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Short Communication

MMP-14 Is Expressed in Preeclamptic Placentas and Mediates Release of Soluble Endoglin

Tu'uhevaha J. Kaitu'u-Lino,*† Kirsten R. Palmer,*† Clare L. Whitehead,*† Elizabeth Williams,‡ Martha Lappas,† and Stephen Tong*†

From the Translational Obstetrics Group, and the Department of Obstetrics and Gynaecology,† Mercy Hospital for Women, University of Melbourne, Heidelberg; and the Centre for Cancer Research,‡ Monash Institute of Medical Research, Monash University, Clayton, Australia*

Soluble endoglin is an anti-angiogenic protein that is released from the placenta and contributes to both maternal endothelial dysfunction and the clinical features of severe preeclampsia. The mechanism through which soluble endoglin is released from the placenta is currently unknown; however, recent work in colorectal cancer identified matrix metalloproteinase 14 (MMP-14) as the cleavage protease of endoglin. To determine whether this is also the mechanism responsible for soluble endoglin release in preeclampsia, we investigated the expression of MMP-14 within the placenta and the effects of its inhibition on soluble endoglin release. Placentas were obtained from severe, early onset preeclamptic pregnancies ($n = 8$) and gestationally matched preterm controls ($n = 8$). MMP-14 was predominately localized to the syncytiotrophoblast. Results from a proximity ligation assay showed protein interactions between endogenous MMP-14 and endoglin within the preeclamptic placenta. To demonstrate that this interaction produces soluble endoglin, we treated trophoblastic BeWo cells with either a broad-spectrum MMP inhibitor (GM6001) or MMP-14 siRNA. Both treatments produced a decrease in soluble endoglin ($P \leq 0.05$). Treatment of mice bearing BeWo xenografts with GM6001 decreased circulating soluble endoglin levels in mouse serum ($P \leq 0.05$). These findings indicate that MMP-14 is the likely cleavage protease of endoglin in the setting of preeclampsia. This approach provides a novel method for the development of potential therapeutics to reduce circulating soluble endoglin and ameliorate the clinical features of severe preeclampsia. ***(Am J Pathol 2012, 180:888–894; DOI: 10.1016/j.ajpath.2011.11.014)***

Preeclampsia affects 3% to 5% of pregnancies and is one of the leading causes of maternal and fetal mortality and morbidity worldwide.[1,2] It is characterized by endothelial dysfunction, causing hypertension and significant maternal multisystem end-organ injury.[1,3] Currently, the only definitive treatment is delivery. However, if the pregnancy is considerably preterm, this inflicts severe prematurity on the baby.

In recent years the anti-angiogenic factors soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng) have been identified as factors centrally responsible for the endothelial dysfunction seen in preeclampsia.[1,4,5] Indeed, both factors are increased in women with preeclampsia, with a dose-dependent relationship seen with increasing disease severity.[6,7] Furthermore, adenoviral coexpression of sEng and sFlt1 in rats produces a severe preeclamptic/hemolysis, elevated liver enzymes, and low platelets (HELLP syndrome) phenotype.[4]

sFlt-1 shares sequence similarity with the extracellular domain of the vascular endothelial growth factor receptor type 1 (Flt1). sFlt-1 antagonizes circulating vascular endothelial growth factor and placental growth factor, both pro-angiogenic factors required for mediating vessel health. sEng shares sequence homology with the extracellular domain of full-length membrane bound endoglin, a co-receptor for transforming growth factor-β, which is highly expressed on both endothelial cells and the syncytiotrophoblast.[8] sEng antagonizes the pro-angiogenic factors transforming growth factor-β1 and transforming growth factor-β3.[4]

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Address reprint request to Stephen Tong, Ph.D., Department of Obstetrics and Gynaecology, Mercy Hospital for Women, 163 Studley Rd., Heidelberg 3084, Victoria, Australia. E-mail: stong@unimelb.edu.au.

The identification of the role of sFlt1 and sEng in preeclampsia provides potential drug targets. Conceivably, neutralizing the biological activity of either or both factors could quench maternal disease, allowing pregnancies to advance to a later gestation when risks of prematurity for the baby are diminished.

sFlt1 is produced by alternative splicing of the Flt1 premRNA.[9] The splicing machinery generates a unique C-terminal amino acid sequence, providing a target to generate therapeutics that could selectively bind sFlt-1, but not Flt1.

In contrast, there is no obvious strategy to selectively neutralize circulating sEng because it is totally homologous with endoglin. Blocking release might be an alternative strategy, but the mechanism through which the placenta produces sEng is currently unknown. A recent study using an overexpression system in COS-7 cells found matrix metalloproteinase-14 (MMP-14, also known as MT1-MMP) cleaves membrane-bound endoglin at close proximity to the transmembrane domain to produce sEng.[10] If preeclamptic placentas produce sEng through the same mechanism, this interaction may represent a possible therapeutic target to prevent sEng production and ameliorate the clinical features of severe preeclampsia.

Here we investigated whether MMP-14 is the cleavage protease that mediates the release of sEng from preeclamptic placental tissue.

Materials and Methods

Tissue Collection

Women presenting to two tertiary women's hospitals in Melbourne, Australia, between 2008 and 2009 gave informed consent for placental tissue collection. Placenta was obtained from preterm pregnancies not complicated by preeclampsia ($n = 8$) and those complicated by severe early-onset preeclampsia ($n = 8$). The preterm cohort does not consist of normal pregnancies, although they do provide the most suitable gestationally age-matched controls. The medical records of these cases were reviewed to ensure no evidence of placental insufficiency (no head/abdominal fetal asymmetry and birthweight appropriate for gestation) or hypertensive disease. Severe preeclamptics were diagnosed in accordance with American Congress of Obstetricians and Gynecologists (ACOG) guidelines and included the presence of hypertension >160/110 on two occasions greater than 6 hours apart and any of the following: proteinuria >5 g/day, oliguria <500 mL/day, visual disturbance, pulmonary edema, right upper quadrant pain, abnormal liver function, thrombocytopenia, or fetal growth restriction.[11] Early-onset preeclampsia was defined as requiring delivery <34 weeks based on maternal indications. Preterm control placentas were selected from women presenting with preterm rupture of membranes or in spontaneous preterm labor without evidence of infection, hypertensive disease, or maternal comorbidities. Patient characteristics are outlined in Table 1.

Placental tissue was obtained immediately following delivery via Cesarean section from women who were not in labor. To standardize collection, the same investigator collected all samples. Placental tissue (excluding fetal membranes) was removed and washed briefly in sterile PBS. Samples for protein extraction were then frozen within 15 minutes 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 both Monash Medical Centre and Mercy Hospital for Women.

Table 1. Baseline Characteristics

	Preterm control ($n = 8$)	Preeclamptic ($n = 8$)
Maternal age mean years (±SEM)	31.0 (1.2)	32.6 (1.6)
Gestation at delivery mean weeks (±SEM)	30.9 (0.9)	32.2 (1.3)
Ethinicity No. (%)		
Caucasian	7 (87.5)	5 (62.5)
Asian	1 (12.5)	3 (37.5)
BMI (kg/m^2) mean (±SEM)	30.9 (1.7)	26.4 (1.7)
Primiparous n (%)	2 (25)	5 (62.5)
SBP at delivery mean mm Hg (±SEM)	121.0 (3.3)	172.1 (5.8)*
DBP at delivery mean mm Hg (±SEM)	73.4 (3.1)	110.7 (4.7)*
Birthweight mean grams (±SEM)	1622 (176.4)	1551 (268.3)

Student's *t*-test was used for continuous variables, and the chi square test was used for categorical variables.

*$P < 0.001$ were statistical comparisons made between preterm controls and preeclamptics.

BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; SEM, standard error of the mean.

Western Blot Analysis and ELISA

Placental lysates (20 μg) were separated on 10% polyacrylamide gels with wet transfer to polyvinylidene difluoride membranes (Millipore, Billerica, MA). Membranes were blocked before blotting overnight with antibodies targeting MMP-14 (1:1000; R&D Systems, Minneapolis, MN), endoglin (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA) or glyceraldehydes-3-phosphate dehydrogenase (1:5000; Cell Signaling Technology, Danvers, MA). Membranes were then visualized using an enhanced chemiluminescence detection system (Santa Cruz Biotechnology) and ChemiDoc XRS (BioRad, Hercules, CA). Glyceraldehydes-3-phosphate dehydrogenase was used as a loading control. Relative densitometry was determined using Image Lab (BioRad).

Soluble endoglin and human chorionic gonadotrophin levels were measured in conditioned culture media or murine serum using the human endoglin enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN) or Elegance hCG ELISA kit (Bioclone, Sydney, Australia), respectively. Both were performed according to the manufacturer's instructions. Optical density was determined using a BioRad X-Mark microplate spectrophotometer (BioRad) and endoglin or hCG levels determined using BioRad Microplate Manager 6 software.

Immunohistochemistry

Endoglin and MMP-14 immunhistochemistry was conducted on placental tissue collected from either pre-

eclamptic or preterm control pregnancies. Paraffin sections (5 μm) of formalin-fixed tissues were dewaxed in Xylene and rehydrated through descending grades of ethanol. Sections were then heated for 20 minutes on defrost in a 700-W microwave, followed by cooling to room temperature for 30 minutes. They were washed for 10 minutes in PBS pH 7.6, and immersed in 3% H_2O_2 in methanol for 10 minutes at room temperature. Sections were then washed with PBS before immersion into Dako blocking buffer for 10 minutes and incubated for 1 hour at room temperature with either rabbit polyclonal endoglin (Santa Cruz Biotechnology) at 4 μg/mL or mouse anti-human MMP-14 (R&D Systems) at 10 μg/mL in 1% bovine serum albumin/PBS. For isotype controls, primary antibody was substituted with either rabbit (endoglin) or mouse (MMP-14) IgG. The SuperPicTure kit (Invitrogen, Carlsbad, CA) was applied according to the manufacturer's instructions to reveal the endoglin and MMP-14 staining. Sections were lightly counterstained with Harris hematoxylin (Accustain; Sigma Diagnostics, Castle Hill, NSW, Australia), dehydrated, and mounted using DPX mounting medium (BDH Laboratory Supplies, Poole, England).

Proximity Ligation Assay

Placental tissue sections from both preeclamptic placenta and preterm control placenta were analyzed using the Duolink proximity ligation assay (Olink, Uppsala, Sweden), which allows identification of protein:protein interaction.

Proximity ligation assay was performed according to manufacturer's instructions. Briefly, sections were pretreated and primary antibodies for MMP-14 (R&D Systems) and endoglin (Santa Cruz Biotechnology) were applied as detailed for immunohistochemistry. For isotype controls, primary antibody was substituted with either rabbit (endoglin) or mouse (MMP-14) IgG. Sections were then washed for 2 × 5 minutes in Duolink wash buffer A (Olink, Uppsala, Sweden) before PLA PLUS and MINUS probes (Olink) were applied for 1 hour at 37°C. Following washing (as previously) a Ligation-Ligase solution (Olink) was applied to each sample for 30 minutes at 37°C. Sections were washed again and an amplification-polymerase solution (Olink, Uppsala, Sweden) applied for 100 minutes at 37°C. Sections were then washed for 2 × 10 minutes in 1 × Duolink wash buffer B (Olink, Uppsala, Sweden), and for 1 minute in 0.01 × Duolink wash buffer B before being allowed to air dry. Once dried sections were mounted with Duolink II Mounting Medium with DAPI (Olink, Uppsala, Sweden) before images were captured using the DeltaVision Deconvolution System (Applied Precision, Inc., Issaquah, WA).

Small Molecule MMP Inhibition (GM6001) in BeWo Cells

Following screening of numerous placental cell lines for soluble endoglin secretion (see Supplemental Figure S1A at *http://ajp.amjpathol.org*). The BeWo choriocarcinoma cell line was chosen as an appropriate *in vitro* model of placental cells. Cells were grown in 10% fetal calf serum in Dulbecco's modified Eagle medium: Nutrient Mixture F12 (DMEM/F12; Invitrogen). To induce syncytialization cells were treated with 40 μmol/L Forskolin (Sigma-Aldrich, St. Louis, MO) for 48 hours, before treatment with 50 μmol/L GM6001 (Millipore, MA), 10 μmol/L MMP2/9 inhibitor (Merck, Darmastadt, Germany), 8 nmol/L MMP13 inhibitor (Merck), or vehicle alone (dimethyl sulfoxide) for 48 hours. Conditioned culture media was collected for endoglin ELISA and protein lysates for Western blot. Experiments were repeated 3 times with a minimum of 3 replicates per treatment. BeWo syncytialization was confirmed by hCG ELISA (Bioclone, Sydney, Australia) (see Supplemental Figure S1B at *http://ajp.amjpathol.org*).

siRNA Knockdown of MMP-14 in BeWo Cells

Syncytialized BeWo cells were transfected with MMP-14 siRNA at 40 nmol/L (Qiagen, Valencia, CA), negative control siRNA at 40 nmol/L (Qiagen), or transfection reagent alone (Lipofectamine, Invitrogen) for 48 hours before conditioned culture medium was collected for endoglin ELISA. Cell lysates were collected for mRNA extraction. Experiments were repeated four times with a minimum of three replicates per treatment.

RT-PCR

RNA was extracted from BeWo lysates using RNeasy mini kit (Qiagen). RNA (0.2 μg) was then converted to cDNA using SuperScript III (Invitrogen) and random hexamers (Invitrogen), as per the manufacturer's guidelines. Taqman gene expression assays for MMP-14 and glyceraldehydes-3-phosphate dehydrogenase were used (Applied Biosystems, Carlsbad, CA). RT-PCR was performed on the CFX 384 (Biorad, Hercules, CA) using FAM-labeled Taqman universal PCR mastermix (Applied Biosystems) with the following run conditions: 50°C for 2 minutes; 95°C for 10 minutes; 95°C for 15 seconds, and 60°C for 1 minute (40 cycles). The PCR product was confirmed by gel electrophoresis. Relative quantification was determined using the comparative C_T method.

Administration of MMP Inhibitor GM6001 to Mice with BeWo Xenografts

Female nonobese diabetic/severe combined immunodeficiency mice of 6 to 10 weeks of age were housed in standard conditions with food and water provided *ad libitum* and a constant light cycle of 12 hours (lights on from 8:00 AM to 8:00 PM). Mice were given a subcutaneous injection of 10^6 BeWo cells on the right flank. Once tumors had reached a volume of 50 to 70 mm^3, GM6001 (100 mg/kg; $n = 9$) or vehicle control (10% dimethyl sulfoxide in PBS; $n = 9$) was administered intraperitoneally. Mice were left for 48 hours before being anesthetized with a xylazine (10 mg/kg)/ketamine (80 mg/kg) anesthetic and blood collected via cardiac puncture. The serum was analyzed for soluble endoglin by ELISA. Ethics approval for this project was granted by the Monash Medical Centre Animal Ethics Committee A.

Statistical Analysis

Continuous variables were compared using either an unpaired *t*-test to assess parametric data or a Mann Whitney *U* test for nonparametric data. Categorical values were compared using the χ^2 test. $P \leq 0.05$ was considered significant. All statistical analyses were undertaken using GraphPad Prism (GraphPad Software, La Jolla, CA).

Results

Endoglin Is Increased in Preeclamptic Placenta and Co-Localizes with MMP-14 to the Syncytiotrophoblast

Expression of endoglin and MMP-14 levels in placenta from women with severe early-onset preeclampsia and gestationally matched preterm controls were determined. Western analysis confirmed that both MMP-14 and endoglin were expressed in the placenta (Figure 1). There was an increased expression of endoglin ($P \leq 0.05$), but not MMP-14 in preeclamptic placentas compared to preterm controls.

Endoglin has been shown to be present on the syncytiotrophoblast, although the placental location of MMP-14 has not been described. For MMP-14 to mediate cleavage of endoglin to produce sEng, it should also be present on the syncytiotrophoblast. Indeed, immunohistochemistry confirmed that MMP-14 was localized predominantly to the syncytiotrophoblast, with mild staining also visualized in the villous stroma, in both preeclamptic and control placentas, as was endoglin (Figure 2, A–F).

MMP-14 and Endoglin Interact on the Surface of the Syncytiotrophoblast

Next we examined whether there was protein-protein interaction between endogenous MMP-14 and endoglin *in situ* using the proximity ligation assay. This technique uses modified secondary antibodies that bind the Fc portion of two different primary antibodies that target candidate proteins of interest. These secondary antibodies are linked to a system producing oligonucleotides by rolling nucleotide amplification if they are in close proximity. Probes are then added fluorescing any oligonucleotides present. Thus, each fluorescent signal represents a point of endogenous protein:protein interaction.

Analysis of both preterm control and preeclamptic placenta by proximity ligation assay and deconvolution microscopy indicated that endogenous MMP-14 and endoglin interact mainly on the syncytiotrophoblast surface (Figure 2, G–I). This finding demonstrates that the interaction between MMP-14 and endoglin is occurring on the placental surface directly abutting the maternal vasculature, possibly promoting release of sEng directly into the maternal circulation.

MMP Inhibition Decreases Soluble Endoglin Release from Placental Cells in Vitro

Having shown endogenous interaction in the placenta, we investigated whether inhibiting MMP-14 decreases sEng *in vitro*. We first screened a panel of seven placental cell lines to see which released sEng and could be used as an *in vitro* model. Syncytialized BeWo cells produced the highest amounts of sEng by far (see Supplemental Figure S1 at *http://ajp.amjpathol.org*). This is of further importance, as BeWo is also the placental cell line that most accurately models the syncytiotrophoblast, supporting the contention that sEng is released from this placental cell layer.

We then administered a broad-spectrum matrix metalloproteinase (MMP) inhibitor (GM6001) to syncytialized BeWo cells. GM6001 caused a significant decrease (Figure 3A) ($P \leq 0.05$) in sEng release. Given GM6001 also inhibits other MMPs besides MMP-14, we administered MMP-13 or MMP-2/9 specific inhibitors. These are other major MMPs found in placenta that are also inhibited by GM6001. None

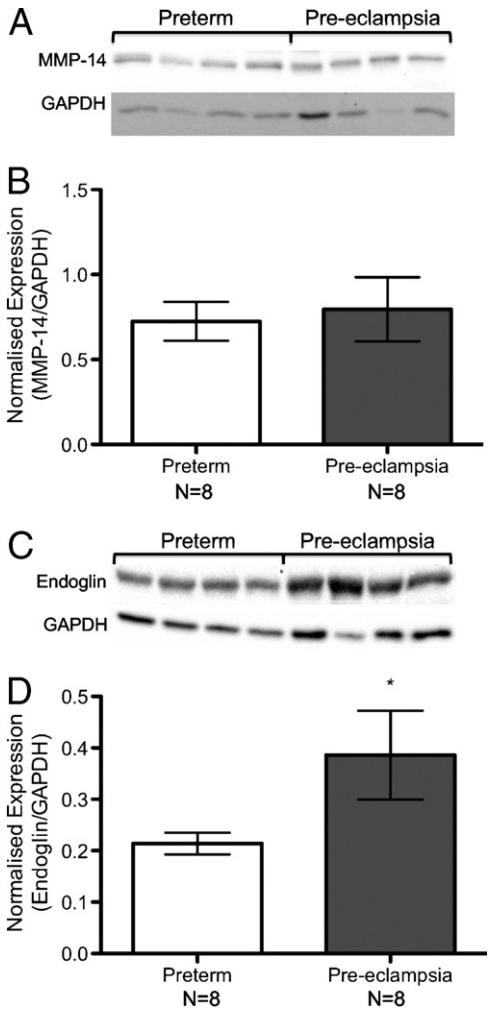

Figure 1. Matrix metalloproteinase (MMP)-14 and endoglin expression in preeclamptic compared to preterm control placenta. Representative Western blots and quantitation for MMP-14 (**A**, **B**) and endoglin (**C**, **D**). Expression levels were confirmed by densitometry (**B**, **D**). Endoglin expression was significantly increased in preeclamptic placentas ($n = 8$) compared to preterm controls ($n = 8$). $^*P \leq 0.05$.

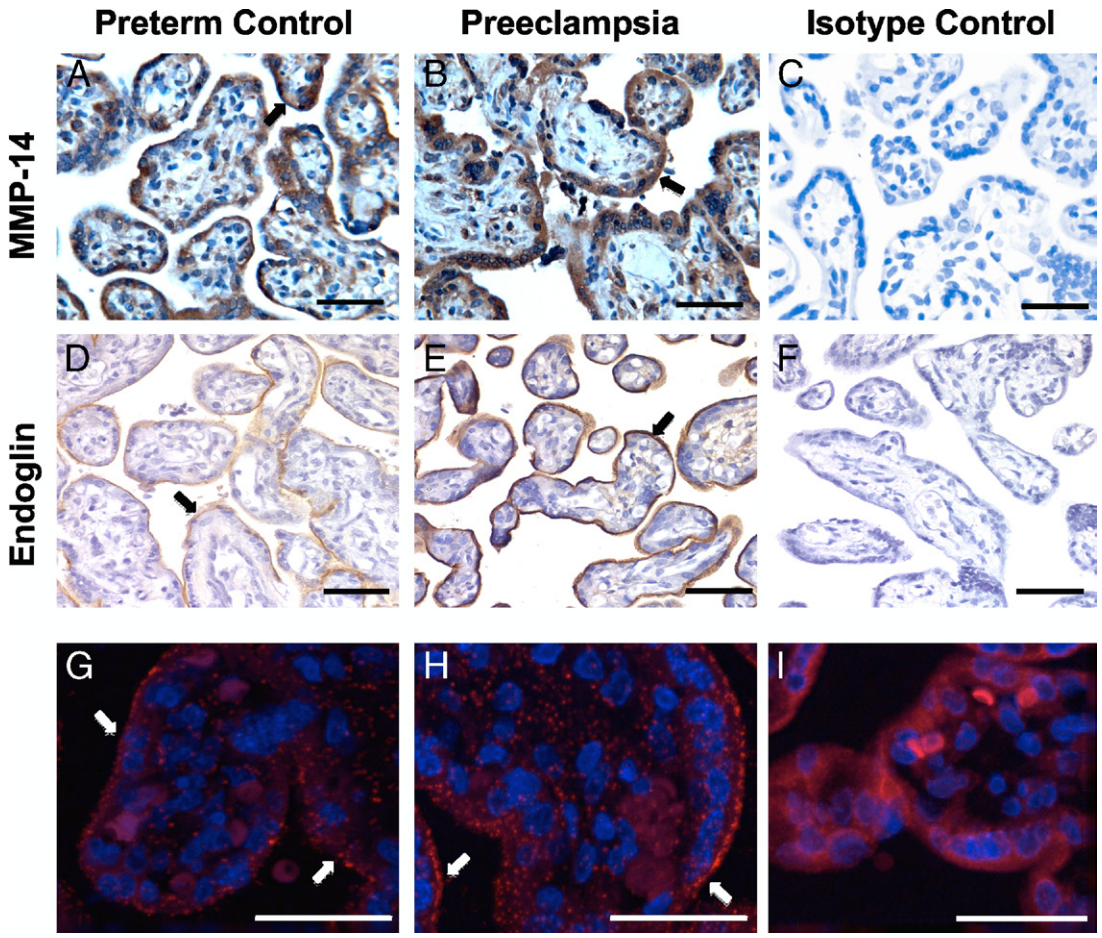

Figure 2. Matrix metalloproteinase (MMP)-14 and endoglin are expressed on, and interact within the syncytiotrophoblast. Immunohistochemistry for MMP-14 (**A**, **B**) and endoglin (**D**, **E**) shows the presence of both proteins on the syncytiotrophoblast layer of placentas from both normotensive preterm controls (**A**, **D**), and preeclamptic placentas (**B**, **E**) (**black arrows**). Isotype controls for MMP-14 (**C**) and endoglin (**F**) are also shown. All placentas were counterstained with H&E. Scale bars: 20 um. Proximity ligation assay was used to demonstrate direct protein:protein interaction between MMP-14 and endoglin on the surface of the syncytiotrophoblast of preterm (**G**) and preeclamptic (**H**) placentas (**white arrows**). Each red dot represents a point of protein:protein interaction. Nuclear staining (DAPI) is shown in blue. Isotype controls (**I**) have no red dots representing protein:protein interaction. Scale bars: 40 μm.

of these decreased sEng (Figure 3A), suggesting the effects of GM6001 were not mediated through these other MMPs.

To further verify that placental sEng release was mediated through MMP-14, we examined whether MMP-14 knockdown inhibited sEng release. In a long experimental protocol requiring prior syncytialization of BeWo cells before siRNA transfection, our most optimized method induced 68% knockdown of MMP-14 mRNA ($P < 0.0001$) (see Supplemental Figure S2 at *http://ajp.amjpathol.org*). BeWo cells treated with MMP-14 siRNA showed a significant reduction in sEng production compared to scrambled siRNA treated cells (Figure 3B) ($P \leq 0.05$). This finding confirms that MMP-14 inhibition reduces sEng release from placental cells *in vitro*.

GM6001, a Small Molecule MMP Inhibitor, Decreases Soluble Endoglin Release in Vivo

Finally, we tested the ability of systemically administered GM6001 to inhibit sEng release from human placental cells *in vivo*. We established a xenograft model using nonobese diabetic/severe combined immunodeficiency mice where we injected 10^6 BeWo cells subcutaneously. In initial experiments establishing the model, we found significant amounts of serum sEng in mice sacrificed once palpable tumors were present (approximately 10 days after injection). In addition, there were significant levels of serum human chorionic gonadotrophin, suggesting BeWo xenografts spontaneously syncytialize.

Using this BeWo xenograft model, we systemically administered GM6001 or vehicle alone once tumors were palpable, then sacrificed the mice after 48 hours, and measured serum sEng. Mice given GM6001 had significantly decreased circulating sEng levels by 58% compared to controls (Figure 3C) ($P \leq 0.05$). Thus, we conclude administration of GM6001 inhibits sEng from BeWo cells *in vivo*. These findings, in combination with our *in vitro* experiments, strongly indicate that sEng release is mediated through the actions of MMP-14.

Discussion

Here we show that MMP-14 indeed appears to be the cleaving protease of endoglin within the placenta. Our

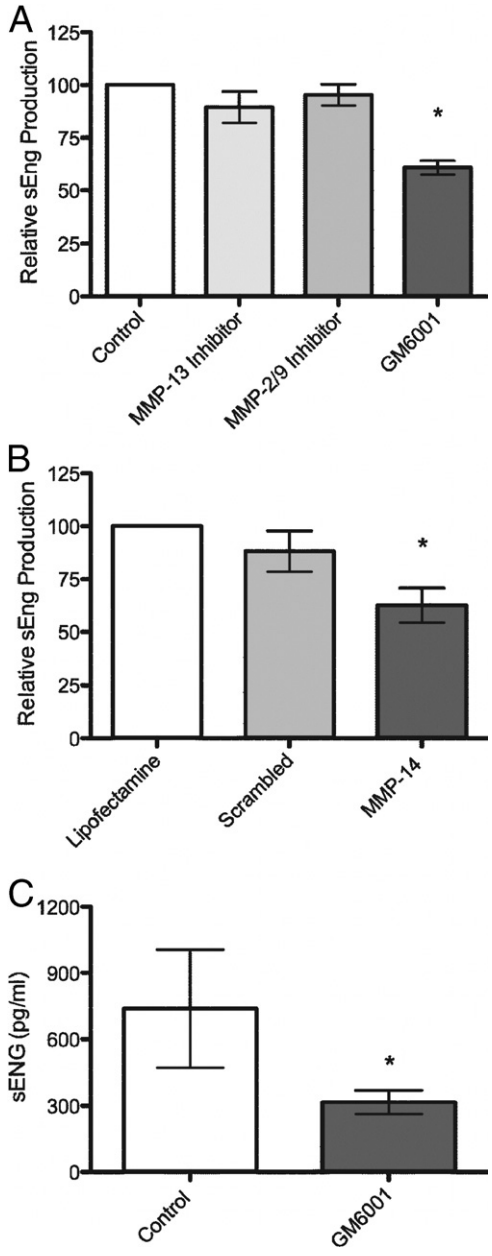

Figure 3. Matrix metalloproteinase (MMP)-14 inhibition inhibits soluble endoglin release *in vitro* and *in vivo*. (**A**) Treatment of syncytialized BeWo cells *in vitro* with the broad-spectrum MMP inhibitor GM6001 significantly decreased soluble endoglin release compared to controls, MMP-13 and MMP-2/9 specific inhibitors (*$P \leq 0.05$) (GM6001 versus all other treatments). **B:** Treatment of syncytialized BeWo cells with MMP-14 siRNA also decreased release of soluble endoglin into the media compared to controls or scrambled siRNAs (*$P \leq 0.05$ versus all other treatments). Results shown in (**A**) and (**B**) are those from three and four combined experiments, respectively, with each experiment done at least in triplicates. Data are expressed as a percentage change relative to control to correct for differences between experiments. **C:** Systemic treatment of nonobese diabetic/severe combined immunodeficiency mice xenografted with BeWo cells using GM6001 significantly decreased serum endoglin levels compared to vehicle controls ($n = 9$ per group) (*$P = 0.05$).

findings indicate that endoglin is significantly upregulated in the preeclamptic placenta with expression colocalizing with MMP-14 predominately in the syncytiotrophoblast. It is likely that the cleavage of endoglin by MMP-14 within this placental layer, which forms the maternal-fetal interface, releases the extracellular domain into the maternal circulation as sEng. This occurs within the normal placenta, although the increased expression of endoglin is likely responsible for the significantly elevated levels of sEng were clinically seen in women with severe preeclampsia.

There is now a body of evidence from *in vivo* animal studies and humans suggesting sEng has a key role in the maternal end-organ damage that occurs in severe preeclampsia. Yet the final molecular step in the placenta that produces sEng has not been experimentally elucidated until now. Cudmore et al[12] recently published a comprehensive set of experiments in endothelial cells showing increased Akt decreased sEng release, whereas phosphatase and tensin homolog (the upstream inhibitor of Akt) increased sEng release. Heme oxygenase-1 also decreased sEng release mediated through Akt. Reporting important insights into the signaling mechanisms behind sEng release, however, they did not map the pathway to final sEng cleavage.

In this work we demonstrated that MMP-14 mediates sEng production through the use of two novel techniques. The proximity ligation assay was used to demonstrate endogenous endoglin and MMP-14 interaction *in situ* on the syncytiotrophoblast surface. Proximity ligation assay has distinct advantages over other existing techniques to show protein-protein interaction. Co-immunoprecipitation or mass spectroscopy methods do not allow simultaneous visualization of cellular location and fluorescence resonance energy transfer technology requires protein overexpression. Secondly, we developed a mouse xenograft model using human-derived BeWo cells to show systemic administration of MMP inhibitors reduced serum sEng.

The siRNA experiments were challenging, given we had to first syncytialize the BeWo cells (adding forskolin/cAMP to induce cellular fusion), transfect siRNAs, and harvest the media 48 hours later. Both transfection reagents and forskolin are damaging to cells and BeWos were inherently difficult to transfect. Using our most optimized protocol, we achieved a 68% mRNA MMP-14 knockdown, which significantly decreased sEng production by 26%. It is unclear whether further decreases in sEng production could be achieved with greater MMP-14 silencing. Alternatively, it is also possible that a second unidentified mechanism also contributes to sEng production independent of MMP-14. Nevertheless, our results from the two complementary approaches of small molecule and siRNA inhibition provide good evidence that MMP-14 mediates sEng production in placental tissue.

Given high sEng levels correlate with the severe end of the preeclamptic spectrum (HELLP syndrome), we believe identification of its cleavage mechanism represents a significant step forward in the possibility of developing targeted therapeutics for this disease. Importantly, the cleavage probably occurs on the cell surface, which is far more accessible than intracellular targets. Notably, a monoclonal antibody that inhibits MMP-14 has already been developed for therapeutic purposes and is being used in clinical trials to treat breast carcinoma.[13] Alternatively, developing agents that specifically block the interaction between MMP-14 and endoglin may be a

strategy that would not require general systemic MMP-14 inhibition.

A therapeutic agent that decreases circulating sEng could potentially decrease the substantial maternal and fetal morbidity and mortality inflicted by severe pre-eclampsia. Given sEng is also present but at lower levels in normal pregnancies, it may only be necessary to decrease levels of sEng, but not completely abrogate production, to meaningfully quench clinical disease.

Acknowledgments

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