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Loss of Akt increases soluble endoglin release from endothelial cells but not placenta

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ABSTRACT

Introduction: Preeclampsia is a serious pregnancy complication for which there are no medical treatments. Soluble endoglin is an anti-angiogenic factor implicated in the pathogenesis of the disease, however little is known about its molecular regulation. The PI3K/Akt pathway is down regulated in preeclamptic placentas and decreased PI3K/Akt signaling has been linked to increased soluble endoglin release from endothelial cells. MMP14 is a key protease that functions to release soluble endoglin from the placental surface.

Objective: This study aimed to determine whether reduced placental PI3K/Akt causes elevated release of soluble endoglin via MMP14.

Study design: Akt mRNA and protein expression were assessed in early onset preeclamptic and preterm control placentas (delivered <34 weeks gestation). PI3K/Akt inhibition was achieved by administering PI3K inhibitor wortmannin, a specific Akt inhibitor or Akt siRNA to primary human umbilical vein endothelial cells, primary trophoblast and placental explants. The effect of PI3K/Akt inhibition on soluble endoglin release, MMP14, endoglin and TIMP-3 mRNA expression was determined.

Results: We identified significantly reduced pAkt and total Akt in preeclamptic placentas relative to preterm control. Inhibition of PI3K/Akt resulted in significantly elevated soluble endoglin release from HUVECs, had no effect on MMP14 mRNA expression but resulted in significantly reduced TIMP3. In contrast inhibiting PI3K/Akt in placental explants or primary trophoblast did not change soluble endoglin release.

Conclusion: This study confirms that the PI3K/Akt cell protection pathway is down regulated in preeclampsia, but demonstrates that this dysregulation is unlikely to be responsible for the excessive placental soluble endoglin release characteristic of preeclampsia.

1. Introduction

Preeclampsia (PE) is a serious complication of pregnancy associated with multi-system organ involvement. It affects 2–7% of all pregnancies and is responsible for 70,000 maternal deaths globally each year and far greater numbers of perinatal losses [1]. The pathogenesis of preeclampsia is reasonably poorly understood. It is believed to result from inadequate placental invasion during implantation and poor placental perfusion [2–4]. The poorly perfused placenta then releases a raft of factors including soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng), anti-angiogenic factors that play a key role in causing the endothelial dysfunction seen in preeclampsia [5–7]. Both factors are increased in women with preeclampsia, and levels correlate with disease severity [8]. Previous studies have nicely demonstrated that whilst over-expression of sFlt1 alone in pregnant rats produced hallmarks of preeclampsia including hypertension and proteinuria, adenoviral co-expression of sEng and sFlt1 in combination phenocopied severe preeclampsia, with hemolysis, liver dysfunction, thrombocytopenia, cerebral edema and fetal growth restriction observed [7,8]. Such studies highlight the critical role of sEng in severe preeclampsia.

Abbreviation: Akt, MMP14 and soluble endoglin.

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Currently the only efficacious treatment for preeclampsia is delivery of the placenta and baby, which imposes risks of disability and death on the baby if the disease is early onset (less than 34 weeks gestation) [8]. Therefore, in order to develop new therapeutics, better understanding of the regulation of sFlt-1 and sEng is essential.

MMP14 is a widely studied metalloproteinase within the field of cancer development and metastasis [9–11]. Of interest to the field of preeclampsia research, in 2010, Hawinkels [12] demonstrated using an over-expression system in COS-7 cells that matrix metalloproteinase 14 (MMP-14; MT1-MMP) cleaved membrane bound endoglin to produce sEng [12]. We subsequently published data demonstrating that the same mechanism occurs in placental tissue [13]. In that study, we showed that MMP14 and endoglin interact at the placental surface, and that knockdown of MMP14 resulted in significantly reduced release of soluble endoglin from placental cells. However, understanding of MMP14 regulation in placenta is still lacking, even within the field of cancer research. One recent paper suggested that E2F transcription factors directly regulate MMPs in metastatic disease, however, we tested the possibility that this same mechanism occurs in placental cells, but found no significant effects of silencing E2F1 or 3 on MMP14 expression [14].

In 2012, Cudmore [15] et al. published a manuscript to demonstrate down-regulation of the endogenous protective pathway, the phosphoinositide 3-kinase (PI3K)/Akt pathway in preeclamptic placentas. They also provide compelling evidence using primary endothelial cells and an animal model to show that Akt activation inhibits soluble endoglin release, and down-regulation of the pathway enhances soluble endoglin release [15]. Given this exciting find, we were intrigued to assess whether loss of PI3K/Akt signaling also produces elevated sEng release from placental cells/tissue given the placenta is the predominant source of sEng in preeclampsia. In addition, given our data suggesting that MMP14 can cleave endoglin at the placental surface to release soluble endoglin we sought to assess whether inhibition of the PI3K/Akt pathway alters MMP14 expression, to determine whether this mechanism might contribute to the enhanced sEng observed in preeclampsia.

2. Materials and methods

2.1. Tissue collection

Women presenting to the Mercy Hospital for Women gave informed written consent for placental tissue collection. Placenta was obtained from preterm pregnancies not complicated by preeclampsia (PT, $n = 18$) and those complicated by severe early-onset PE ($n = 17$). Preeclamptics were diagnosed in accordance with ACOG guidelines 2013[16]. In addition, all samples were obtained from cases of early-onset preterm PE, defined as requiring delivery <34 weeks gestation. Pre-term control placentas were selected from women presenting with pre-term rupture of membranes, placental previa or anti-partum hemorrhage and did not have any evidence of infection (histopathological examination of the placentas), hypertensive disease or maternal co-morbidities. All patients delivered by caesarian section. Patient characteristics are outlined in Table 1.

Placental tissue was obtained immediately following delivery. Maternal and fetal surfaces were removed and a sample was then washed briefly in sterile phosphate-buffered saline (PBS). Samples for RNA or protein 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 the Mercy Health Human Research Ethics Committee.

Table 1
Patient clinical characteristics. Clinical details of the two cohorts from whom placentas were obtained for analysis. The preeclamptic cohort all had preeclampsia necessitating preterm delivery (<34 weeks gestation). Preterm controls were normotensive, delivered prematurely for indications other than preeclampsia of fetal growth restriction.

	Preterm ($n = 17$)	Preeclamptics ($n = 17$)
Maternal age median (range)	35 (20–43)	33 (19–42)
Gestation at delivery median (range)	29.6 (25–33.3)	29.6 (25.4–33)
BMI[†] (kg/m^2) median (range)	28.4 (19.5–32)	28 (21–39)
Parity No. (%)		
0	23.5%	52.32%
1	41.2%	35.32%
⩾2	35.3%	11.8%
Gravidity No. (%)		
Primiparous	5.9%	47%*
Multiparous	94.1%	53%
Highest SBP prior to delivery (mmHg) Median (range)	120 (105–140)	170 (140–200)****
Highest DBP prior to delivery (mmHg) Median (range)	70 (49–98)	98 (90–120)****
Birth weight (g) Median (range)	1395 (654–2450)	1051 (597–2345)

* $p < 0.05$.
**** $p < 0.0001$.
[†] Data on BMI (body mass index at the first pregnancy visit) available for 13 out of 17 preterm controls, 11 out of 17 preeclamptic patients. SBP = systolic blood pressure, DBP = diastolic blood pressure.

2.2. RT-PCR

RNA was extracted from placental tissue, primary trophoblast and human umbilical vein endothelial cells using the RNeasy mini kit (Qiagen, Valencia, CA) and quantified using a Nanodrop ND 1000 spectrophotometer (NanoDrop technologies Inc, Wilmington, DE). 1 μg of RNA was converted to cDNA using Applied Biosystems high capacity cDNA reverse transcriptase kit (Life technologies, Mulgrave, Australia) as per manufacturer guidelines.

Taqman gene expression assays for Akt, Endoglin, MMP14 and TIMP3 were used (Life Technologies). RT-PCR was performed on the CFX 384 (Biorad, Hercules, CA, USA) using FAM-labeled Taqman universal PCR mastermix (Life Technologies) with the following run conditions: 50 °C for 2 min; 95 °C for 10 min, 95 °C for 15 s, 60 °C for 1 min (40 cycles).

All mRNA data were normalized to an appropriate housekeeping gene (Topoisomerase 1 and cytochromes c-1 for placental tissue [17] or GAPDH for *in vitro* studies) as an internal control and calibrated against the average C_t of the control samples. The results were expressed as fold change relative to controls.

2.3. Western blot

20 μg of placental lysates ($n = 18$ PT and $n = 17$ PE) were separated on 10% polyacrylamide gels with wet transfer to PVDF membranes (Millipore, Billerica, MA). Membranes were blocked prior to blotting overnight with an antibody targeting Akt or pAkt (both 1:1000, Sigma, St Louis, MO, USA), or GAPDH (1:5000, Cell Signaling Technology, Danvers, MA, USA). Membranes were then visualized using an enhanced chemiluminescence detection system (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and ChemiDoc XRS (BioRad, Hercules, CA, USA). GAPDH was used as a loading control. Relative densitometry was determined in all samples using Image Lab (BioRad).