Definitive Molecular Evidence of Renin-Angiotensin System in Human Uterine Decidual Cells

Chunbiao Li, Rais Ansari, Ziming Yu, Dinesh Shah

Abstract—The tissue renin-angiotensin system (RAS) has been suggested to be present in human gestational tissues, but uncertainty exists about the authenticity of this RAS, and the cellular origin of this RAS has not been defined. In the present study, we confirmed the presence of authentic renin and angiotensinogen in the prolactin-producing decidual tissue by sequencing the cDNAs generated through reverse transcription–polymerase chain reaction, confirming cDNA product sizes, and by performing Northern blot analysis of the RNA. Our comparative data demonstrate that prolactin has the highest expression in the decidual tissue, followed by renin, and that angiotensinogen has the least expression. We demonstrated with fluorescent in situ hybridization that prolactin-expressing endocrine decidual cells are the same cells that express both renin and angiotensinogen. These results have implications in regard to how the decidual RAS may be regulated and what potential role this local RAS may have in the pathogenesis of preeclampsia. (Hypertension. 2000;36:159-164.)

Key Words: renin-angiotensin system ■ human ■ pregnancy ■ preeclampsia

It is now well recognized that in addition to a circulating renin-angiotensin system (RAS), there is a tissue RAS in several extrarenal sites.1–2 The concept of tissue RAS is based on the detection of renin mRNA in several extrarenal tissues, namely, adrenal gland,1 testes,4 brain,5 ovary,6 vasculature,7 heart,7 and uterus.8 Angiotensinogen also has been shown to be expressed in several extrahepatic tissues, such as kidney,9 brain,5 aorta,10 heart,10 adrenal gland,10 and ovary.11 The tissue RAS has been proposed to play a role in the local regulation of blood flow and vasculature.2

Gravid uterus in nephrectomized rabbits was shown through enzymatic assay for renin to be a source of renin.12 Although it has been shown that human placenta expresses renin13 and that choriocidal cells secrete renin, mostly in the form of prorenin,14,15 current evidence suggests that the placental prorenin may not enter the maternal circulation.16 Therefore, renin production must occur on the maternal side for tissue renin to have a role in the local regulation of uterine blood flow. Decidua has been shown through Northern blot analysis to be a major source of renin in the gravid human uterus.8 The decidua is the highly specialized and modified endometrium in pregnancy17 that is formed through prolonged stimulation of the endometrium by estrogens and progesterone and through stimuli provided by the implanting blastocyst.17 The production of prolactin by decidua is considered to be a specific biochemical marker of the process of decidua18. Such prolactin-producing decidual cells may be called endocrine decidual cells. In addition to the endocrine decidual cells, the decidua contains numerous cells of hemopoietic origin.19–21 The cells of hemopoietic origin have been demonstrated as various lymphocytes and macrophages.20,21 However, the type of decidual cells that are the sources of renin and angiotensinogen is not well defined. Jikihara et al22 suggested that decidual macrophages produce renin on the basis of Southern blot analysis of a cDNA fragment produced through reverse transcription/polymerase chain reaction (RT-PCR). The limitation of the study by Jikihara et al is that it is not possible to purify decidual macrophages completely free of native nonhemopoietic decidual cells22 (J.M. Banu and D.M. Shah, unpublished observations, 1996). Therefore, the presence of a few copies of renin mRNA from nonhemopoietic decidual cells would give positive results in Southern blot analysis of RT-PCR for the presence of renin in decidual macrophage preparation. Furthermore, renin and angiotensinogen expression has been localized in the unremodeled decidual vessels of early gestation decidua.23 It is important to define the cellular origin of RAS in late gestation because many disorders of gestation with restriction of uterine blood flow manifest in late gestation. It is critical to define the cellular origin of renin and angiotensinogen in the decidua because of the implications regarding the understanding of how this renin/angiotensinogen production may be regulated. For example, an endocrine decidual cell origin of renin and angiotensinogen may place regulatory control for renin and angiotensinogen on the process of decidualization itself and therefore may be related to the role of sex steroids estrogen and progesterone.24 On the other hand, decidual macrophage renin may be regulated by the biochemical mechanisms for recruitment and activation of the macrophages.
There are several methodological considerations to unequivocally establish the presence of tissue RAS. Renin-like enzymatic activity to release angiotensin I (Ang I) has been demonstrated in cathepsin D, pepsinogen, other aspartyl proteases, and other renin-like enzymes. Therefore, the demonstration of enzymatic activity may not be adequate evidence for local renin production. Furthermore, some of the monoclonal anti-renin antibodies have been found to cross-react with other renin-like proteases; therefore, immunohistochemical studies with these antibodies cannot provide categoric evidence for the presence of renin. Northern blot analysis is a highly specific method that should be performed under high stringency conditions. However, because renin gene has a high sequence homology with cathepsin D, the size of the renin transcript must be accurately defined in Northern hybridization analysis. The similarity of the uterine decidual renin to the renal renin can be highly reliably demonstrated by sequencing the cDNA generated with RT-PCR from RNA samples isolated from decidual tissues. In addition, angiotensinogen is the only known precursor of angiotensin peptides; therefore, to definitively establish an independent tissue RAS, one must demonstrate evidence for the local production of angiotensinogen in addition to the presence of renin production.

The aims of the current investigation were (1) to establish definitive evidence of the presence of authentic renin and angiotensinogen in the maternal decidual tissue in late gestation through the use of molecular methods and (2) to demonstrate the cellular origin of their expression in the specific maternal cells of defined identity by using prolactin expression as a marker of endocrine decidual cells.

### Methods

**Decidual Tissue and Cell Cultures**

We used normal-term (37 to 42 weeks) placentas obtained after vaginal deliveries. Decidual tissue was obtained from placental membranes according to the method described by Handwerger et al. Fetal membranes were collected, placed immediately into cold Hanks’ solution, and taken to the laboratory. The membranes were washed with Hanks’ solution, and the maternal decidual tissue attached to the membranes was gently peeled off. Some of the decidual tissue was frozen in liquid nitrogen to extract total RNA for RT-PCR. For the preparation of decidual cell cultures, the tissue was minced into small pieces and subjected to enzymatic digestion as follows: the tissue was washed in fresh medium through centrifugation (200g) and then suspended in DME/F-12 containing 0.1% collagenase, 0.02% DNase, and 2% FBS. This suspension was incubated at 37°C with constant shaking for 1 hour. After aspiration of the supernatant cell suspension, the tissue was further digested for an additional hour, and the supernatant was collected. The cell suspension was filtered through a 150-μm filter of nylon mesh, and the cells were pelleted through centrifugation. After being washed twice, the pellet was resuspended in 6 mL of culture medium. Cell separation was performed to enrich prolactin-producing decidual cells through isopycnic centrifugation on a linear density gradient of 40% Percoll, as described by Handwerger et al. The Percoll gradient was established with the centrifugation of 30 mL of 40% Percoll at 13 000g for 15 minutes. The dispersed cells, suspended in 2 mL of medium, were gently layered onto a preformed gradient. Cell separation was carried out through centrifugation at 800g for 20 minutes. Dextran marker beads (Pharmacia Biotech) were used to monitor density gradient. The cell suspension in 1,017 to 1,050 density fraction was aspirated, pooled in a 50-mL tube, and diluted to 50 mL with culture medium. The cells were pelleted through centrifugation at 100g for 7 minutes, and then the cells were resuspended in DME/F-12 with 10% FBS. A cell count with trypan blue dye exclusion was performed in a hemocytometer, and the cell suspension was diluted to 0.25×10⁶ cells/mL. The cells were either plated onto collagen III–coated culture dishes with DME/F-12 medium that contained 2% FBS and then incubated at 37°C in a 5% CO₂/95% air atmosphere or fixed with 3% paraformaldehyde for 1 hour at 4°C and then plated onto SuperFrostPlus microscope slides (Fisher Scientific) and air dried for in situ hybridization. Some cell cultures were used for total RNA extraction with an Ultraspec-II RNA Isolation Kit (Biotex Labs Inc).

### Synthesis of Renin, Angiotensinogen, and Prolactin cDNA for Sequencing

Primers for the amplification of cDNA fragments were designed according to the published sequences of human renal renin gene, human hepatic angiotensinogen gene, and human placental prolactin gene from the GenBank database (www.ncbi.nlm.nih.gov). The regions of the DNA sequences of interest were chosen where there was low homology with related proteins. The primer pairs for PCR were selected from different exons to ensure that DNA fragment amplified from genomic DNA would not be of the same size. A summary of the primers, their site on the GenBank sequence and exon location of respective genes, GenBank database accession number, sequence from 5’ to 3’, and their PCR product size are shown in Table 1. All of the primers were synthesized at the molecular biology core laboratory at Case Western Reserve University with an ABI 394 DNA synthesizer (PE Applied Biosystems).

The total RNA was extracted from decidual tissue with an Ultraspec-II RNA Isolation Kit (Biotex Laboratory Inc). For RT-PCR, the GeneAmp RNA PCR kit (PE Applied Biosystems) was used according to the manufacturer’s instructions. Total RNA (1 μg) was used for RT with random primers. The conditions for the PCR that were different from the manufacturer’s instructions or specific for the reaction (ie, magnesium concentration and temperature) are also shown in Table 1. The RT-PCR products were separated on agarose gel and purified with a QIAquick Gel Extraction Kit (Qiagen Inc). The fragments were sequenced with an ABI 377 Prism DNA

### Table 1. RT-PCR Primers for Amplifying cDNAs

<table>
<thead>
<tr>
<th>Primer</th>
<th>Site/Exon</th>
<th>Accession</th>
<th>No.</th>
<th>Sequence (5’ to 3’)</th>
<th>Product Size, bp</th>
<th>[Mg²⁺], mmol/L</th>
<th>Temperature, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>hRN-F</td>
<td>521–540/5</td>
<td>E00286</td>
<td>AGGACATCATCACCGTGGGT</td>
<td>292</td>
<td>3</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>hRN-R</td>
<td>812–793/6</td>
<td>TGGAAATTCTCCCTGTAAATG</td>
<td>56</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hPRL-F</td>
<td>140–161/2</td>
<td>D00411</td>
<td>CATCCATAACCTCTCGAGAA</td>
<td>222</td>
<td>2</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>hPRL-R</td>
<td>361–340/4</td>
<td>ACGTCGTCAGCAGTATCA</td>
<td>59</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hANG-F</td>
<td>1068–087/3</td>
<td>K02215</td>
<td>GGTGATCCAGCTCACTATG</td>
<td>320</td>
<td>2</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>hANG-R</td>
<td>1387–368/5</td>
<td>CAGCGTGTGTAAAGCCTTGGG</td>
<td>60</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

hRN indicates human renal renin; hPRL, human decidual prolactin; hANG, human hepatic angiotensinogen; F, forward primer; and R, reverse primer.
automated sequencer at the molecular biology core laboratory of Case Western Reserve University.

Northern Analysis

Decidual tissues were collected from normal-term placentas as described earlier. Total cellular RNA was extracted from the tissues with guanidine isothiocyanate, followed by ultracentrifugation in cesium chloride gradient. Twenty micrograms of total RNA was fractionated by electrophoresis, the RNA was transferred and cross-linked to nylon membrane. Three membrane blots were prepared from 2 RNA samples. These 3 blots were prehybridized in ULTRAhyb buffer (Ambion Inc) at 42°C for 5 hours and then hybridized in the same buffer, respectively, with radiolabeled (3P) cDNA probes of renin, angiotensinogen, and prolactin at 42°C overnight.33 After hybridization, the blots were washed at 65°C successively twice (30 minutes each) in 2 x SSC/0.1% SDS. The washed blots were exposed overnight to x-ray films at 30°C in 0.1% x-ray films at 30°C. The blots were developed with glycine and hydrogen peroxide.34 Slides were washed in PBS and air dried. Hybridization buffer (Boehringer-Mannheim Biochemicals) with riboprobe (500 ng/slide) was added. Slides were covered, sealed, and incubated overnight in a humidified chamber at 55°C. RNase A (Sigma Chemical Co) treatment was performed at 37°C, followed by the TSA multicolor detection procedure. Biotin- and dig-labeled probes were detected sequentially on the same slides as described later. TNB (Tris hydrochloride/sodium chloride/blocking reagent) was used as a blocking reagent. Biotin-labeled probes were detected first. Streptavidin-HRP (1:100 dilution) in TNB buffer was used for biotin detection through streptavidin-biotin reaction. Biotinyl tyramide (1:50 dilution) was added for biotin signal “amplification.” Streptavidin-Cy3 (1:100 dilution) was used for fluorescence labeling of biotin. The residual peroxidase activity at the end of the first set of reactions was inactivated by the incubation in 0.01 N HCl. Next, dig-labeled probes were detected on the same slides as follows: anti-dig antibody conjugated with HRP (anti-dig-HRP, 1:100 dilution) was added, and biotin was precipitated by biotinyl tyramide (1:50 dilution) treatment for signal amplification. The detection of dig-labeled probes was performed with streptavidin-fluorescein (1:200 dilution). After incubation at 37°C for 30 minutes, slides were washed with TNT (Tris hydrochloride/sodium chloride/Tween-20) and mounted with antifading mounting medium (Vector Laboratories, Inc). Slides were inspected under the fluorescent microscope (Axiphoto; Carl Zeiss Inc) through appropriate filters for green (fluorescein) and red (Cy3) fluorescence detection. Computer-generated images were merged as indicated and stored on disks.

**Double Color Fluorescent In Situ Hybridization**

With nested PCR, primers were designed/tailed with either SP6 or T7 promoter sequences for the synthesis of labeled riboprobes. The riboprobes were synthesized and labeled with digoxigenin (dig) for renin and angiotensinogen and with biotin for prolactin with an in vitro transcription kit (Promega). Detailed information on the various primers with SP6 and T7 promoter sequences is given in Table 2. To confirm the probes, 2 μl of each probe and 2 μl of positive transcription control were loaded onto a 1% formaldehyde agarose gel. After electrophoresis, the RNAs were transferred to nylon membrane, and the probes were detected with anti-dig or anti-biotin horseradish peroxidase (HRP) (Boehringer-Mannheim Biochemicals) and visualized with an enhanced chemiluminescence kit (Amersham Pharmacia Biotech Inc.).

The protocol used was modified from the protocol for the RNA Color Kit (Amersham Pharmacia Biotech Inc) and the protocol for TSA Multicolor Detection (NEN Life Science Products). Fixed cells on slides were treated with Triton and rinsed with acetic anhydride, followed by glycine and hydrogen peroxide.34 Slides were washed in PBS and air dried. Hybridization buffer (Boehringer-Mannheim Biochemicals) with riboprobe (500 ng/slide) was added. Slides were covered, sealed, and incubated overnight in a humidified chamber at 55°C. RNase A (Sigma Chemical Co) treatment was performed at 37°C, followed by the TSA multicolor detection procedure. Biotin- and dig-labeled probes were detected sequentially on the same slides as described later. TNB (Tris hydrochloride/sodium chloride/blocking reagent) was used as a blocking reagent. Biotin-labeled probes were detected first. Streptavidin-HRP (1:100 dilution) in TNB buffer was used for biotin detection through streptavidin-biotin reaction. Biotinyl tyramide (1:50 dilution) was added for biotin signal “amplification.” Streptavidin-Cy3 (1:100 dilution) was used for fluorescence labeling of biotin. The residual peroxidase activity at the end of the first set of reactions was inactivated by the incubation in 0.01 N HCl. Next, dig-labeled probes were detected on the same slides as follows: anti-dig antibody conjugated with HRP (anti-dig-HRP, 1:100 dilution) was added, and biotin was precipitated by biotinyl tyramide (1:50 dilution) treatment for signal amplification. The detection of dig-labeled probes was performed with streptavidin-fluorescein (1:200 dilution). After incubation at 37°C for 30 minutes, slides were washed with TNT (Tris hydrochloride/sodium chloride/Tween-20) and mounted with antifading mounting medium (Vector Laboratories, Inc). Slides were inspected under the fluorescent microscope (Axiphoto; Carl Zeiss Inc) through appropriate filters for green (fluorescein) and red (Cy3) fluorescence detection. Computer-generated images were merged as indicated and stored on disks.

**Results**

Renin and Angiotensinogen Expression in Prolactin-Expressing Decidual Tissue

The results of RT-PCR from decidual tissue RNA are presented in Figure 1. As predicted from the position of the primers, the RT-PCR produced a 292-bp cDNA fragment for human renin. The prolactin and angiotensinogen cDNA fragments were also in agreement with the predicted sizes (222 and 320 bp, respectively). No cDNA fragments were generated from the negative control. The size of the cDNAs indicates that these fragments were generated from the respective mRNA species but not from the genomic DNAs. The synthesis of cDNAs consistent with predicted product

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**TABLE 2. PCR Primers for Amplifying Templates for In Vitro Transcription of Riboprobes**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Site/Exon</th>
<th>Sequence (5’ to 3’)</th>
<th>Product Size, bp</th>
<th>[Mg2+]</th>
<th>Temperature, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>REN sense F</td>
<td>644–662/6</td>
<td>SP6-ccaaaagggtgcttaaaga</td>
<td>151</td>
<td>2</td>
<td>58</td>
</tr>
<tr>
<td>REN sense R</td>
<td>770–751/6</td>
<td>tgaasaatccctctgtaatg</td>
<td>57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>REN antisense F</td>
<td>644–662/5</td>
<td>cccaaagggtgcttaaaga</td>
<td>151</td>
<td>2</td>
<td>58</td>
</tr>
<tr>
<td>REN antisense R</td>
<td>770–751/6</td>
<td>T7-cgaasaatccctctgtaatg</td>
<td>57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANG sense F</td>
<td>1068–1087/3</td>
<td>SP6-ctgatcctacccctactatg</td>
<td>342</td>
<td>2</td>
<td>62</td>
</tr>
<tr>
<td>ANG sense R</td>
<td>1387–1368/5</td>
<td>caggcttgtaacgcttggg</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANG antisense F</td>
<td>1068–1087/3</td>
<td>gctgatcctacccctactatg</td>
<td>342</td>
<td>2</td>
<td>62</td>
</tr>
<tr>
<td>ANG antisense R</td>
<td>1387–1368/5</td>
<td>T7-caggcttgtaacgcttggg</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRL sense F</td>
<td>268–289 /3–4</td>
<td>SP6-aggccaaagatgaatctaaa</td>
<td>117</td>
<td>2</td>
<td>56</td>
</tr>
<tr>
<td>PRL sense R</td>
<td>361–340/4</td>
<td>acttcgatcagcagatpataca</td>
<td>64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRL antisense F</td>
<td>268–289 /3–4</td>
<td>Aagccaaagatgaatctaaa</td>
<td>117</td>
<td>2</td>
<td>56</td>
</tr>
<tr>
<td>PRL antisense R</td>
<td>361–340/4</td>
<td>T7-acttcgatcagcagatpataca</td>
<td>64</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SP6 promoter indicates cgtattgtaggctacatagata; T7 promoter, tgaatccgctccatagata; ANG, angiotensinogen; F, forward; R, reverse; and REN, renin.
sizes also suggests that respective mRNAs were present in the decidual tissue preparations. Sequencing of the cDNAs is the most definitive evidence of the presence of renin, angiotensinogen, and prolactin mRNA in the decidual tissue. Blast search analysis (www.ncbi.nlm.nih.gov) revealed that the renin cDNA sequence matched 99%, prolactin cDNA sequence matched 100%, and angiotensinogen cDNA matched 100% of their respective gene sequences in the GenBank database.

To further confirm the expression of renin and angiotensinogen in prolactin-producing decidual tissues, we performed Northern hybridization analysis on normal human decidual RNA samples. To demonstrate the specificity of hybridization signal, full Northern blots are presented in Figure 2. Single bands with the predicted mRNA sizes were clearly detected for renin, angiotensinogen, and prolactin, respectively.

Northern blot analysis indicates that the prolactin message was expressed in higher amounts compared with renin or angiotensinogen and that the relative expression of renin is substantially higher than that of angiotensinogen. We also performed RT-PCR of RNA extracted from cultured decidual cells derived through Percoll density separation. The relative amount of the cDNA indicated that RNA from cultured decidual cell contained more renin mRNA species than that from tissue on a comparative basis (results not shown).

Localized of Renin and Angiotensinogen Expression in Dispersed Enriched Endocrine Decidual Cells by In Situ Hybridization

The results of in situ hybridization are shown in Figure 3. Renin and angiotensinogen expression is demonstrated to be present in the endocrine decidual cells that express prolactin as well. No fluorescence was demonstrated for any of the mRNA when sense probes were used. Furthermore, the authenticity of the demonstration of renin, angiotensinogen, and prolactin expression by in situ hybridization is highly definitive, with the negative cells not exhibiting any fluorescence. Examination of the groups of cells showed that all cells positive for prolactin expression are also positive for renin and angiotensinogen expression, and vice versa (data not shown). In situ hybridization data definitively localize renin and angiotensinogen expression in endocrine decidual cells that also express prolactin.

Discussion

In the present study, we definitively demonstrated that both renin and angiotensinogen mRNAs were present in decidual tissue along with prolactin expression. The sequences of the cDNAs confirmed that renin and angiotensinogen produced by decidua were the same as authentic renal renin and hepatic angiotensinogen. Vascular endothelial cells ubiquitously produce ACEs. Therefore, the gravid human uterus has a complete tissue-based RAS on the maternal side.

Our in situ hybridization results demonstrate renin and angiotensinogen expression in the endocrine decidual cells that also express prolactin. The data of Morgan et al.\textsuperscript{23} that demonstrate the localization of renin expression in small unremodeled vessels of spiral arteries may be a finding specific to the early gestation (5 to 13 weeks) decidua.\textsuperscript{23} Furthermore, we examined renin and angiotensinogen expression in dispersed decidual cell preparation enriched for prolactin-producing cells, which did not contain vascular components. We used the TSA method for enhancement of the fluorescent signal, which is a highly sensitive method. Our data demonstrate that decidua has a tissue RAS in addition to a widely distributed vascular RAS.

Decidua is composed of several types of cells. The type of decidual cell that expresses renin and angiotensinogen may have implications in regulation of the expression. Our results demonstrate the expression of both renin and angiotensinogen in the prolactin-expressing decidual cells. The expression of prolactin appears to be regulated by the sex steroids estrogen and progesterone.\textsuperscript{35} Our previous data show that progesterone treatment of endometrial stromal cells from a nongravid uterus increases renin activity\textsuperscript{36} and that the action of pro-
gesterone may be mediated by increasing both the renin gene expression and the processing of prorenin to mature renin (D.M. Shah, J.M. Banu, and J.M. Chirgwin, unpublished observations, 1995). The localization of renin and angiotensinogen to decidual cells that usually surround the uterine terminal vessels has implications that this RAS may be involved in the regulation of uterine blood flow. Experiments in primates have shown that the infusion of low-dose Ang II may actually increase uterine blood flow.37 Rosenfeld et al38 have shown that the low-dose infusion of Ang II has minimum effect on the uterine blood flow but that the pharmacological-dose (10 μg/min) infusion decreases uterine blood flow in gravid ewes. For prorenin to participate in the regulation of blood flow, it must be available locally in a biologically active form. Our previous observation that decidual renin is enzymatically active36,39 supports the role of this renin in the regulation of blood flow. We have previously reported that the coculture of endothelial cells with decidual cells increases renin secretion.39 The observation that endothelial cells may further increase decidual renin secretion affirms the role of decidual RAS in vasomotor regulation.

Recently, Takimoto et al40 reported the development of a preeclampsia-eclampsia syndrome by crossbreeding transgenic mice with the introduction of human renin (h-ren) and human angiotensinogen (h-ang) in the mouse genome. Specifically, when male mice carrying h-ren were mated with female mice carrying h-ang, preeclamptic syndrome developed and h-ren overexpression was demonstrated on the fetal side in the placenta. One unique aspect of the mouse model of preeclampsia is that fetal renin from the placenta appeared to transfer to maternal circulation much more readily than in humans.36 Data on renin (5%) versus prorenin (95%) in mouse model40 suggest that renin in the uteroplacental interphase need not be in large proportion for this renin to mediate the pathogenesis of preeclampsia in the transgenic mouse model. We have recently demonstrated that renin gene expression is increased in decidua vera ≈3-fold compared with controls in human preeclampsia (D.M Shah, J.M. Banu, J.M. Chirgwin, and R.R. Tekmal, unpublished observations, 2000). Our data on increased decidual renin gene expression in human preeclampsia and data on complete RAS on the maternal side are correspondent with the pathogenesis of the mouse model but distinct in the role of fetal renin in the mouse model versus the maternal renin role in human preeclampsia. However, pathogenic processes must occur on the maternal side even in the mouse model, because human angiotensinogen is present on the maternal side and human renin cannot cleave mouse angiotensinogen. Collectively, these data strongly corroborate the role of uterine RAS in the pathogenesis of human preeclampsia. Women homozygous for the angiotensinogen T235 variant (Met235→Thr) have been suggested to be at an increased risk for preeclampsia.41 A promoter mutation (−10 A/G) associated with T235 variant is thought to be responsible for increased Ang II production. These observations emphasize the role of locally produced Ang II in vasomotor ischemia in selected populations. The role of renin overexpression, angiotensinogen homozygous state for T235 variant, −10 A/G promoter mutation, or a combination deserves further investigation to elucidate the pathogenesis of human preeclampsia.

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References


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