European Heart Journal (2012) **33**, 1150–1158
doi:10.1093/eurheartj/ehr065

BASIC SCIENCE

Loss of Akt activity increases circulating soluble endoglin release in preeclampsia: identification of inter-dependency between Akt-1 and heme oxygenase-1

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Received 3 December 2010; revised 21 January 2011; accepted 31 January 2011; online publish-ahead-of-print 16 March 2011

Aims Endothelial dysfunction is a hallmark of preeclampsia. Desensitization of the phosphoinositide 3-kinase (PI3K)/Akt pathway underlies endothelial dysfunction and haeme oxygenase-1 (HO-1) is decreased in preeclampsia. To identify therapeutic targets, we sought to assess whether these two regulators act to suppress soluble endoglin (sEng), an antagonist of transforming growth factor-β (TGF-β) signalling, which is known to be elevated in preeclampsia.

Methods and results Vascular endothelial growth factor-A (VEGF-A), fibroblast growth factor (FGF-2), angiopoietin-1 (Ang-1), and insulin, which all activate the PI3K/Akt pathway, inhibited the release of sEng from endothelial cells. Inhibition of the PI3K/Akt pathway, by overexpression of phosphatase and tensin homolog (PTEN) or a dominant-negative isoform of Akt (Akt_{dn}) induced sEng release from endothelial cells and prevented the inhibitory effect of VEGF-A. Conversely, overexpression of a constitutively active Akt (Akt^{myr}) inhibited PTEN and cytokine-induced sEng release. Systemic delivery of Akt^{myr} to mice significantly reduced circulating sEng, whereas Akt_{dn} promoted sEng release. Phosphorylation of Akt was reduced in preeclamptic placenta and this correlated with the elevated level of circulating sEng. Knock-down of Akt using siRNA prevented HO-1-mediated inhibition of sEng release and reduced HO-1 expression. Furthermore, HO-1 null mice have reduced phosphorylated Akt in their organs and overexpression of Akt^{myr} failed to suppress the elevated levels of sEng detected in HO-1 null mice, indicating that HO-1 is required for the Akt-mediated inhibition of sEng.

Conclusion The loss of PI3K/Akt and/or HO-1 activity promotes sEng release and positive manipulation of these pathways offers a strategy to circumvent endothelial dysfunction.

Keywords Endothelium • Soluble endoglin • HO-1 • PI3K/Akt • HO-1 • Preeclampsia

Introduction

Neutralization of transforming growth factor (TGF)-β leads to endothelial dysfunction characterized by impaired endothelium-mediated vasodilatation and elevated expression of surface adhesion molecules, resulting in increased leucocyte adhesion.[1] Endoglin (CD105), a transmembrane co-receptor for TGF-β1 and TGF-β3, is predominantly expressed by activated, proliferating

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endothelium during angiogenesis[2,3] and regulates the activity of endothelial nitric oxide synthase (eNOS).[4,5] Proteolytic cleavage of the extracellular domain of endoglin gives rise to soluble endoglin (sEng), which functions to neutralize TGF-β signalling.[6]

It has been shown that an increase in circulating sEng has direct, significant, negative effects on endothelial health *in vivo*.[1] Soluble endoglin was also shown to abrogate *in vitro* tube formation, prevent TGF-β1 induction of eNOS phosphorylation, and abolish activation of TGF-β1-mediated Smad 2/3-dependent luciferase reporter activity.[6] In addition, sEng was shown to enhance lung and liver microvascular permeability, cause focal endotheliosis in kidney glomeruli, and block TGF-β-induced rat arterial vasodilation.[6] High levels of plasma sEng have been associated with vascular disorders, such as systemic sclerosis,[7] atherosclerosis,[8] familial hypertension,[7] malaria,[9] and most notably preeclampsia;[10] a novel risk factor for cardiovascular disease in women.[11] Recently, sEng was implicated as a likely cause of the reduced number of regulatory T cells observed in the systemic circulation of preeclamptic women.[12] In addition, sEng was shown to act synergistically with soluble Flt-1 (sFlt-1), the natural antagonist of vascular endothelial growth factor (VEGF), to induce maternal endothelial dysfunction and severe preeclampsia in animal studies.[6]

Haeme oxygenase-1 (HO-1) is an inducible, cytoprotective, and anti-inflammatory enzyme. It is widely acknowledged to provide a defence against oxidant damage[13,14] and to be protective against ischaemia-reperfusion injury.[15–18] Haeme oxygenase-1 null mice have systemic endothelial damage and have greatly elevated circulating sEng.[19] Haeme oxygenase-1 inhibits sEng release, from the placenta and the endothelium, mediated by proinflammatory cytokines, such as, tumour necrosis factor (TNF-α) and interferon-γ (IFN-γ).[19] Significantly, a recent publication showed that the angiotensin receptor agonistic auto-antibody stimulates sEng, *in vivo*, by upregulation of TNF-α and this upregulation can be prevented by induction of HO-1 using haemin[20] confirming our earlier study.

Cellular systems employ a number of endogenous protective mechanisms to defend against cell damage and death. The phosphoinositide 3-kinase (PI3K)/Akt and HO pathways are two important examples. Activation of the PI3K/Akt pathway is crucial for endothelial cell homeostasis and survival after vascular injury.[21] Numerous growth factors, including VEGF-A,[22] basic fibroblast growth factor (FGF-2),[23] angiopoietin-1 (Ang-1),[24] and insulin,[25] exert their protective effect via activation of the PI3K/Akt pathway.

To date, the mechanism responsible for sEng release has not been addressed. In this study, we sought to understand the mechanistic regulation of sEng release and investigated the involvement of two central regulators of vascular homeostasis; the PI3K/Akt and inducible HO-1 pathways.

Methods

Reagents and antibodies

Recombinant VEGF and FGF-2 were purchased from RELIA*Tech* (Brauschweig, Germany). Angiopoietin-1 was purchased from R&D Systems (Abingdon, UK). Monoclonal antibody, anti-PTEN (A2B1) and polyclonal antibodies, anti-Endoglin (C-term), anti-Endoglin (N-term) were from Autogen Bioclear Ltd (Wiltshire, UK). Monoclonal antibody, anti-HO-1 was purchased from Abcam (UK). Intracellular signalling protein antibodies anti-Akt and anti-phospho-Akt (ser 473) antibodies were purchased from New England Biolabs Ltd (Hertfordshire, UK). Polyclonal rabbit anti-HO-1 antibody was purchased from StressGen Biotechnologies Corporation (Canada). Human TNF-α and IFN-γ, monoclonal anti-β-actin, insulin, and all other cell culture reagents and chemicals purchased from Sigma-Aldrich Company Ltd (Dorset, UK).

Soluble endoglin ELISA

Soluble endoglin was measured in culture supernatants using the commercial ELISA kits according to manufacturer's instructions (R&D Systems, UK).

Cell culture

Human umbilical vein endothelial cells (HUVECs) were isolated, characterized, and cultured as previously described.[26] Experiments were performed on third or fourth passage cells. Human umbilical vein endothelial cells were stimulated with VEGF (20 ng/mL), TNF-α (10 ng/mL), or IFN-γ (10 ng/mL) and media collected and assayed for sEng by ELISA.

Adenoviral gene transfer

Recombinant, replication-deficient adenoviruses directing the expression of wild-type (WT) human PTEN (AdPTEN), catalytically inactive human PTEN ($AdPTEN_{dn}$), dominant-negative Akt (Thr308 to Ala and Ser473 to Ala, $AdAkt_{dn}$), and constitutively active, myristoylated Akt ($AdAkt^{myr}$) were generously provided by Dr Christopher Kontos (Duke University, USA) and AdCMV (empty vector used for control infections) adenoviruses were amplified in HEK-293A cells and purified using the BD Adeno-X^{TM} purification kit (BD Biosciences). Viral titres were estimated by using the BD Adeno-X^{TM} rapid titer kit. Human umbilical vein endothelial cells were infected by incubation with adenovirus in M199 containing 5% fetal calf serum (FCS) overnight at 37°C prior to addition of stimulants or vehicle control for up to 24 h. Optimal multiplicity of infection for the adenoviruses was determined by western blotting. The recombinant, replication-deficient adenovirus encoding rat HO-1 (AdHO-1) was used as described previously.[27]

siRNA transfection

Human umbilical vein endothelial cells were trypsinized and ~1×10^6 cells electroporated with ~3 μg of HO-1,[28] Akt-1, or control siRNA using the HUVEC kit II and Nucleofector (Amaxa GmbH, Cologne, Germany) as described previously.[29]

Quantitative real-time PCR

Sample preparation and real-time PCR was performed as described previously.[29] Briefly, mRNA was prepared using TRIzol and DNase-1 digestion/purification on RNAeasy columns (Qiagen), and reverse transcribed with the cDNA Synthesis Kit (Promega). Triplicate cDNA samples and standards were amplified in SensiMix containing SYBR green (Quantace) with primers specific for endoglin (Forward: GTC-TCA-CTT-CAT-GCC-TCC-AGC-T; Reverse: GG-CTG-TCC-ATG-TTG-AGG-CAG-T) or β-actin. The mean threshold cycle (C_T) for HO-1 was normalized to β-actin and expressed relative to control.

Western blotting

Proteins were extracted from HUVEC with RIPA buffer and subjected to SDS–PAGE on 10% gels, transferred to nitrocellulose membranes (Amersham-Pharmacia, UK). Membranes were incubated with

appropriate antibodies at 4°C overnight. Antibody reactions were detected using the ECL detection kit (Amersham-Pharmacia, UK). Ratios of protein expression to loading control were determined by densitometry using ImageJ software.

Placental tissue and serum collection and preparation

Institutional Ethics Committee approved the tissue and serum collection and written informed consent was obtained. Eligible cases were singleton pregnancies with a diagnosis of preeclampsia. Preeclampsia as diagnosed if a previously normotensive woman had two repeat (4 h apart) diastolic blood pressure measurements of $\geq$90 mmHg after week 20 of gestation, together with proteinuria of $>$300 mg in a 24-h urine specimen or 2+ protein dipsticks in two repeat measurements (4 h apart). Human placental tissue and serum were obtained from normal pregnancies and gestationally matched pregnancies complicated by preeclampsia.

Animals

All procedures and animal care were approved by Institutional Ethics Committees and were in accordance with UK Home Office licensing regulations. C57/Bl6J animals with targeted deletion of the HO-1 gene by neomycin resistance gene insertion[30,31] were supplied by Prof Anupam Agarwal (University of Alabama, Birmingham, USA) and rederived in accordance with local regulations. Mice were injected in the tail vein with AdAkt^{myr} (5×10^9 pfu), AdAkt$_{dn}$ or control Adβgal. Five days post-injection blood was harvested by cardiac puncture and organs collected for histology, western blotting, and liver explant culture.

Ex vivo liver explant culture

Mice were sacrificed and their livers excised and cut into 1 mm^2 pieces. Six to ten pieces of liver were equilibrated for 4 h in phenol red-free DMEM containing 5% FCS in 24-well plate. Medium was changed to fresh phenol red-free DMEM containing 5% FCS and after 24 h conditioned medium was collected and stored at and liver explants were collected and stored at −80°C prior to assay for sEng by ELISA. The explant protein was also harvested and protein content assayed.

Statistical analysis

All data are expressed as the mean (±SEM). Statistical comparisons were performed using one-way ANOVA followed by the Student–

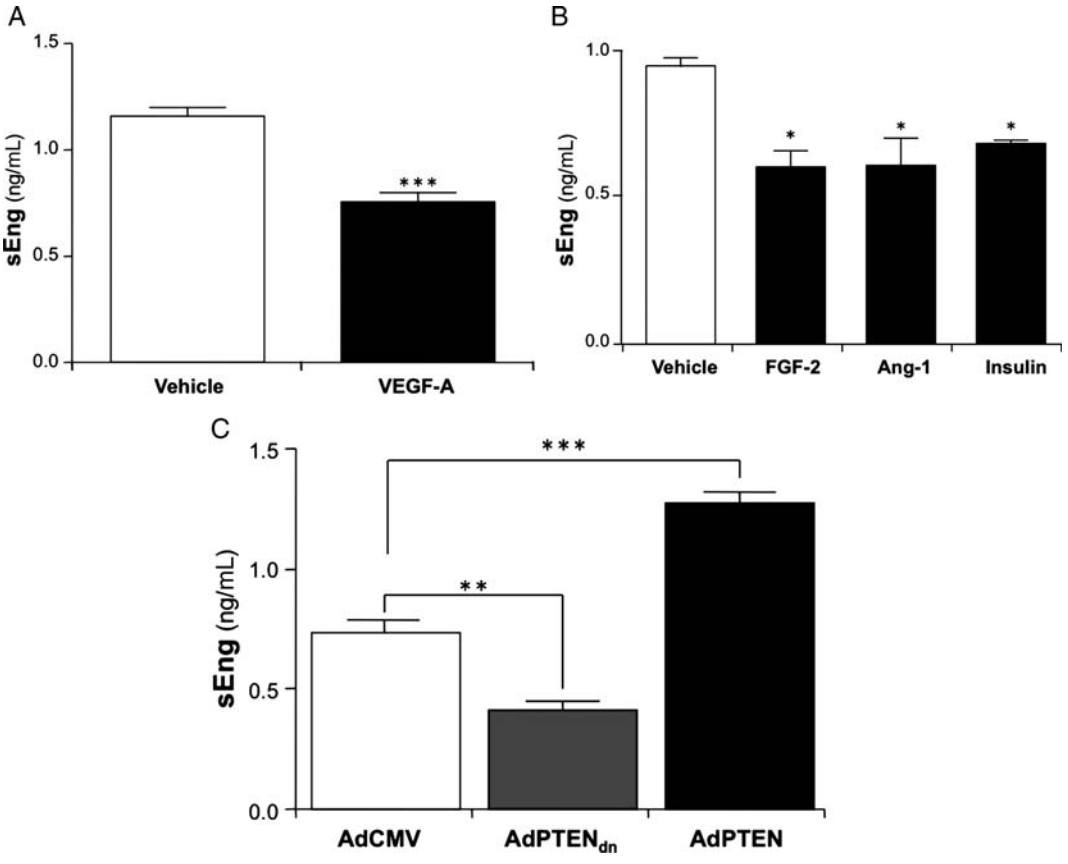

Figure 1 Survival factors and Akt activation repress soluble endoglin release from endothelial cells. All experiments were conducted using confluent human umbilical vein endothelial cells. Cells were incubated with (*A*) VEGF-A (20 ng/mL), (*B*) FGF-2 (20 ng/mL); angiopoieitn-1 (Ang-1;400 ng/mL), or insulin (200 nmol/L). (*C*) Human umbilical vein endothelial cells infected with adenoviruses encoding PTEN (AdPTEN), a dominant-negative PTEN mutant (AdPTEN$_{dn}$) or empty vector (AdCMV). All experiments were conducted in M199/5%FCS for 24 h and cell supernatants collected for soluble endoglin (sEng) quantification by ELISA. All results are the mean (±SEM) of three experiments performed in triplicate ($n = 9$). ***$P < 0.001$, *$P < 0.05$ vs. vehicle. **$P < 0.01$ and ***$P < 0.001$ vs. AdCMV.

Newman–Keuls test as appropriate. Statistical significance was set at a value of $P < 0.05$.

Results

Soluble endoglin release is suppressed by survival factors via activation of the phosphoinositide 3-kinase/Akt pathway in endothelial cells

To assess the impact of pro-survival factors on sEng release in a model system, isolated endothelial cells were incubated with VEGF-A, FGF-2, Ang-1, and insulin, which all activate the PI3K/Akt signalling.[22–25] These factors reduced the release of sEng from endothelial cells (*Figure 1A* and *B*) suggesting that vascular protection reduces shedding of endothelial membrane-bound endoglin. To examine whether the PI3K/Akt pathway regulates endoglin shedding, HUVEC were infected with adenoviruses encoding phosphatase and tensin homolog (PTEN), the phosphatase that inhibits PI3K signalling (AdPTEN) or inactive PTEN ($AdPTEN_{dn}$)[32] (see *Figures 3C* and *5A* for overexpression). $AdPTEN_{dn}$, which potentiates the PI3K pathway, thus activating Akt, significantly decreased the release of sEng (*Figure 1C*), whereas overexpression of PTEN, which depletes the cell of phosphatidylinositol 3,4,5-trisphosphate, the substrate required for Akt activation, induced a two-fold increase in sEng release (*Figure 1C*).

Inhibition of Akt activity using an adenovirus encoding a dominant-negative Akt construct, ($Ad\text{-}Akt_{dn}$)[33] increased endoglin mRNA expression in HUVEC (*Figure 2A*) and prevented VEGF-A-mediated repression of sEng release (*Figure 2B*). However, when $AdAkt_{dn}$ was co-infected with $AdPTEN_{dn}$, the ability of $AdPTEN_{dn}$ to inhibit sEng release was lost (*Figure 2C*) suggesting that PI3K is acting via Akt to modulate sEng release. Tail vein injection of $AdAkt_{dn}$ into mice resulted in increased Akt expression in liver tissue after six days (*Figure 2D* inset). Liver explants established from $AdAkt_{dn}$ infected mice showed increased secretion of sEng (*Figure 2D*). These data demonstrate that inhibition of the survival protein, Akt, *in vitro* and *in vivo*, augments the levels of cleaved endoglin released from cells.

To further investigate this phenomenon, we went on to examine whether positive modulation of Akt, could suppress sEng release. Interestingly, overexpression of constitutively active myristilated Akt (Akt^{myr}) did not inhibit endoglin mRNA expression, nor did it inhibit basal sEng release from HUVEC (*Figure 3A* and *B*). However, when co-expressed with PTEN, Akt^{myr} completely abrogated PTEN-mediated upregulation of endoglin mRNA

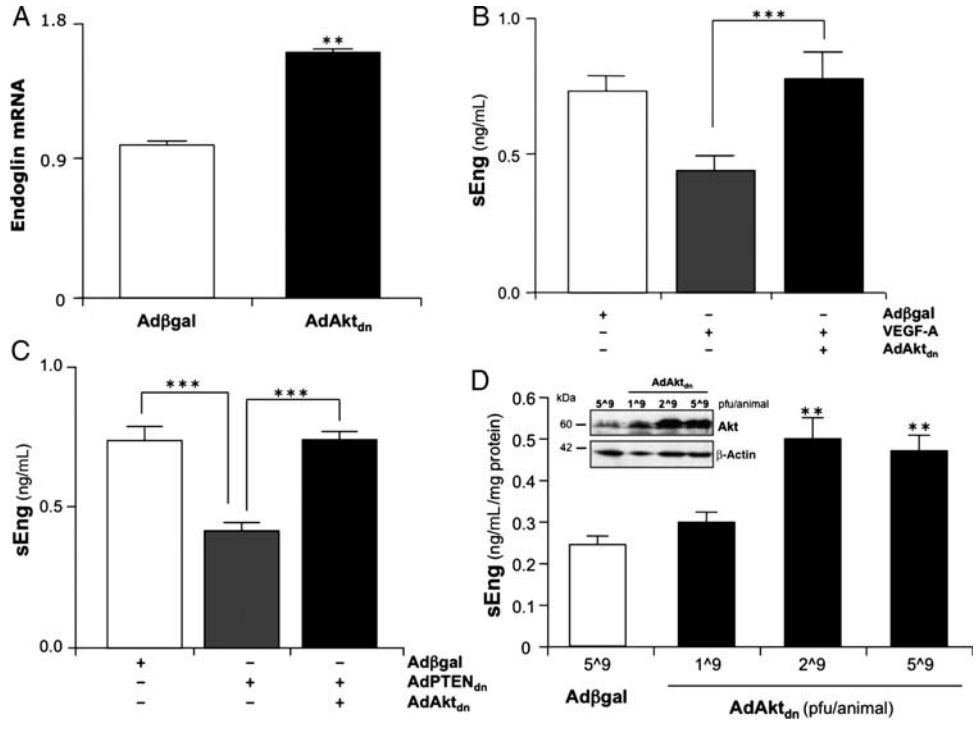

Figure 2 Akt inhibition induces the release of soluble endoglin. (*A*) Relative endoglin mRNA levels in human umbilical vein endothelial cells after infection with an adenovirus encoding dominant-negative Akt ($AdAkt_{dn}$) or β-galactoside control adenovirus (Adβgal). $^{**}P < 0.01$ vs. Adβgal. Soluble endoglin (sEng) level from Akt_{dn} overexpressing human umbilical vein endothelial cells (*B*) stimulated with VEGF-A (20 ng/mL) or (*C*) co-infected with $AdPTEN_{dn}$.(*D*) C57/B6J mice were injected i.v. with $AdAkt_{dn}$ or Adβgal and 5 days later blood and organs analysed. Soluble endoglin levels in conditioned medium of liver explants from mice at 24 h. Soluble endoglin release was normalized to total protein content of the explants and soluble endoglin quantified by ELISA. All results are the mean (±SEM) of three experiments performed in triplicate ($n = 9$). $^{**}P < 0.01$ vs. Adβgal. Inset—immunoblot of mouse liver lysates for Akt.

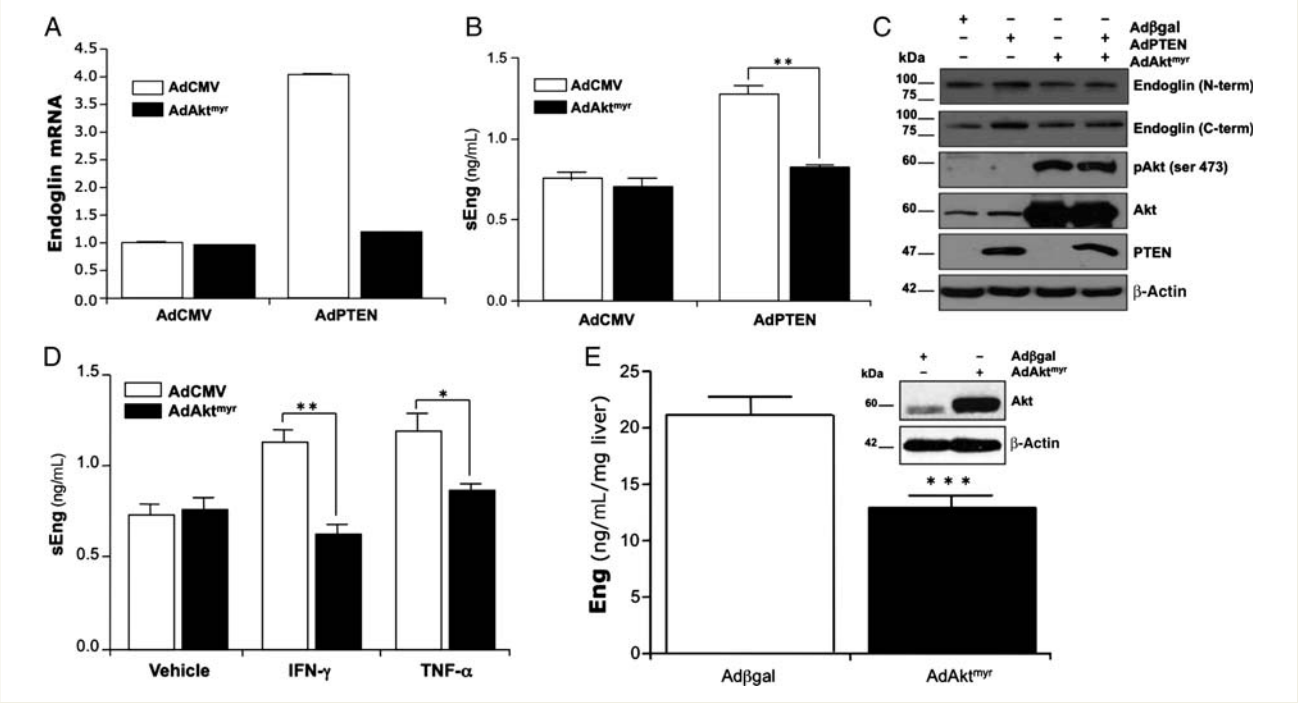

Figure 3 Akt activation inhibits the release of soluble endoglin. (*A*) Relative endoglin mRNA and (*B*) soluble endoglin protein levels after human umbilical vein endothelial cells were infected with AdCMV, AdPTEN, and/or myristylated Akt (AdAkt^{myr}). (*C*) Immunoblot with antibodies against; endoglin (N-terminus), endoglin (C-terminus), phosphorylated Akt [pAkt (ser 473)], Akt, PTEN, and β-actin. (*D*) Akt^{myr} overexpressing cells incubated with interferon-γ (IFN-γ;10 ng/mL) or tumour necrosis factor-α (TNF-α; 50 ng/mL). All experiments (unless stated otherwise) were conducted in M199/5%FCS for 24 h and cell supernatants collected for soluble endoglin quantification by ELISA. All results are the mean (±SEM) of three experiments performed in triplicate ($n = 9$). *$P < 0.05$, **$P < 0.01$. Mice were injected i.v. with AdAkt^{myr} or Adβgal and 5 days later blood and organs analysed. (*E*) Endoglin levels in liver from mice at 24 h. Endoglin was normalized to total protein and quantified by ELISA. All results are the mean (±SEM) of three experiments performed in triplicate ($n = 9$). ***$P < 0.001$ vs. Adβgal.

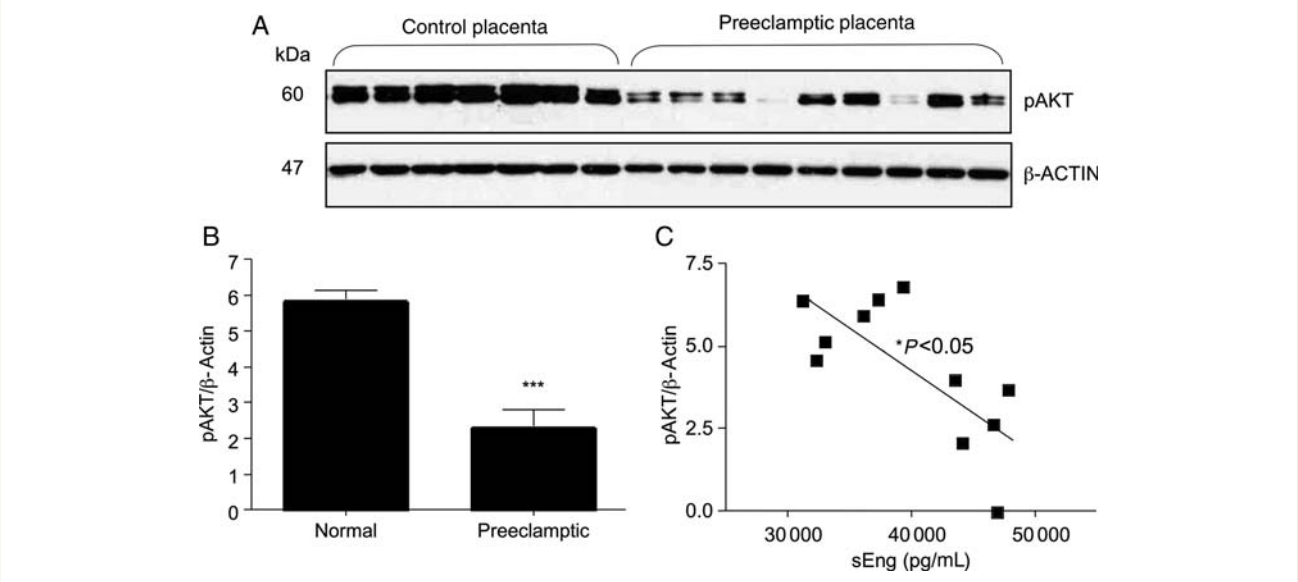

Figure 4 Phosphorylation of Akt is decreased in preeclamptic placenta and correlates inversely with soluble endoglin. (*A*) Lysates of placenta from normal or preeclamptic pregnancies were immunoblotted with antibodies against phosphorylated Akt (pAkt-ser 473) and β-actin. (*B*) Densitometric analysis showing ration of pAkt:β-actin in A. (*C*) Correlation between plasma soluble endoglin and pAkt:β-actin ratio of placenta. *$P < 0.05$.

(*Figure 3A*) and release of the soluble protein (*Figure 3B*). Human umbilical vein endothelial cell lysates immunoblotted for the N and C termini of endoglin confirmed the upregulation of endoglin following PTEN overexpression and Akt^{myr}, which increased the level of phosphorylated Akt, inhibited this upregulation (*Figure 3C*). Consistent with these findings, the pro-inflammatory cytokine-mediated release of sEng was inhibited by Akt^{myr} (*Figure 3D*) and systemic administration of $AdAkt^{myr}$ to mice resulted in decreased endoglin protein in liver tissues compared with control animals (*Figure 3E*).

Akt is reduced in the preeclamptic placenta and inversely correlates with maternal soluble endoglin

Preeclampsia is characterized by widespread endothelial cell dysfunction and the progressive elevation of circulating sEng. Western blot analyses of placental lysates demonstrated that phosphorylation of Akt was significantly reduced in preeclamptic placenta compared with placenta from normal pregnancies (*Figure 4A* and *B*). Furthermore, the level of placental Akt phosphorylation inversely correlated with maternal plasma sEng levels (*Figure 4C*). Thus, the rise in circulating sEng paralleled the fall in Akt activity.

Haeme oxygenase-1 suppresses soluble endoglin release via Akt

We previously demonstrated that HO reduces the release of sEng under basal and cytokine-stimulated conditions.[19] This has been recently confirmed in a study showing that TNF-α-induced sEng release from endothelial cells and placental explants could be abrogated by upregulation of HO activity by haemin.[20] Interestingly, activation of Akt by overexpression of $AdPTEN_{dn}$, which induced Akt phosphorylation, also upregulated HO-1 protein in endothelial cells (*Figure 5A*). Knockdown of Akt1, using siRNA, significantly induced sEng release (*Figure 5B*) and more importantly, prevented the inhibition of sEng caused by overexpression of HO-1 (*Figure 5B*). In addition, HO-1 expression in HUVEC was also prevented by siRNA-mediated knockdown of Akt1 (*Figure 5C*). We previously demonstrated that knockdown of HO-1, using siRNA, induced sEng release,[19] here we show that knockdown of HO-1 potentiates the IFN-γ and TNF-α-induced sEng release (*Figure 5D*). In addition, overexpression of Akt^{myr} could not prevent the upregulation of sEng after loss of HO-1 (*Figure 6A*), suggesting that HO-1 and Akt regulate sEng release interdependently.

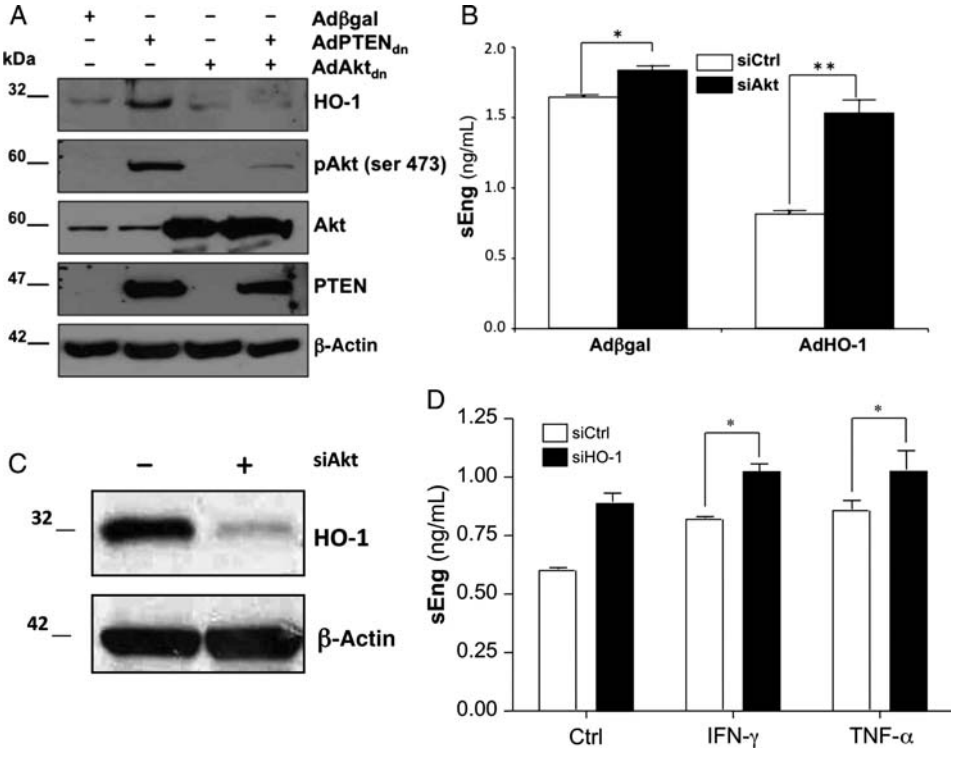

Figure 5 Haeme oxygenase-1 requires Akt to inhibit soluble endoglin release. (*A*) Lysates from human umbilical vein endothelial cells infected with AdCMV, $AdPTEN_{dn}$, and/$AdAkt_{dn}$ were immunoblotted with antibodies against phosphorylated Akt [pAkt (ser 473)], Akt, PTEN, HO-1, and β-actin. (*B*) Knockdown of Akt1 (siAkt) in human umbilical vein endothelial cells overexpressing HO-1 or βgal. (*C*) Immunoblot of Akt siRNA treated human umbilical vein endothelial cells for HO-1 and β-actin. (*D*) Knockdown of HO-1 (siHO-1) in human umbilical vein endothelial cells and stimulation with interferon-γ (10 ng/mL) or tumour necrosis factor-α (TNF-α; 50 ng/mL). All experiments were conducted in M199/5%FCS for 24 h and cell supernatants collected for soluble endoglin (sEng) quantification by ELISA. All results are the mean (±SEM) of three experiments performed in triplicate ($n = 9$). *$P < 0.05$, **$P < 0.01$.

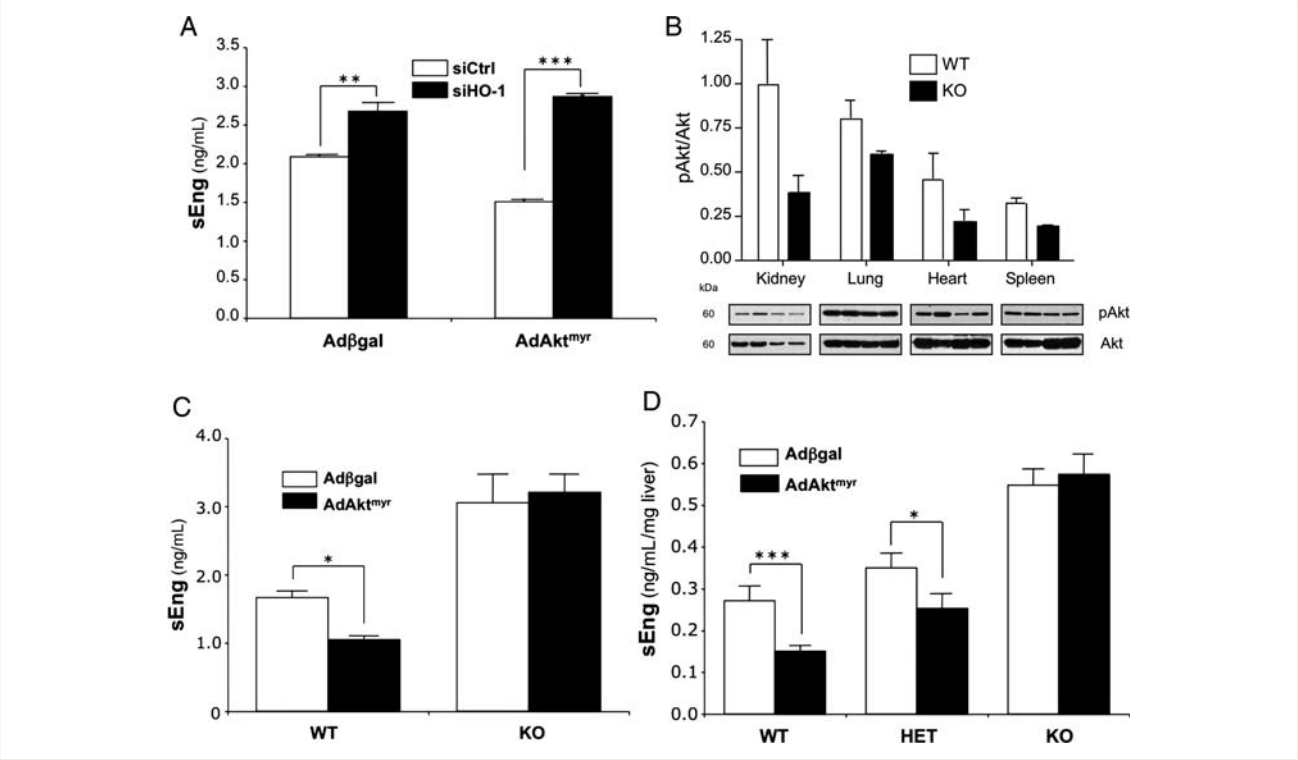

Figure 6 Akt requires haeme oxygenase-1 to inhibit soluble endoglin release. (*A*) Soluble endoglin (sEng) release from human umbilical vein endothelial cells after knockdown of haeme oxygenase-1 (siHO-1), and control (siCtrl) in human umbilical vein endothelial cells overexpressing Akt^{myr} or βgal. (*B*) pAkt levels in organs of haeme oxygenase-1 wild-type and haeme oxygenase-1-deficient mice. Mice were injected i.v. with $AdAkt^{myr}$ or Adβgal and 5 days later blood and organs analysed. (*B*) Plasma soluble endoglin levels in wild-type and haeme oxygenase-1-deficient mice. (*C*) Soluble endoglin levels in conditioned medium of liver explants from haeme oxygenase-1 wild-type, heterozygous, and haeme oxygenase-1-deficient mice at 24 h. In explant studies, soluble endoglin release was normalized to total protein content of the explants and soluble endoglin quantified by ELISA. All results are the mean (±SEM) of three experiments performed in triplicate ($n = 9$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

HO-1-null mice exhibit elevated circulating sEng[19] and western blotting of organs from HO-1 null mice showed a decrease in phosphorylation of Akt (*Figure 6B*). Furthermore, Akt^{myr} overexpression in WT animals resulted in reduced circulating sEng (*Figure 6C*). Moreover, Akt^{myr} overexpression failed to suppress the circulating levels of sEng in HO-1 null mice (*Figure 6C*). To investigate this further, liver explants were established from adenoviral-infected mice, cultured for 24 h and supernatants assayed for sEng. Explants from $AdAkt^{myr}$ infected WT and HO-1 heterozygous mice, produced significantly less sEng compared with Adβgal-infected controls (*Figure 6D*). Liver explants from HO-1 null mice released significantly more sEng than WT and heterozygous mice and consistent with our *in vitro* studies, overexpression of $AdAkt^{myr}$ had no effect on sEng release from liver explants in HO-1 null mice (*Figure 6D*) demonstrating that HO-1 and Akt play pivotal, interdependent roles in suppressing the release of sEng *in vivo*.

Discussion

Serum sFlt-1 and sEng are increased in pregnant women prior to the clinical symptoms of preeclampsia.[10] Inhibition of VEGF or TGF-β signalling by high circulating sEng activates the endothelium to promote vascular dysfunction.[1,6] The salient finding highlighted by this study is the identification of PI3K/Akt signalling, in concert with HO-1, as a central negative regulator of endoglin shedding *in vivo*. The significance of this finding is reinforced by the observation that Akt phophorylation is decreased in the preeclamptic placenta and inversely correlates with the maternal circulating levels of sEng. In addition, survival factors that exert their protective effects via Akt, such as VEGF-A, FGF-2, Ang-1, and insulin, all negatively regulate sEng release from endothelial cells. Our finding that VEGF suppresses sEng release from endothelial cells suggests that in preeclampsia, the increase in placental sEng[6,19] is likely to be further compounded by the loss of VEGF-A activity due to the concomitant rise in its antagonist, sFlt-1, in the maternal circulation.

Knockdown of HO-2 reduces Akt phosphorylation *in vivo*[34] and carbon monoxide, the gaseous product of HO, stimulates Akt phosphorylation in hepatocytes[35] and endothelial cells[36] supporting a positive feedback loop between the HO and PI3K/Akt pathways. In this regard, it is important to remember that the loss of HO activity may be a causative factor in preeclampsia, as HO-1 protects against TNF-α-induced placental damage[37] and

suppresses cytokine-mediated sEng and sFlt-1 release.[19] The most compelling evidence for this comes from a recent study using faetal placental cells from women at 11 weeks gestation. Farina *et al.*[38] showed that the expression of HO-1 mRNA decreased in chorionic villous samples (faetal cells) from women who went on to develop preeclampsia. This very early decrease in HO-1 could explain, at least in part, the elevated levels of anti-angiogenic factors seen later in pregnancy in preeclamptic women. Transforming growth factor-β1 stimulates HO-1 expression via the PI3K/Akt pathway in human lung epithelial cells.[39] Thus, loss of TGF-β1 signalling, due to the rise in sEng in preeclampsia, may further compromise maternal endothelial HO activity. Our data show that PI3K/Akt activation is decreased in the organs of HO-1 null mice and that increased PI3K/Akt activation induces HO-1 expression in endothelial cells and loss of such a positive feedback system may lead to greater loss of endothelial integrity under conditions of high circulating sEng, observed in a number of vascular disorders. The predominant upstream regulator of HO-1 expression is Nuclear factor-like 2 (Nrf-2). Nuclear accumulation of Nrf-2 and HO-1 expression was shown to be PI3K-dependent and MEK-MAPK independent in the endothelium.[40] The interdependency between PI3K/Akt and HO-1 identified in this study needs further investigation to determine whether they are regulated at the level of Nrf-2.

It has been shown that an increase in circulating sEng has direct, significant, negative effects on endothelial health *in vivo*.[1] It abrogates TGF-β-mediated signalling, enhances lung and liver microvascular permeability, causes focal endotheliosis in kidney glomeruli and blocks TGF-β1-induced vasodilation.[6] The involvement of sEng in a number of wide-ranging pathologies demonstrates that sEng is not only marker of endothelial integrity but also a contributing factor of endothelial dysfunction. Our discovery of a co-dependency between HO-1 and Akt in relation to sEng release implies that dysfunction of only one of these factors in the endothelium may explain the resultant increase in sEng in these disorders. Interestingly, both TNFα and sEng are elevated in malaria[41] and the level correlates with disease severity,[9] whereas HO-1 and CO protect against malaria progression.[42]

In conclusion, the discovery that PI3K/Akt and HO-1 provide key co-dependent and inhibitory signals required to suppress sEng release strongly suggests that the positive manipulation of PI3K/Akt and/or HO pathways would provide potential therapeutic targets in preventing excessive sEng release in vascular disorders including preeclampsia.

Funding

This work was supported by grants from the Medical Research Council (G0601295 and G0700288) and British Heart Foundation (RG/09/001/25940). Funding to pay the Open Access publication charges for this article was provided by Medical Research Council.

Conflict of interest: none declared.

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