Increased cystatin C expression in the pre-eclamptic placenta

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Trophoblast invasion is regulated by proteinases and their inhibitors. Cystatin C inhibits cysteine proteinases. The serum concentration of cystatin C is increased in late pregnancy and pre-eclampsia. We aimed to investigate whether the expression of cystatin C is increased in the pre-eclamptic placenta and to investigate the expression pattern of cystatin C mRNA and protein in placental tissue. Tissue samples from the central part of the placenta from 13 normal and 22 pre-eclamptic pregnancies were included. We used real-time polymerase chain reaction (RT-PCR) and in situ hybridization for mRNA expression analysis and immunohistochemistry and Western blotting for protein expression analysis. RT-PCR showed a significantly higher expression of cystatin C mRNA in pre-eclampsia than in normal pregnancy, with the highest expression in cases with severe pre-eclampsia. In situ hybridization revealed a distinct pattern of high expression in the extravillous trophoblast cells of the basal plate and low expression in the syncytiotrophoblast covering villi. The cystatin C protein distribution matched the mRNA expression pattern. Western blot analysis revealed an increased protein expression in cases with severe pre-eclampsia and confirmed the presence of cystatin C in amniotic fluid samples. The high expression of cystatin C mRNA in the extravillous trophoblast cells of the basal plate suggests a role for cystatin C in the regulation of proteinases in placentation. Placental expression and secretion of cystatin C could contribute to the elevated maternal plasma levels seen in pre-eclampsia.

Key words: amniotic fluid/placentation/proteinase inhibitor/trophoblast

Introduction

Pre-eclampsia is a leading cause of maternal and fetal morbidity and mortality. The condition is characterized by hypertension, proteinuria and a generalized systemic vasoconstriction (Kingdom, 2000; Redman and Sargent, 2005). The clinical syndrome arises from circulatory disturbances secondary to a generalized endothelial dysfunction caused by inflammation (Kingdom, 2000).

Although the aetiology of the disease is still unclear, pre-eclampsia is considered to result from an insufficient function of the placenta (Redman and Sargent, 2005; van den Brule et al., 2005). The migration and invasion of trophoblast cells into the uterine wall and maternal vasculature is pivotal to the success of placentation. Interstitial trophoblasts invade the uterine tissues, anchoring the placenta to the uterus, and the extravillous trophoblasts migrate into the wall of the maternal spiral arteries. This process converts the uterine spiral arteries into low-resistance, high-capacitance vessels allowing sufficient blood flow to meet the demands of the growing fetus. Pre-eclampsia has been linked to a deficiency in the trophoblast invasion of the maternal spiral arteries, leading to a poorly perfused feto-placental unit (Kingdom, 2000; Redman and Sargent, 2005; van den Brule et al., 2005).

Trophoblast invasion is a complex, multi-step process involving the concerted action of adhesion, degradation and migration processes. The degradation of the extracellular matrix requires specific enzymes, proteases, controlled by respective inhibitors (Redman and Sargent, 2005). The cysteine-proteases (cathepsins) have been studied in implantation and placentation of various species and are believed to be important for trophoblast invasion (Salamonsen, 1999; Mason et al., 2002; Ishida et al., 2004). Cathepsins B and L and their inhibitor cystatin C have been reported to be expressed by trophoblasts and decidual macrophages in early human placentation and implantation (Divya et al., 2002; Nakanishi et al., 2005).

Cystatin C is the strongest extracellular inhibitor of the cysteine proteases and may also have regulatory effects on other proteases (Ray et al., 2003). Owing to its small size (13.3 kDa) and steady production, the serum level of cystatin C is a reliable marker for the glomerular filtration rate (GFR) in the non-pregnant setting (Grubb, 2000). The serum level of cystatin C is increased in pregnancy and further so in pre-eclampsia, closely correlated to functional and structural changes in the kidneys (Strevens et al., 2002, 2003). Consequently, the serum level of cystatin C has been proposed as a marker for the transition from normal pregnancy to pre-eclampsia and for the severity of pre-eclampsia (Strevens et al., 2001).

Increased serum levels of cystatin C in late pregnancy and pre-eclampsia have been explained by changes in renal handling of the protein. However, increased synthesis and secretion of the protein is another possibility (Strevens et al., 2001; Akbari, 2004), which would explain increased levels seen in dupplex pregnancies without pre-eclampsia. Even though cystatin C was originally cloned from placental cDNA (Abrahamson et al., 1987), expression of cystatin C has not been studied in placental tissue from late pregnancy or pregnancy complicated by pre-eclampsia.

In the present study, cystatin C expression was analysed at the mRNA and protein level in human placental tissue from normal and
nulliparity, gestational age (weeks) 39.4 (35.7–40.9) 38.7 (36.4–40.4) 32.4 (29.1–34.4) 37.7 (36.1–40.7) +

Diastolic pressure (mmHg) 74

Serum-cystatin C (mg/L) 10

Nulliparity, n (%) 10 (77) 6 (67) 6 (77)

Body mass index (kg/m²) 22.9 ± 4.0 22.4 ± 3.0 6 (67)

Systolic pressure (mmHg) 123 ± 12 162 ± 6 22.4 ± 3.0

Diastolic pressure (mmHg) 74 ± 7 100 ± 4 162 ± 6

Proteinuria (g/L) ± 1

Placental weight (g) 3240 (2475–4220) 3180 (2530–4000) 633 ± 112

Gender (F:M) 7:6 6:3 6 (67)

Birth weight (g) 3420 (2475–4220) 3180 (2530–4000) 633 ± 112

Appgar score (1 min) 9 (8–10) 9 (9–10) 8 (9–10) 6 (6–9)

Appgar score (5 min) 10 (9–10) 9 (9–10) 8 (9–10) 6 (9–10)

Mode of delivery

VD 11 9 0 0

PS 2 0 0 0

ES 0 0 0 0

Serum-urate (µmol/L) 280 ± 38 362 ± 97 * 417 ± 62 ** 419 ± 96 **

Serum-cystatin C (mg/L) 1.12 ± 0.16 1.51 ± 0.16 ** 1.43 ± 0.19 ** 1.73 ± 0.29 ***

Continuous data are presented as median (range) or mean ± SD. Categorical data are presented as n (%). ND, not detected; F, female; M, male; VD, vaginal delivery; PS, planned caesarean section; ES, emergency caesarean section. ANOVA and Bonferroni/Dunn test showed significant differences between normal pregnancy and mild/severe pre-eclampsia. All women were white Caucasians except one in the severe group (African). Two fetuses in the early-onset severe PE group were small for gestational age. *P < 0.05, **P < 0.01, ***P < 0.001.

Table 1. Clinical characteristics of pregnant women and their offspring

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pre-eclamptic pregnancies. Placental cystatin C expression was related to the time of onset and to the clinical degree of pre-eclampsia. Western blot analysis was used to confirm the presence of cystatin C in placental tissue and amniotic fluid samples.

Materials and methods

Collection of placental tissue

Placental tissue was collected at the Department of Obstetrics and Gynecology at the University Hospital in Lund, Sweden. All participants received oral and written information about the study and informed consent was obtained. The study was approved by the Ethics Committee at Lund University, and all procedures were performed in accordance with the Helsinki Declaration (Helsinki, 2004). We included 13 normal pregnancies and 22 pre-eclamptic pregnancies (13 severe) according to definitions below. All pregnancies were dated according to routine ultrasound measurements at 17–18 weeks of gestation. Only women with no history of hypertension, renal disease or diabetes were included (Table I).

The control group included 13 uncomplicated singleton pregnancies of which 11 were spontaneous vaginal deliveries and 2 were planned caesarean sections at term.

Pre-eclampsia was defined as follows: a systolic and diastolic blood pressure of ≥140 and ≥90 mmHg, respectively, and proteinuria defined as ≥300 mg protein in a 24-h urine specimen or ≥1+ protein by dipstick urine sample later confirmed using dipstick evaluation or 24-hour urine specimen occurring after 20 weeks of gestation in a woman with previously normal blood pressure. Severe pre-eclampsia was defined as follows: a systolic and diastolic blood pressure of ≥160 and ≥110 mmHg, respectively, on two occasions at least 6 h apart while bed resting and/or proteinuria of ≥5 g in a in a 24-h urine specimen or ≥3+ on two random urine samples collected at least 4 h apart (Anonymous, 2000). Women with severe pre-eclampsia were separated into early-(<35 weeks) and late-onset cases (≥35 weeks).

Venous blood samples were collected, centrifuged (~2000 g) at 3500 rpm for 10 min. and frozen at −80°C in aliquots and stored for later analysis. Placental tissue samples were taken immediately after delivery. A cube, 10 x 10 x 10 mm, from the central part of the placenta consisting of villi was collected and stored at −80°C. Tissue sections, 12-µm thick, were cut and thaw-mounted onto silanized slides and stored at −80°C until in situ hybridization.

Real-time polymerase chain reaction

RNA extraction

Total RNA was extracted from frozen placental tissue as previously described (Bottalico et al., 2004) using Trizol® (Gibco BRL, Gaithersburg, USA). Proteoglycan and polysaccharide were removed by adding isopropanol followed by salt precipitation with 0.8 mol L⁻¹ sodium citrate and 1.2 mol L⁻¹ sodium chloride. The quality of RNA samples was determined by electrophoresis through a denaturing gel (1.5%, agarose/2% formalin) using a 1× MOPS buffer (Intergen Company, Norcross, USA). RNA loading mix (GenHunter, Nashville, USA) was used to verify the 18S and 28S RNA bands under UV light. All samples were controlled for distinct 18S and 28S bands prior to further analysis.

cDNA synthesis

Total placental RNA was reverse transcribed according to protocols from Applied Biosystems, Foster City, USA, in a 50 µl reaction mixture containing 0.5 µg total RNA and final concentrations of 1×TaqMan RT buffer, 5.5 mmol L⁻¹ MgCl₂, 500 µmol L⁻¹ dNTPs, 2.5 µmol L⁻¹ random hexamers, 0.4 U µl⁻¹ RNase inhibitor and 1.25 U µl⁻¹ MultiScribe Reverse Transcriptase. The reactions were incubated at 25°C for 10 min, at 48°C for 30 min and then at 95°C for 5 min of inactivation (Applied Biosystems).

Real-time polymerase chain reaction

Cystatin C mRNA was quantified using Real-time polymerase chain reaction (RT-PCR) on ABI PRISM® 7000 sequence detection system (Applied Biosystems). Primers and probes were obtained from Assays on-Design/Demand™ (Applied Biosystems) (Table II). Each primer pair was located on different exons of the investigated gene. Oligonucleotide probes were labelled with fluorogenic dye, 6-carboxyfluorescein (Fam). The PCR reactions were carried out in a 25 µl final volume containing final concentrations of ×1 Universal PCR Master Mix (Applied Biosystems), ×1 Assaymix (Applied Biosystems), 0.25 µmol L⁻¹ probe, 0.9 µmol L⁻¹ of forward and reverse primers, respectively, and 1 µl of the DNA template (10 ng/µl⁻¹). The thermal cycling conditions were initiated by uracil-N-glycosylase activation at 50°C for 2 min and an initial denaturation at 95°C for 10 min, and then 40 cycles at 95°C for 15 s, annealing at 60°C for 1 min. Two negative controls, without template, were included for every amplification. For each reaction, a duplicate assay was carried out. Transcripts of β-actin, a housekeeping gene, were quantified to normalize each sample. Quantification was achieved through a calibration curve obtained by serial 10-fold dilutions of the template DNA (0.08–80 ng). Results are expressed as relative values.
Cystatin C expression in the placenta

In situ hybridization

RNA probes

DNA templates were generated by PCR from cDNA cloned in pUC18 (Abrahamson et al., 1987) using bipartite primers consisting of an anti-sense T7 RNA promoter and a downstream gene-specific sequence primer or a sense T3 RNA promoter and an upstream gene-specific primer, NT (360–710). PCR reactions using 3 ng cDNA, 1 μmol L⁻¹ primers, 200 μmol L⁻¹ dNTPs, 3 mmol L⁻¹ MgCl₂, 10 mmol L⁻¹ Tris, pH 8.3, 50 mmol L⁻¹ KCl and 2.5 units Taq polymerase (Boehringer Mannheim, Mannheim, Germany) were amplified at 95°C for 1 min, 66.9°C for 1 min and 72°C for 1 min for 30 cycles, with a final extension at 72°C for 10 min. The templates were purified from agarose gels using QIA-Quick Gel Extraction Kit (Qiagen, Hilden, Germany) and thereafter sequenced using a cycle sequencing reaction kit ABI PRISM, BigDye™ (Applied Biosystems). Complementary RNA (cRNA) probes were transcribed from 25 ng of gel-purified DNA templates using 35S-UTP (800 Ci mmol⁻¹) (Amersham Biosciences Europe GmbH, Freiburg, Germany) and either T3 or T7 RNA polymerase according to the manufacturers instruction (Ambion MaxiScript, Ambion, Austin, USA) to generate sense and antisense probes as previously described (Bottalico et al., 2004).

In situ hybridization

The tissue sections were fixed, dehydrated and delipidated as previously described (Bradley et al., 1992). Sections were hybridized at 55°C for 24 h, with 2 × 10⁶ cpm of denatured 35S-cRNA probe per 80 μl of hybridization buffer (Bottalico et al., 2004). Slides were then washed to remove excess probe and thereafter apposed to Hyperfilm Biomax MR (Kodak, Rochester, USA) for 3 days before coating with nuclear track emulsion NTB (Kodak). After a 4-week exposure at 4°C, the slides were developed in Dektol (Kodak), fixed and counterstained with a Giemsa stain.

Immunohistochemistry

A polyclonal rabbit anti-human cystatin C (rabbit antiserum 8206) purified from urine was used for immunohistochemistry in a 1 : 2000 dilution (Abrahamson et al., 1986). A ready-to-use peroxidase-based kit EnVision + System-HRP (DAB) (Dako Denmark A/S, Glostrup, Denmark) was used for visualization according to the manufacturer’s instructions. The presence of cytotakin, an epithelial (trophoblast) marker, was visualized using a 1 : 1000 dilution of a mouse monoclonal anti-human cytotakin clone MNF 116 (Dako Denmark A/S).

Frozen sections were fixed with 4% formaldehyde in 0.1 mol L⁻¹ phosphate buffer for 5 min at room temperature. The primary and secondary antibodies were diluted in 1× PBS containing 1.5% normal goat serum and 0.25% Triton X-100. After a final rinse in PBS, followed by 0.1 mol L⁻¹ Tris–HCl (pH 8), the immune complexes were visualized in 0.5 mg ml⁻¹ 3,3’-diaminobenzidine (DAB) containing 0.03% H₂O₂ and counterstained with haematoxylin. Sections were dehydrated with serial ethanol washes and then coveredslipped with Mountex® (Histolab, Gothenburg, Sweden). All slides were evaluated by two independent investigators (S.H. and K.K.) blinded to the experimental condition (normal versus pre-eclampsia).

Western blot analysis

Placental tissue samples from five cases with severe pre-eclampsia and five controls were analysed. We also included amniotic fluid from three cases with severe pre-eclampsia and three controls.

Protein extraction

Tissue samples (150–200 mg) were crushed on dry ice and 1 ml of cold (4°C) lysis buffer was added. Samples were homogenized using a TissueLyser (Qiagen) at 4°C and then centrifuged at 8000 g for 10 min at 4°C. Supernatants were frozen and stored at −20°C. The amniotic fluid samples were analysed after brief centrifugation.

Protein concentration was measured using a spectrophotometric procedure (Peterson, 1977). Samples and the kaleidoscope mw-marker Bio-Rad 161-0324 (Bio-Rad, Hercules, USA) were diluted in LDS sample buffer (4×) (Invitrogen NP0007) (Invitrogen, Carlsbad, CA, USA) and MilliQ H₂O to yield a protein concentration of 10 ng μl⁻¹ and a total of 50 ng was added to each well. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) was carried out on a 12% Bis–Tris NuPAGE gel (Invitrogen NPO342BOX) on an Xcell Surelock™ MiniCell (Invitrogen E0002) at 200 V for 35 min. For quantification, we included serial dilutions (100, 50, 25 and 10 ng) of recombinant cystatin C (a gift from Dr Katarina Hakansson, Lund, Sweden).

After electrophoretic transfer to a PVDVF membrane (Bio-Rad 162-0184) at 30 V for 60 min, the membrane was blocked with dry milk (diluted in TBS-Tween) (Bio-Rad 170-6404) at 4°C over night. The blots were then incubated with primary polyclonal antibodies (rabbit antiserum 8206) 1 : 4000 for 1 h at room temperature. The blots were rinsed in TBS-Tween for 1 × 15 min followed by 3 × 5 min and incubated with secondary anti-rabbit IgG-HRP sc-2030 (SDS Santa Cruz Biotechnology, USA) diluted 1 : 10000 for 1 h at room temperature. The blots were rinsed as above and then subjected to enhanced chemiluminescence ECL+ (GE Healthcare Biosciences, USA). Autoradiographic film (Hyperfilm ECL, Amersham, USA) was applied to the blot for 30 s when satisfactory exposure was obtained. The film was scanned (GeneFlash) and the digitized images were quantified by densitometry (GeneTools) according to manufacturer’s instructions (Syngene, Cambridge, UK).

Serum cystatin C

Serum cystatin C was measured by an automated particle-enhanced immuno-turbidimetric method on a Hitachi Modular P analysis system with reagents (code No. LX002) obtained from Dako Denmark A/S, Glostrup, Denmark and according to the procedure recommended by the reagent producer. Total coefficient of variation was 2.1%.

Serum urate

Serum urate was measured by an enzymatic method on a Hitachi Modular P analysis system with reagents (code No. 1873426) obtained from Roche Diagnostics (Mannheim, Germany) and according to the procedure recommended by the reagent producer. Total coefficient of variation was 1.9%.

Microphotograph and figure preparation

Microphotographs were prepared using an Olympus BX 60 microscope equipped for dark-field and bright-field microscopy with a digital camera (Olympus DP50-CU). Captured images were assembled using Adobe Photoshop 7.0 and printed electronically.

Statistics

ANOVA and the Bonferroni/Dunn test were used to test for difference in characteristics between the groups (Table I). The Kruskal–Wallis and the Mann–Whitney U-test were used to evaluate the differences of relative cystatin C mRNA and to evaluate the differences of cystatin C protein between the groups, p-value < 0.05 (two-tailed) was considered statistically significant.

Table II. TaqMan probes used for real-time polymerase chain reaction

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Accession numbers</th>
<th>Size (nt)</th>
<th>Primers</th>
<th>Reporter</th>
<th>Quencher</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystatin C</td>
<td>NM_000099</td>
<td>&lt;150</td>
<td>Hs00969174_m1</td>
<td>Fam</td>
<td>None</td>
</tr>
<tr>
<td>β-Actin</td>
<td>NM_001101</td>
<td>&lt;150</td>
<td>Hs99999903_m1</td>
<td>Fam</td>
<td>None</td>
</tr>
</tbody>
</table>
Cystatin C antibody identified a 13.3 kDa protein corresponding to the size of cystatin C. Dense bands corresponding to cystatin C and faint double-bands believed to represent modified (truncated) cystatin C (Abrahamson et al., 1991) could be seen in both normal and pre-eclamptic placental tissues. Cystatin C was also detected in the amniotic fluid from both normal and pre-eclamptic pregnancy; however, no double-bands were seen in the amniotic fluid (Figure 3).

Quantification of the bands revealed significantly ($P < 0.05$) higher amounts of cystatin C protein for the five severe pre-eclamptic cases with a median of 30.9 ng (range 18.49–61.28 ng) compared with the five normal pregnancy cases with a median of 17.16 ng (range 9.59–20.66 ng). The median for the six amniotic fluid samples was 8.6 ng.

**Discussion**

In this study, we demonstrate an increased placental expression of cystatin C in pre-eclampsia, with a good correlation to the clinical severity of the disease.

An elevated serum level of cystatin C in pregnancy has been explained by altered renal handling of low molecular weight proteins in conjunction with a decreased GFR seen in late pregnancy and pre-eclampsia (Strevens et al., 2001, 2002). The maternal plasma level of cystatin C was found to be a good marker for the onset and severity of pre-eclampsia (Strevens et al., 2001). In a renal biopsy study, the serum level of cystatin C correlated to the degree of renal structural changes (glomerular endotheliosis) typically seen in pre-eclampsia (Strevens et al., 2003). In late pregnancy, the serum level of cystatin C has been closely correlated to third trimester changes in the GFR in both normal pregnancy and pre-eclampsia (Strevens et al., 2002).

However, since the serum concentration of cystatin C remains virtually unchanged throughout the first and second trimester, when renal perfusion and filtration undergo dramatic increases (Conrad and Lindheimer, 1999), it is hard to believe that the serum level of cystatin C in pregnancy solely should depend on the renal filtration rate. An increased synthesis of the protein, either by the feto-placental unit or in a more generalized matter, has been proposed as alternative explanations. Our present findings suggest that placental synthesis contributes to the elevated serum levels of cystatin C seen in pre-eclampsia.

In the second and third trimesters the placenta increases in size and weight to be able to meet increasing demands from the fetus, coinciding with a gradual increase in the maternal serum concentration of cystatin C. In this study, except for the severe early-onset group, the groups did not differ with respect to placental weight.

In normal pregnancy, the serum level of cystatin C remains unchanged in early- and mid-pregnancy before a gradual increase to a mean of 1.16 mg l$^{-1}$ at term (Cataldi et al., 1999; Galteau et al., 2001; Strevens et al., 2001). The fetal plasma level of cystatin C is 30–40% above the maternal level throughout pregnancy (Cataldi et al., 1999; Finney et al., 2000). In amniotic fluid, the concentration of cystatin C decreases throughout pregnancy to a mean of 0.44 mg l$^{-1}$ at term (Mussap et al., 2002). The amniotic fluid level of cystatin C has previously not been investigated in pre-eclampsia. In the present study, amniotic levels were on average 1.15 mg l$^{-1}$.

The increased fetal plasma level of cystatin C has been explained by immature (decreased) renal degradation and trans-placental passage of the protein has been rendered unlikely (Finney et al., 2000; Mussap et al., 2002). We speculate that cystatin C synthesized and secreted by trophoblast cells could enter both fetal and maternal circulation and thereby contribute to elevated maternal and fetal plasma levels in late pregnancy and pre-eclampsia. Increased levels of, the less active, truncated form of cystatin C may be due to the inflammation seen in the pre-eclamptic placenta. The absence of truncated cystatin...
C in amniotic fluid indicates that the native form may be more protected against inflammatory truncation (Abrahamson et al., 1991; Buttle et al., 1991). Future studies will have to confirm the origin, form and significance of cystatin C in amniotic fluid.

Since cystatin C is known to be expressed in most human tissues including kidney, liver, pancreas, intestine, stomach, lung and placenta (Abrahamson et al., 1990), an increased expression in cells outside the placenta could also contribute to the elevated circulatory levels seen in normal pregnancy and pre-eclampsia.

The role of proteinases and their inhibitors in pre-eclampsia has been sparsely studied, involving predominantly matrix metalloproteinases (MMPs) and their inhibitors, tissue inhibitors of metalloproteinases (TIMPs). A protease:inhibitor imbalance has been suggested to explain failure of the trophoblasts to invade maternal decidual blood vessels in pre-eclamptic pregnancy (Vettraino et al., 1996; Gallery et al., 1999; Pang and Xing, 2003; Merchant and Davidge, 2004). Recent studies report imbalance in the MMP-2:TIMP-1 ratio and the cathepsin:cystatin C ratio in patients who subsequently developed pre-eclampsia (al-Hameri et al., 2001; Myers et al., 2005).

In early placentation the cysteine proteinases, cathepsins B and L, have been localized to the mature invasive trophoblast giant cells and cystatin C has been localized as a major product in the decidualizing stroma, indicating that cathepsins B and L are necessary for normal implantation/placentation controlled by a coordinated expression of cystatin C within the implantation site (Afonso et al., 1997; Divya et al., 2002). In a study of early human placentation, cystatin C and several cathepsins were highly expressed on the surface of cells of decidua and by trophoblasts, indicating cystatin

![Figure 2. In situ hybridization showing cystatin C mRNA expression in normal placental tissue. Bright field (A and D) and dark field (B and E). The figures show chorionic villi and parts of the basal plate containing extravillous trophoblast (evt) cells from the central part of placenta in lower (A and B) and higher (D and E) magnification. The villous trophoblasts show low or moderate expression of cystatin C mRNA, whereas the evts show high expression of cystatin mRNA (arrows). Immunohistochemical staining for cystatin C protein of adjacent sections (C and F) showed a similar pattern of distribution. Immunohistochemical staining of adjacent sections for cytokeratin (G and H) verified that the cystatin C mRNA positive cells were not of mesenchymal origin. Scale bars: C = 100 μm, F = 30 μm, H = 50 μm.](https://academic.oup.com/molehr/article-abstract/13/3/189/1024129)

![Figure 3. Western blotting of cystatin C in placental tissue from normal pregnancy and severe pre-eclampsia and amniotic fluid from severe pre-eclampsia. The bands are seen at 13.3 kDa corresponding to the size of the protein. Standards of recombinant cystatin C were used for quantification of the bands by densitometry (Figure 4). Double bands (arrow) were only seen in placental samples.](https://academic.oup.com/molehr/article-abstract/13/3/189/1024129)
C-cystatin interaction to be important for the control and regulation of the invasiveness of the trophoblast (Nakanishi et al., 2005).

Our findings suggest that the placental production is an important source of cystatin C in the maternal circulation. The finding of specific cystatin C expression in the extravillous trophoblasts could indicate a specific role for this protein in the pathogenesis of pre-eclampsia. Future studies are needed to relate cystatin C expression to the expression of cathepsins in normal and pre-eclamptic placentation and to determine the significance of placental expression in relation to the increased serum concentration of the protein seen in late pregnancy and pre-eclampsia.

In conclusion, placental expression of cystatin C is increased at the mRNA and protein level in pre-eclampsia, suggesting an increased synthesis and secretion of cystatin C protein that could contribute to the elevated maternal plasma levels observed in pre-eclampsia.

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References


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