Angiotensin II Inhibits Human Trophoblast Invasion through AT1 Receptor Activation*

Yang Xia, Hong Y. Wen, and Rodney E. Kellems‡

From The Department of Biochemistry and Molecular Biology, The University of Texas Medical School, Houston, Texas 77030

Trophoblast implantation depends, in part, on the controlled production of plasmin from plasminogen, a process regulated by plasminogen activators and plasminogen activator inhibitors. We have determined that angiotensin II (Ang II) stimulates plasminogen activator inhibitor-1 (PAI-1) synthesis and secretion in human trophoblasts in a time- and concentration-dependent manner. Our results indicate that Ang II activates PAI-1 gene expression through the AT1 receptor and involves the calcium-dependent activation of calcineurin and the nuclear translocation of NFAT. Increased PAI-1 synthesis and secretion is associated with reduced trophoblast invasion as judged by an in vitro invasion assay. These studies are the first to link the renin-angiotensin system with the fibrinolytic system to regulate trophoblast invasion.

The fibrinolytic system is best known for its role in the regulated digestion of fibrin clots. A key component of this system is plasminogen, an inactive zymogen, which is converted into the active protease, plasmin, by the action of a plasminogen activator termed tissue-type plasminogen activator (tPA). The ability of tPA to activate plasminogen is substantially enhanced by its association with the substrate, fibrin (1). Plasminogen can also be activated (i.e. converted to plasmin) by the action of another plasminogen activator, urokinase-type plasminogen activator (uPA) (2–6). In contrast to tPA, uPA is not associated with fibrin clots but instead is often associated with a specific cell surface receptor (uPAR) (7), which is thought to play a key role in cell migration and invasion (8). In this role, uPA is synthesized as an inactive single-chain proenzyme that can be stored or secreted. Secreted uPA is cleaved into the active two-chain molecule upon binding to uPAR on the surface of cells. Following activation, receptor-bound uPA is capable of converting plasminogen into plasmin, the latter of which is able to degrade several key components of the extracellular matrix (ECM) (9). It is widely accepted that uPA initiates a cascade of proteolysis at the cell surface, which in turn leads to the degradation of the ECM, thereby promoting cellular migration. It is now clear that the uPA-regulated aspect of the fibrinolytic system plays a key role in mediating ECM degradation and cell invasion. Plasminogen activator activity is controlled by plasminogen activator inhibitors (PAIs) of which PAI-1 is the predominant physiological inhibitor (1, 10, 11). PAI-1 is an ECM glycoprotein that is capable of inhibiting both free and receptor-bound uPA through the formation of an irreversible covalent complex (12). In this way PAI-1 is believed to control cell migration and invasion in tumor growth and angiogenesis (13–16).

Numerous studies suggest that PAI-1 production by human trophoblasts plays an important role in trophoblast invasion and placental development (17, 18). The human hemochorial placenta is a highly invasive structure in which a subpopulation of placental trophoblast cells invades the uterus, its underlying stroma, and local blood vessels to achieve a physiological union between maternal and fetal circulatory systems (19). Plasminogen activators and plasminogen activator inhibitors are believed to regulate trophoblast invasion of the uterus. Key players in this process include uPA, PAI-1, and PAI-2 that together govern the production of plasmin from circulating plasminogen (20–24). Recent studies suggest that the renin-angiotensin system (RAS) exerts an important role in the regulation of PAI-1 gene expression in human mesangial cells (25–28), vascular smooth muscle cells (29, 30), and rat aortic and cardiac cells (31). Renin, angiotensinogen, angiotensin-converting enzyme, and angiotensin receptors are all present in the human placenta, suggesting that angiotensin II (Ang II) synthesized in the placenta may serve as a local modulator of placental function (32, 33). Although it is clear that PAI-1 is elevated during normal pregnancy and even higher in pre-eclampsia (34–36), the direct correlation of the RAS with PAI-1 production during human pregnancy is not well studied. We show here that Ang II stimulates PAI-1 synthesis and secretion in human trophoblasts in a time- and concentration-dependent manner. Our results indicate that Ang II activates PAI-1 gene expression through the AT1 receptor and involves the calcium-dependent activation of the phosphatase, calcineurin, and the nuclear translocation of the transcription factor, nuclear factor of activated T cells (NFAT). We show that increased PAI-1 synthesis and secretion result in reduced trophoblast invasion as judged by an in vitro Matrigel invasion assay. These studies are the first to link the renin-angiotensin system with the fibrinolytic system to regulate trophoblast invasion.

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† To whom correspondence should be addressed: Dept. of Biochemistry and Molecular Biology, University of Texas Medical School, 6431 Fannin, Houston, TX 77030, Tel.: 713-500-6124; Fax: 713-500-0652; E-mail: Rodney.E.Kellems@uth.tmc.edu.
‡ The abbreviations used are: tPA, tissue plasminogen activator; uPA, urokinase plasminogen activator; uPAR, urokinase plasminogen activator receptor; Ang II, angiotensin II; RAS, renin-angiotensin system; PAI-1, plasminogen activator inhibitor-1; PAI-2, plasminogen activator inhibitor-2; ECM, extracellular cellular matrix; CSA, cyclosporin A; NFAT, nuclear factor of activated T cells; PKC, protein kinase c; PLC, phospholipase C; ELISA, enzyme-linked immunosorbent assay; IP3, inositol 1,4,5-triphosphate, TGF-β, transforming growth factor-β; GFP, green fluorescence protein; CMV, cytomegalovirus; PBS, phosphate-buffered saline.
EXPERIMENTAL PROCEDURES

Materials—Tissue culture medium, fetal bovine serum, antibiotic-antimycotic (100×), Superscript II RNase H reverse transcriptase, Platinum Taq DNA polymerase, and a total RNA isolation kit were purchased from Invitrogen (Grand Island, NY). The PAI-1 enzyme-linked immunosorbent assay (ELISA) kit was obtained from American Diagnostic, Inc. (Greenwich, CT). The nylon membranes used in RNA transfer were purchased from Bio-Rad Laboratories (Hercules, CA). Radiolabeled RNA probes were obtained from ICN Radiochemicals (Irvine, CA). BD Biocat Matrigel invasion chambers and control inserts were obtained from Becton Dickinson Labware (Bedford, MA). Ang II and bovine calf serum were obtained from Sigma Chemical Co. (St. Louis, MO). Polyclonal antibodies specific for human PAI-1, AT1, and β-actin were obtained from Santa Cruz Biotechnologies (Santa Cruz, CA). Losartan and prazosin were obtained from Research Bio-Bridge (Hoehne, Rheinheyn (Rahway, NJ)). The expression constructs pCMV-NFAT and pCMV-ΔNFAT plasmids were digested by AgeI and NotI to release the GFP fragment. The correct size fragments of CMV-ΔNFAT and CMV-NFAT were isolated and purified from the gel, blunted-ended, and self-ligated as described before (35).

Results—Preparation of Plasmids for Transfection—Plasmids used to express NFAT-GFP and ΔNFAT-GFP were constructed in pEGFP-C1 (CLON-TECH, Palo Alto, CA) and were generous gifts from Dr. R. Sanders (Duke University, Durham, NC). The expression constructs pCMV-NFAT and pCMV-ΔNFAT Transient Transfection—HTR-8/SVneo transfected cells were cultured and transfected with pCMV-NFAT and pCMV-ΔNFAT plasmid (2 μg) in the absence of Ang II. The pCMVlacZ plasmid (2 μg) was used as an internal control to monitor transfection efficiency. After 48 h, the cellular extract was isolated, and Western blot analysis was performed to detect PAI-1 protein production using rabbit polyclonal anti human PAI-1 antibody as described before (35).

NFAT-GFP Transient Transfection and Microscopic Techniques—For cells expressing the NFAT-GFP fusion protein, HTR-8/SVneo human trophoblasts were cultured on laminin-coated (10 μg) glass coverslips inserted into the multiwell dishes. After 24 h cells were transfected with 2 μg of NFAT-GFP construct and 6 h later washed twice with PBS. Half of the cells were treated by 100 nm Ang II for 48 h, and the other half were maintained under the same conditions in the absence of Ang II. After 48 h all cells were washed with 1× PBS, and the cells on the coverslips were viewed using an Olympus BX 60 fluorescence microscope equipped with darkfield optics with a green filter and photographed using a SPOT digital camera (Diagnostic Instruments Inc., Sterling Height, MI).

Matrigel Transwell Invasion Assay—Invasion of HTR-8/SVneo was measured with a Matrigel Invasion Chamber (Becton Dickinson Labware, Bedford, MA) as described before (40). Briefly, confluent cells were harvested with 0.25% trypsin and 0.2% EDTA then centrifuged at 1000 × g for 10 min. Cells were washed with 1× phosphate-buffered saline (PBS) once and resuspended in RPMI 1640 containing 0.5% bovine serum albumin. The lower compartment of the invasion chamber was filled with RPMI 1640 containing 5% fetal bovine serum as chemotactic. The lower compartment was overlaid with an 8-μm pore size polyethylene phthalate membrane precoated with Matrigel basement membrane matrix at 125 μg/ml. The cells (2.5 × 10^5) per well plate were loaded in the upper compartment of the prehydrated Matrigel-coated invasion chambers in the absence or presence of Ang II. The control insert chambers contained 8-μm pore size polyethylene phthalate membrane without being precoated with Matrigel basement membrane were seeded at the same concentration of cells and treated the same as the invasion chamber. Percent invasion is expressed as the percent invasion through the Matrigel matrix-coated membrane relative to cell migration through the control membrane. The upper surface of the filter was scraped with moist cotton swabs to remove Matrigel and non-migrated cells. Then both the control insert membranes, which were not precoated with Matrigel, and the Matrigel precoated insert membrane were stained with Diff-Quick stain (Biochemical Sciences Inc., Swedesboro, NJ) for 3 min. The membranes were washed three times with water, and the cells in the membranes were viewed by using an Olympus BX 60 fluorescence microscope equipped with brightfield optics and photographed using a SPOT digital camera (Diagnostic Instruments Inc., Sterling Height, MI). In some experiments, losartan (1 μM), CSA (1 μM), and human anti PAI-1 antibody (1:100) were added to the cells at the initiation of Ang II treatment.

Statistics—All values are expressed as mean ± S.E. Data were analyzed for statistical significance using GraphPad Prism software. Statistical significance was determined by analysis of variance test. A value of p < 0.05 was interpreted to mean that observed experimental differences were statistically significant.

RESULTS

PAI-1 mRNA Abundance Is Increased by Ang II in Human Trophoblast Cells—Because of the potentially important role of PAI-1 in regulating trophoblast invasion, it is important to identify signal transduction pathways regulating PAI-1 gene expression. Realizing that the maternal-fetal interface has a locally active renin-angiotensin system (32, 41) and that PAI-1 gene expression is regulated by Ang II in other systems (25, 28, 29) we conducted experiments to determine if the abundance of PAI-1 mRNA is induced by Ang II in human trophoblasts. For
the abundance of PAI-1 in human trophoblasts. These cells were incubated with various concentrations of Ang II (A) or with 100 nM Ang II for various times (B) as indicated. PAI-1 mRNA abundance was determined by Northern blot hybridization using a PAI-1 cDNA probe. A and B (upper panels): Total RNA was isolated from cells treated with various concentrations of Ang II or with 100 nM Ang II for various times (B) as indicated. PAI-1 mRNA abundance was determined by Northern blot hybridization using a PAI-1 cDNA probe. A and B (upper panels): Total RNA was isolated from cells treated with various concentrations of Ang II or with 100 nM Ang II for various times (B) as indicated. PAI-1 mRNA abundance was determined by Northern blot hybridization using a PAI-1 cDNA probe. A and B (lower panels): Densitometric analysis of three Northern blot analyses was performed. The results are shown as PAI-1/28 S rRNA optical density ratios. Data are expressed as mean ± S.E. *p < 0.05 versus control.

PAI-1 protein presumably exerts its physiological effects following secretion from cells where it is associated with plasminogen activators such as uPA (42). To determine the effect of Ang II on the secretion of PAI-1, we determined the concentration of PAI-1 in cell culture medium following 24 h of incubation with 100 nM Ang II. The concentration of PAI-1 in cell culture medium was determined using an enzyme-linked immunosorbent assay (ELISA) specific for human PAI-1. The results (Fig. 2C) show that the concentration of PAI-1 in cell culture fluid increased 2.5-fold in response to 24-h incubation with 100 nM Ang II. These findings show that trophoblasts secrete increased amounts of PAI-1 in response to Ang II treatment.

Ang II Acts through AT1 Receptors on Human Trophoblasts—To determine if induction of PAI-1 production is through the AT1 receptor on HTR-8/SVneo cells, losartan, an AT1 receptor antagonist, was used to treat the cells. Treatment with losartan (1 μM) completely prevented PAI-1 mRNA abundance (Fig. 3A), protein synthesis (Fig. 3B), and protein secretion (Fig. 3C) in response to treatment with 100 nM Ang II. These results indicate that HTR-8/SVneo cells possess the AT1 receptor and that PAI-1 mRNA abundance and protein synthesis and secretion are enhanced as a result of AT1 receptor activation.

Ang II-mediated Induction of PAI-1 Gene Expression and Protein Secretion Is Inhibited by CSA—Intracellular signaling downstream of AT1 receptor activation in cardiomyocytes involves calcium mobilization and the activation of calcineurin, a calcium/calmodulin-dependent cytoplasmic phosphatase (43). To determine if calcineurin is involved in AT1 receptor signaling in human trophoblasts we used the calcineurin inhibitor, CSA. The effect of CSA on Ang II-induced stimulation of PAI-1 gene expression and protein secretion was determined. PAI-1 gene expression was assessed by Northern analysis to determine the abundance of PAI-1 mRNA and by Western analysis to determine the abundance of cellular PAI-1 protein. PAI-1 secretion was quantified by the use of an ELISA to determine the concentration of PAI-1 in cellular fluid following incubation with Ang II. The results show that Ang II induction of PAI-1 mRNA abundance (Fig. 4A), PAI-1 protein abundance (Fig. 4B), and PAI-1 protein secretion (Fig. 4C) were consistently inhibited by about 45–55% in the presence of 1 μM CSA. These results indicate that the induction of PAI-1 gene expression by...
cells were treated with 100 nM Ang II for 24 h, and secreted PAI-1 in serum-free medium was measured using an enzyme-linked immunosorbent assay (ELISA), which specifically detects human PAI-1. Data are expressed as the mean ± S.E. *, p < 0.05 versus control; **, p < 0.05 versus Ang II treatment. The results are compiled from three independent experiments.

**Fig. 3.** Losartan blocks Ang II induction of PAI-1 gene expression. A (right panel): PAI-1 mRNA abundance in control and Ang II-treated cells with/without losartan was determined by Northern blot hybridization using a PAI-1 cDNA probe. A (left panel): Densitometric analysis of two Northern blots was performed. The results are shown as PAI-1/28S rRNA optical density ratios. The results are expressed as the mean ± S.E. *, p < 0.05 versus control; **, p < 0.05 versus Ang II treatment. B (right panel): The abundance of PAI-1 protein and β-actin was determined by Western blot analysis using polyclonal antibody directed against human PAI-1 and β-actin, respectively. B (left panel): Densitometric analysis of three Western blots was performed. The results are shown as PAI-1/β-actin protein optical density ratios. The results are expressed as the mean ± S.E. *, p < 0.05 versus control; **, p < 0.05 versus Ang II treatment. C. secreted PAI-1 in serum-free medium was measured using an enzyme-linked immunosorbent assay (ELISA), which specifically detects human PAI-1. Data are expressed as the mean ± S.E. *, p < 0.05 versus control; **, p < 0.05 versus Ang II treatment. The results are compiled from three independent experiments.

NFAT did not activate PAI-1 gene expression and did not result in an increase in PAI-1 protein concentration. However, transfection of the constitutively active NFATc did result in increased PAI-1 protein levels (Fig. 5A). These results provide strong evidence that NFAT transcriptional activators are involved in activation of PAI-1 gene expression.

Based on the results presented above, we propose that Ang II treatment results in dephosphorylation of NFAT by following its translocation to the nucleus. To test this hypothesis, we transfected an expression construct encoding an NFAT-GFP fusion protein into control and Ang II-treated human trophoblast cells. After 48 h, cells were washed with phosphate-buffered saline and viewed by fluorescence microscopy to determine the cellular localization of green fluorescence. In control cultures, green fluorescence was cytoplasmic and was excluded from nuclei (Fig. 5B). However, in Ang II-treated cells, the green fluorescence became concentrated in nuclei in 65% of transfected cells. In control cells, the GFP fluorescence in the transfected cells was only found in the cytosol. These results indicate that human trophoblasts are capable of appropriate compart-
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**DISCUSSION**

Trophoblast invasion depends, in part, on the regulated production of proteolytic enzymes such as plasmin, which function to degrade the extracellular matrix and to activate other proteases such as matrix metalloproteinases (24, 45). Trophoblasts are equipped to activate plasminogen, leading to the production of plasmin, though the action of urokinase-type plasminogen activator (uPA) associated with specific cell surface receptors, uPARs (24). This system appears to be well controlled during normal intrauterine pregnancies in which the activity of uPA is regulated by plasminogen activator inhibitor-1 (PAI-1) acting on uPA.

Ang II-induced PAI-1 Production Results in Reduced Trophoblast Invasiveness—Evidence provided above indicates that Ang II induces PAI-1 gene expression and protein secretion. Based on the fact that PAI-1 functions as an inhibitor of plasminogen activators resulting in a decreased degradation of the ECM, we propose that induction of PAI-1 protein synthesis and secretion by Ang II reduces invasiveness of human trophoblast cells. To test this hypothesis, trophoblast invasiveness was measured by an *in vitro* assay based on the ability of cells to penetrate a Matrigel-coated filter in an invasion chamber over a 24-h period. The ability of the trophoblasts to invade Matrigel-coated membranes is presented relative to the migration of cells through a control membrane that was not coated with Matrigel matrix. Ang II treatment decreased the invasion of the cells by 52% compared with control cells without Ang II treatment (Fig. 6, A, B, and C). To test whether the decreased invasion of trophoblasts following Ang II treatment is caused by a calcineurin-dependent PAI-1 induction following AT1 receptor activation, we treated the cells with losartan, CSA, and specific PAI-1 antibody, respectively, plus Ang II. The results (Fig. 6, A, C, and D) show that losartan completely abolished the inhibition of invasion by Ang II. Human PAI-1 antibody and CSA treatment also reduced the inhibition of invasion by Ang II relative to that of control cells without Ang II treatment (Fig. 6, A, D, E, and F). These results suggest that decreased trophoblast invasiveness following Ang II treatment is through AT1-receptor activation via calcineurin-dependent PAI-1 gene activation.

**FIG. 5.** NFAT activation, nuclear translocation, and PAI-1 gene activation. A: *Inset*, expression constructs encoding wild type NFAT or a constitutively active NFAT (NFATc) were cotransfected into cells along with a β-galactosidase plasmid. After 48 h, cellular protein extracts were prepared and Western blot analysis was used to detect PAI-1 protein by anti-human PAI-1 antibody. The level of β-actin was used as a loading control. Densitometric analysis of two Western blots was performed. The results are shown as PAI-1/β-actin protein optical density ratios. Data are expressed as mean ± S.E. *p* < 0.05 versus control. The results are compiled from two independent experiments. B, control and Ang II-treated cells were transfected with an NFAT-GFP expression construct. After 48 h, cells were visualized for green fluorescence. The green fluorescence became nucleus-concentrated in 65% of transfected cells with Ang II treatment. However, in the untreated cells, the green fluorescence in the transfected cells was only found in cytosol. The original photos were taken at 400× magnification. Bars, 400 μm.
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RESULTS

Matrigel invasion assay was performed to evaluate trophoblast cell invasion of control (A) and Ang II (100 nM)-treated cells (B) after 24 h. In some experiments cells were treated with 1 μM losartan (C), 1 μM CSA (D), and anti-human PAI-1 antibody-1:100 (E) in addition to 100 nM Ang II. The percentage of cells migrating through the Matrigel matrix to reach the underlying membrane is indicated in parentheses. The original photographs were taken at 100× magnification. Bars, 100 μM. F, the invasion index of cells under different conditions is normalized to non-treated control cells. Data are expressed as mean ± S.E.; *, p < 0.05 versus control; **, p < 0.05 versus Ang II treatment. The results are compiled from three independent experiments.

production by human trophoblasts in a time- and dosage-dependent manner, acting through the AT1 receptor. We have also shown that activation of AT1 receptors decreases trophoblast invasion. Our studies are the first to link the RAS with the fibrinolytic system to regulate placentation development and function. These findings suggest that the local RAS plays an important role in trophoblast invasion.

The renin-angiotensin system (RAS) is a signaling cascade that plays a key role in regulating blood pressure and electrolyte balance. Traditionally, the RAS has been considered primarily as a circulating system involved in the regulation of blood pressure and salt and fluid homeostasis. In addition to this classic view of the RAS, accumulating evidence indicates that the components of the RAS are synthesized in many tissues, such as brain, heart, ovary, and placenta, and that Ang II levels can be controlled locally, independent of circulating Ang II (32, 33). Renin, angiotensinogen, angiotensin-converting enzyme, and angiotensin receptors are all present in the human placenta. These findings suggest that Ang II synthesized in the placenta may serve as an autocrine/paracrine modulator of placental function (32, 33). Activation of the RAS has previously been shown to induce multiple growth factors that have been implicated in fibrosis, such as transforming growth factor-β (TGF-β) and platelet-derived growth factor-β (46–48). Induction of these growth factors appears to be mediated by Ang II acting at the AT1 receptor (49). TGF-β itself is known to regulate PAI-1 gene expression through the Smad-dependent signaling pathway (50). However, the induction of PAI-1 by Ang II in vitro in mesangial cells is TGF-β-independent (49). Hypoxia, TGF-β, and endothelin-1 are known to regulate PAI-1 gene expression in the trophoblast (51, 52). However, the link between the RAS and the PAI-1 proteolytic pathway has not been recognized and fully understood in physiological and pathological placental development. Our current studies provide strong evidence that a local RAS links with the fibrinolytic system to control key aspects of placental development.

We have also shown that the intracellular signaling pathway linking AT1 receptor activation with PAI-1 gene expression involves calcium-mediated activation of calcineurin, a cytoplasmic phosphatase. Numerous recent studies have shown that a variety of physiological and pathological stimuli elicit cellular responses through calcium-mediated signaling pathways involving the activation of calcineurin (43, 44, 53). This cytoplasmic phosphatase acts to dephosphorylate the phosphorylated form of NFAT, thereby allowing the dephosphorylated NFAT to enter the nucleus and activate genes. A common strategy for probing this pathway involves the use of CSA, a compound that binds with cyclophilin, forming a complex that binds to the catalytic subunit of calcineurin, inhibiting its activity (54). The role of the Ca2+-calcineurin-NFAT signaling pathway in the induction of PAI-1 gene expression in trophoblasts has not been addressed prior to our studies. We have presented three lines of evidence for the involvement of the calcineurin/NFAT pathway in the activation of PAI-1 gene expression in human trophoblasts. First, we have shown that CSA partially blocked the induction of PAI-1 gene expression by Ang II in human trophoblasts. Second, we have shown that a constitutively active mutant of NFAT activates PAI-1 gene expression. Third, we have shown that AT1 receptor activation leads to nuclear translocation of an NFAT-GFP fusion protein. Together, these results provide strong evidence for the role of the calcineurin/NFAT-signaling pathway in PAI-1 gene expression following AT1 receptor activation in human trophoblasts.

CSA only partially blocked the induction of PAI-1 gene expression following AT1 receptor activation. This finding is consistent with previous studies in other systems showing important roles for protein kinase C (PKC) signaling pathways in the...
up-regulation of PAI-1 gene expression following AT1 receptor activation (28). The involvement of calcineurin and PKC signaling downstream of AT1 receptor activation is consistent with the activation of phospholipase C (PLC). The action of PLC generates two intracellular messenger molecules, inositol 1,4,5-trisphosphate (IP3) and diacylglycerol. IP3 contributes to intracellular calcium mobilization, and diacylglycerol activates PKC. The fact that CSA only partially blocks the AT1 receptor-mediated activation of PAI-1 gene expression indicates that signaling downstream of PKC may also contribute to PAI-1 gene activation in human trophoblasts.

Our results are consistent with a molecular pathway for a local RAS regulating trophoblast invasion via PAI-1 production as shown in Fig. 7. According to this model, Ang II from the local RAS leads to mobilization of intracellular Ca2+, resulting in activation of calcineurin. NFAT within the cytoplasm is dephosphorylated by calcineurin, enabling it to translocate to the nucleus where it participates in the activation of PAI-1 gene expression to control trophoblast invasion in normal placental development.

In both human and mouse the RAS system undergoes major changes in response to pregnancy (32, 39). In normal human pregnancy, Ang II is increased in the maternal circulation (41, 55). As we have shown here, one of the local functions of the increased Ang II in the placenta may be to induce local PAI-1 production. Because PAI-1 is the key regulator of uPA activity and uPA is important for cell invasion, our studies suggest that the local RAS regulates PAI-1 production to regulate trophoblast invasion. In this regard, we have recently reported that high levels of renin gene expression occur at the maternal fetal interface throughout pregnancy in mice, initially in the deciduum and subsequently in the placenta (39). Increased renin gene expression in the deciduum could lead to increased Ang II production and in this way could limit trophoblast invasion. Likewise, increased renin gene expression in trophoblasts in near term placentas could lead to increased local Ang II synthesis to reduce trophoblast invasion near the end of gestation. Thus, local renin gene expression at the maternal-fetal interface may regulate trophoblast invasion through the activation of the AT1 receptor.

Pre-eclampsia is one of the leading causes of maternal and fetal morbidity and mortality during pregnancy (56). The condition is characterized by severe maternal hypertension, proteinuria, and is often associated with shallow trophoblast invasion and improper spiral arterial remodeling. Elevated PAI-1 gene expression in human trophoblasts results in increased production and activation of calcineurin. NFAT within the cytoplasm is dephosphorylated by calcineurin, enabling it to translocate to the nucleus where it participates in the activation of PAI-1 gene expression to control trophoblast invasion in normal placental development.

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AT1 receptor. These findings indicate that the AT1 receptor agonistic autoantibody associated with pre-eclampsia is efficient at activating AT1 receptors on human trophoblasts.2 Overall, our studies suggest that the local RAS may play an important role in normal placental development and that abnormalities of the RAS in pre-eclampsia, i.e. the presence of an autoantibody masquerading as Ang II, may be an essential cause of shallow trophoblast invasion, increased maternal proteinuria, and increased thrombosis associated with pre-eclampsia. Our current efforts focus on testing the pathophysiological impact of pre-eclampsia-associated AT1 receptor agonistic autoantibodies on trophoblast physiology and development.

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