co-morbidities. Samples were collected between 2012 and 2016. Of note, for array analysis, pre-term cases included both vaginal and caesarean section delivered patients, whilst for qRT-PCR verification and western blot analysis, only patients delivering via caesarean section were included. Patient characteristics are outlined in Table 1 (array) and Table 2 (qRT-PCR and WB). 7 preterm and 4 preeclamptic patient samples were included in both data sets. Whilst we acknowledge preterm placental samples are not likely to be from strictly normal pregnancies, they represent the most appropriate control for preterm preeclampsia samples as we are investigating changes that may be specific to PE.

Placental tissue was obtained immediately following delivery. Placental tissue (excluding fetal membranes) was removed and washed briefly in sterile phosphate-buffered saline (PBS). Samples for protein and RNA extraction were frozen within 15 min of delivery and stored at −80 °C. A portion of each placenta was also fixed in 10% buffered formalin for histology. Human ethics approval was obtained for this study from Mercy Health Human Research Ethics Committee.

2.2. Western blot analysis

Protein lysates were isolated from placental tissue collected in RNA Later via homogenization in RIPA buffer containing proteinase and phosphatase inhibitors, followed by assessment of protein concentration using a BCA assay (ThermoFisher Scientific). Placental lysate samples (24 μg) were separated on 12% poly-acrylamide gels with wet transfer to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA). Membranes were prepared in methanol and ultrapure H_20 prior to blocking with 5% skim milk powder in TBST before incubation overnight with anti-STS (1:1500, Sigma-Aldrich NSW, Australia) or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a loading control (1:2000, Cell Signalling Technology, Danvers, MA). Membranes were visualized using enhanced chemiluminescence detection system (Santa Cruz Biotechnology) and ChemiDoc XRS (BioRad, Hercules, CA, USA). Relative densitometry was determined using ChemiDoc XRS (BioRad).

2.3. Immunohistochemistry

STS immunohistochemistry was conducted on preeclamptic (n = 6) and gestation-matched preterm (n = 6) control placentas. Paraffin sections were deparaffinised in Xylene and rehydrated through descending concentrations of ethanol. Sections were heated in Tris-buffered saline, 0.1% Tween 20 (TBST) for 20 min s at 150 W in a 700 Watt microwave oven. After cooling to room temperature (RT), sections were washed in a PBS (pH 7.6), immersed in 3% H_20_2 in methanol for 10 min and rinsed with PBS. Blocking buffer (DAKO) was applied for 10 min and sections incubated for 1 h at 37 °C with anti-human STS (1:100) in 1% BSA/PBS. For isotype controls, primary antibody was substituted with rabbit IgG. The SuperPicTure kit (Invitrogen, Carlsbad, CA) was applied according to manufacturer's instructions to reveal STS staining. Sections were counterstained with Harris hematoxalin (Accustain, Sigma Diagnostics, Castle Hill, NSW, Australia), dehydrated and mounted.

2.4. RNA extraction

2.4.1. PAXgene whole blood

The PAX-gene blood miRNA kit (PreAnalytiX) and QIAcube was used according to manufacturer's instructions for automated extraction of total RNA. Genomic DNA was removed using DNAse

Table 1
Patient clinical characteristics. Clinical details of the two cohorts from whom placentas were obtained for array analysis. The preeclamptic cohort all had preeclampsia necessitating preterm delivery (<34 weeks gestation). Preterm controls were normotensive, delivered prematurely for indications other than preeclampsia. Data are presented as median (range) with the exception of Gravida and Parity which are presented as percentages.***p < 0.001****p < 0.0001. SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure.† Data on BMI (body mass index at the first pregnancy visit) available for 18 out of 22 preterm controls, 4 out of 12 preeclamptic patients.

	Preterm Placenta (n = 12)	Preeclamptic Placenta (n = 22)
Maternal Age median (range)	30 (18–39)	30 (22–38)
Gestation at Delivery median (range)	31.5 (30–33)	31 (30–33)
BMI† (kg/m^2) median (range)	25.6 (19–46)	30.5 (23–38)
Parity no. (%)	47.8%	41.6%
0	26.0%	25.0%
1	26.3%	33.4%
≥2		
Gravidity no. (%)	44.4%	34.8%
Primiparous	55.6%	65.2%
Multiparous		
Highest SBP prior to delivery (mmHg) Median (range)	120 (100–138)	160 (145–190)****
Highest DBP prior to delivery (mmHg) Median (range)	65 (50–88)	100 (85–120)****
Mode of delivery	59%	0%
Vaginal	41%***	100%
Caeserean		