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Expression of Collapsin Response Mediator Protein 1 in Placenta of Normal Gestation and Link to Early-Onset Preeclampsia

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Abstract

A human isoform of Collapsin Response Mediator Protein (CRMP) family proteins, CRMP-1, has been identified as a novel invasion suppressor. The aim of this study was to determine CRMP-1 expression pattern in placentas during normal pregnancy and elucidate the clinical significance of CRMP-1 expression in the placentas of women with early-onset preeclamptic pregnancies. We recruited 66 normal healthy pregnant Chinese women and 60 Chinese patients with preeclampsia [early-onset pre-reclampsia(ePE), n = 30 and late-onset preeclampsia(lPE) n = 30]. Gestational age-matched normal healthy pregnant women were used as controls of early-onset and late-onset preeclampsia, which were 23-33 + 6weeks, n = 18 and control B: 34-40 weeks, n = 20). Quantitative RT-PCR, Western blot analysis and immunohistochemistry were used to analyze the expressions of CRMP-1 in placentas. Expression of CRMP-1 was detected in syncytio- and cytotrophoblasts of all groups using immunohistochemistry. CRMP-1 was most abundantly expressed in syncytiotrophoblasts, moderately in cytotrophoblasts and the intermediate trophoblasts especially in the first trimester. The placental expression of CRMP-1 is particularly striking in the first trimester and decreases throughout pregnancy. There is a significant increase in CRMP-1 expression in the placenta of ePE but not of lPE, as compared to gestational-matched controls. The aberrant upregulation of CRMP-1 expression may link to the mechanism of developing ePE.

Keywords

collapsin response mediator protein 1 (CRMP-1), preeclampsia, placenta, early onset

Introduction

Preeclampsia (PE) is a pregnancy-specific syndrome characterized by hypertension and proteinuria, which affects 9.4% of all pregnancies in China and is the second leading cause of maternal and perinatal morbidity and mortality in China.[1] Although the primary mechanism of PE is still unknown, histological examinations of placental bed biopsies from PE women have demonstrated that the PE placentus is characterized by abnormal trophoblast invasion (also termed shallow implantation of placenta), deficient maternal spiral artery modification, and increased apoptosis of trophoblastic cells. In addition, limited invasion of trophoblasts into superficial decidua was responsible for placental ischemia, endothelial cell dysfunction, and subsequent PE.[2,3] However, the understanding of trophoblast invasion is still in its infancy, despite the identification of numerous genes involved in its regulation.

The severity of the clinical manifestations of PE is directly related to the time of onset. According to gestational age of PE onset, PE includes 2 kinds, one is early-onset PE (ePE) with an onset before 34 weeks of gestation and the other is late-onset PE (lPE) with an onset at or after 34 weeks of gestation.[4,5] Emerging evidence suggests that the ePE and lPE may represent distinct entities with different physiopathology and rather different stages of a single disease.[6,7]

The early-onset form is more severe, frequently leading to delivery of growth-retarded premature babies or poor outcomes for the newborns and the mothers.[8] Early-onset PE is strongly associated with dysfunction of trophoblast invasion and subsequently abnormal placental development, whereas lPE is believed to occur secondary to maternal endothelial cell injury.[9] This distinction is further supported by the fact that ePE has been associated with abnormal placental morphology,[10] while placenta from lPE are morphologically similar to those from healthy pregnancies.

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Table 1. Clinical Characteristics of All Pregnant Women.[a,b,c,d,e]

	First Trimester, 5-13^{+6} weeks (n = 20)	Second Trimester, 14-27^{+6} weeks (n = 16)	Third Trimester, 28-40 weeks (n = 30)	Control A, 23-33^{+6} weeks (n = 18)	ePE, 23-33^{+6} weeks (n = 30)	Control B, 34-40 weeks (n = 20)	lPE, 34-39 weeks (n = 30)
Maternal age, years	29.47 ± 0.94	30.44 ± 0.69	30.37 ± 0.62	31.00 ± 0.66	29.47 ± 0.94	29.47 ± 0.94	29.47 ± 0.94
Gestational age, weeks	9.30 ± 0.40	21.75 ± 0.90	34.82 ± 0.72	27.76 ± 0.68	29.38 ± 0.46	37.28 ± 0.45	37.22 ± 0.24
SBP, mm Hg	102.43 ± 1.38	99.77 ± 1.13	106.03 ± 1.58	108.5 ± 2.73	167.5 ± 2.45[f]	112.9 ± 1.81	163.7 ± 2.88[g]
DBP, mm Hg	72.77 ± 1.52	69.60 ± 1.37	74.40 ± 1.50	64.2 ± 7.5	106.4 ± 13.6[f]	68.0 ± 8.2	95.1 ± 6.8[g]
Proteinuria	N/A	N/A	N/A	N/A	++−+++	N/A	+−+++

Abbreviations: PE, preeclampsia; SBP, maximal systolic blood pressure; DBP, maximal diastolic blood pressure; ePE, early-onset preeclampsia; lPE, late-onset preeclampsia.
[a]Values are shown as mean ± SE.
[b]Control A and control B are gestational age-matched controls of normal pregnancies for ePE and lPE, respectively.
[c]There were no significant differences in maternal age and the blood pressure among the 3 groups of normal healthy pregnant women ($P > .05$).
[d]There were no significant differences in maternal age and delivery age between early and late-onset preeclampsia group and the matched control groups ($P > .05$).
[e]There was a marked elevation in systolic and diastolic blood pressure in the preeclampsia groups as compared to the control group ($P < .05$).
[f]$P < .05$, ePE versus control A.
[g]$P < .05$, lPE versus control B.

The collapsing response mediator proteins (CRMPs), also known as turned on after division protein (TOAD), UNC-33-like phosphoprotein (Ulip), dihydropyrimidinase-related protein, and TOAD/Ulip/CRMP (TUC), belong to a family of 5 cytosolic phosphoproteins (CRMP-1, CRMP-2, CRMP-3, CRMP-4, and CRMP-5), which are primarily expressed in the nervous system during embryogenesis.[11,12] They are responsible for semaphoring 3A-mediated growth cone collapse of neuronal cells.[11-13] These phosphoproteins have 50% to 70% sequence homology with one another, being approximately 60 to 66 kDa in size and can form heterotetramers.[12,14-17] Shih et al and Chen et al have reported that CRMP-1 is an invasion suppressor, showing that reduced CRMP-1 expression is associated with early metastasis and poor survival in patients with lung cancer.[13,18,19]

EST (expressed sequence tags) profiling revealed expression of CRMP-1 in placental tissues, but its cell-type localization in human placenta and significance in placental pathologies have not been defined. Given that CRMP-1 has been implicated in inhibiting cancer cell invasion and that PE has been associated with trophoblast shallow invasion,[2] we hypothesized that CRMP-1 might contribute to the pathogenesis of ePE. In this report, we determined the expression and localization of CRMP-1 in placental tissues from gestational age-matched normal and preeclamptic pregnancies and explored the potential pathogenesis of CRMP-1 protein in PE.

Materials and Methods

The study was conducted at Shengjing Hospital, China Medical University, under the policy of the local Ethics Committee of Medical Faculty and with institutional review board approval for tissue collection and preparation. Written informed consent was obtained from each patient included in the study.

Study Population

All patients were recruited from Shengjing Hospital during the time period of August 2011 to July 2012. Only women with single pregnancies were included. Women with chronic hypertension, collagen vascular disease, any evidence of intrapartum infection, or other pregnancy complications such as fetal anomalies, chromosomal abnormalities, or diabetes mellitus were excluded from this study. In each case, gestational age was confirmed by the last menstrual period or, where doubt existed, by an ultrasound examination before 14 weeks of gestation.

The trial enrolled 66 normal healthy pregnant women (gestational age, 5-40 weeks) and 60 patients with PE (gestational age, 23-40 weeks). The normal pregnant women were divided into 3 groups according to gestational weeks (first trimester group: 5-13^{+6} weeks, n = 20; second trimester group: 14-27^{+6} weeks, n = 16; and third trimester group: 28-41 weeks, n = 30). First trimester group women underwent elective (surgical) termination of pregnancy. Second trimester group women gave birth due to cervical incompetence. Third trimester group women gave birth by vaginal or cesarean section.

Two different groups of cases were included (1) ePE, which consisted of women diagnosed and delivered before gestational week 34 (23-33^{+6} weeks, n = 30) and (2) lPE, which consisted of women diagnosed and delivered after gestational week 34 (34-39 weeks, n = 30). Preeclampsia was diagnosed according to international criteria as described previously.[8]

Antihypertensive treatment was commenced in both groups of women with PE if the systolic blood pressure rose above 160 mm Hg, the diastolic rose above 105 mm Hg, or both conditions existed.

As described previously,[8] we used respective gestational age-matched normal healthy pregnant women as controls to ePE and lPE groups (control A: 22-33^{+6} weeks, n = 18 and

control B: 34-40 weeks, n = 20). The clinical characteristics of patients are summarized in Table 1.

Placental Tissue Collections

Immediately after delivery or abortion, chorionic villi and placenta were cut. Tissue treatment, RNA and protein isolation, and immunohistochemical staining were carried out as described previously.[8]

RNA Extraction and Real-Time Polymerase Chain Reaction

These were carried out essentially as described previously.[8] The polymerase chain reaction (PCR) primers for human genes are as follows: collapsin response mediator protein 1 (NM_001313), product size: 203 bp, forward: 5′-ATCGCCAAGGACTGACTGAG-3′, reverse: 5′-GAGGATGCTTCTCTGCAACC-3′ , and β-actin (NM_001101), product size: 101 bp, forward: 5′-TTGCCGACAGGATGCAGAA-3′, reverse: 5′-GCCGATCCACACGGAGTACT-3′.

Western Blot Analysis

These were carried out as described previously.[8] The following primary antibodies were used: rabbit anti-human CRMP-1 monoclonal antibodies (1:400; Abcam, Hong Kong, China) and rabbit polyclonal to β-actin antibody (1:1000; Abcam). β-Actin expression served as a loading control.

Immunohistochemistry

These were done essentially as described previously,[8] except that the sections were incubated in a moist chamber for 1 hour at room temperature with CRMP-1 primary antibody.

Statistical Analysis

Data were expressed as the mean ± standard error. Statistical significance between the groups were assessed by the Mann-Whitney *U* test for nonparametric independent 2-group comparison with the program SPSS 16.0 for Windows (SPSS Inc, Chicago, Illinois). *P* value <.05 was regarded as statistically significant.

Results

Characteristics of Study Population

We recruited 30 women with ePE, 30 women with lPE, and 66 women with uncomplicated pregnancies (gestational age, 5-41 weeks). The control A group included 8 healthy pregnant women in the second trimester (23-27^{+6} weeks) and 10 in the third trimester (28-33^{+6} weeks). The control B group included 20 healthy pregnant women in the third trimester. The baseline characteristics of the 2 groups are outlined in Table 1. There were no significant differences in the maternal age and blood pressure between the groups ($P > .05$). There were no significant differences in maternal age and delivery age between the ePE and lPE groups and the matched control groups ($P > .05$). There was a marked elevation in the systolic and diastolic blood pressure in the PE groups when compared to the control groups ($P < .05$).

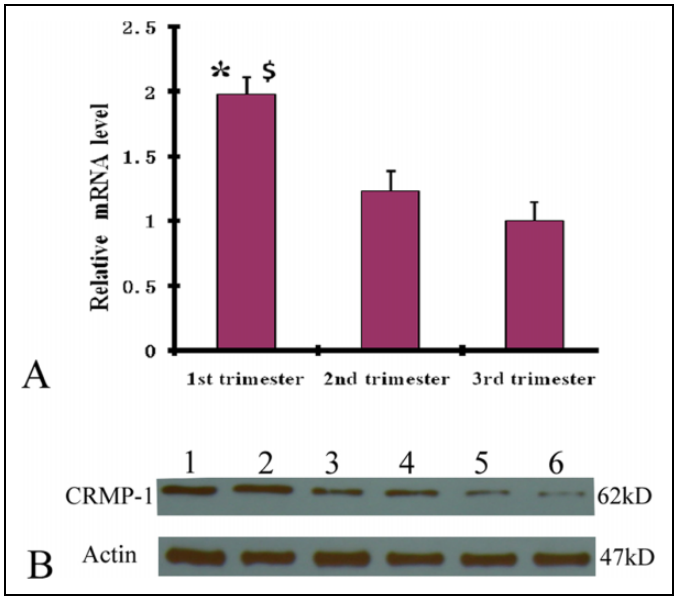

Figure 1. Expression of collapsin response mediator protein 1 (CRMP-1) in chorionic villi or placenta of normal pregnant women. A, Quantitative real-time polymerase chain reaction (RT-PCR) analysis of CRMP-1 messenger (mRNA) in chorionic villi or placenta at different stages of normal pregnancy. The results demonstrated a significant descending tendency with progression of gestation. The level of CRMP-1 mRNA was significantly higher in early pregnancy compared with those of the second (*$P < .05$) and third trimesters ($^{\$}P < .05$). Data are represented as mean ± standard error (SE) after normalization to β-actin. *$P < .05$, significantly decreased/increased compared with the second trimester. $^{\$}P < .05$, significantly decreased/increased compared with the third trimester. B, Representative Western blot results of CRMP-1 in chorionic villi or placenta at different stages of normal pregnancy. Lanes 1 and 2, first trimester; lanes 3 and 4, second trimester; lanes 5 and 6, third trimester. The level of CRMP-1 in chorionic villi was the highest during the first trimester and declined with the progression of gestation. Total protein of 30 μg was loaded onto each lane on a 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel. β-Actin was used as a loading control.

Expression of CRMP-1 in Chorionic Villi and Placenta Showed a Decreasing Tendency During Normal Pregnancy

We examined expression of CRMP-1 using quantitative real-time PCR (RT-PCR) in 20 chorionic villi from women in the first trimester (5-13^{+6} weeks), 16 placenta from women in the second trimester (14-27^{+6} weeks), and 30 placenta from women in the third trimester (28-41 weeks). The messenger RNA (mRNA) level of CRMP-1 was significantly higher ($P = .003$, $P = .001$) in the first trimester compared to that in the other groups. Specifically, the CRMP-1 mRNA level in the first trimester was higher by 1.61- and 1.98-fold when

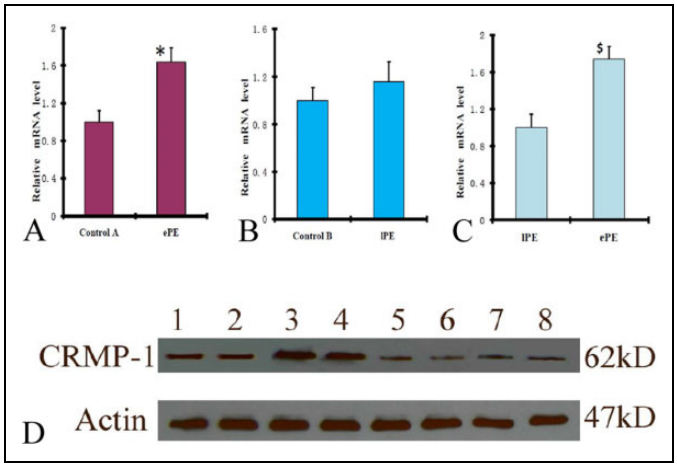

Figure 2. Expression of collapsin response mediator protein 1 (CRMP-1) in early-onset and late-onset preeclamptic placenta. A-C, Quantitative real-time polymerase chain reaction (RT-PCR) analysis of CRMP-1 messenger (mRNA) in the preeclamptic pregnancies compared with the respective matched control groups. A, The results demonstrated a significant upregulation of CRMP-1 transcripts in the placenta of early-onset preeclampsia (ePE) compared with control A. B, There was no significant difference between the late-onset preeclampsia (lPE) and control B. C, The transcript level of CRMP-1 was significantly higher in ePE placenta than that in lPE. Data are represented as mean ± standard error (SE) after normalization to β-actin. *$P < .05$, significantly decreased/increased compared with control A. $^{\$}P < .05$, significantly decreased/increased compared with lPE. D, Representative Western blot results of CRMP-1 in placenta of patients with ePE and lPE compared with matched controls. Lanes 1 and 2, control A; lanes 3 and 4, ePE; lanes 5 and 6, control B; lanes 7 and 8, lPE. The CRMP-1 protein was significantly increased in early-onset preeclamptic placenta. The late-onset preeclamptic placenta revealed no significant change in expression of CRMP-1 protein compared with the matched control. Total protein of 30 μg was loaded into each lane on a 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel. β-Actin was used as a loading control.

compared to that in the second trimester and third trimester ($P < .05$; Figure 1A), respectively.

A significant increase in the level of the CRMP-1 protein was also seen in the first trimester chorionic villi compared to that in the other groups (the second and third trimesters) as determined by Western blot analysis (Figure 1B).

Upregulation of Expression of CRMP-1 in Placenta From Women With ePE

We examined 60 pathological placenta for the expression of CRMP-1 using quantitative RT-PCR: 30 with ePE ($23\text{-}33^{+6}$ weeks, ePE) and 30 with lPE (34-39 weeks, lPE). The expression data of the pathological placenta were compared with those of gestational age-matched control placenta: control A ($n = 18$) was used for ePE and control B ($n = 20$) for lPE.

The mRNA level of CRMP-1 was significantly higher ($P = .004$) in ePE compared to that in control A. Specifically, the CRMP-1 mRNA level in ePE was upregulated by 1.64- and 1.74-fold when compared to control A and lPE (Figure 2A and C), respectively. There was no significant upregulation of CRMP-1mRNA in lPE when compared to control B (Figure 2B). A significant increase in the protein level of CRMP-1 was also seen in the ePE placental tissues compared to those in the other groups (control A, control B, and lPE) as determined by Western blot analysis (Figure 2D).

Localization of CRMP-1 Proteins in Chorionic Villi or Placenta of Normal Pregnancy

Expression of CRMP-1 was detected in syncytio- and cytotrophoblasts of the whole stage of pregnancy using immunohistochemistry (Figure 3A-C). Collapsin response mediator protein 1 was most abundantly expressed in syncytiotrophoblasts, moderately in cytotrophoblasts, and intermediate in trophoblasts in the first trimester (Figure 3A). The expression in trophoblasts was significantly higher in the first trimester than in the second and third trimesters, consistent with Western blot results (Figure 1B).

Localization of CRMP-1 Proteins in ePE and lPE Placenta

Expression of CRMP-1 was detected in syncytio- and cytotrophoblasts of the PE groups and matched controls using immunohistochemistry (Figure 3E-H). In control A and ePE, CRMP-1 was most abundantly expressed in syncytiotrophoblasts and moderately in cytotrophoblasts (Figure 3E and F). Expression of CRMP-1 in trophoblasts was significantly increased in ePE when compared to control A, consistent with Western blot results (Figure 2D). In contrast, expression of CRMP-1 in syncytiotrophoblasts was not different between lPE and control B (Figure 3G and H), consistent with Western blot results (Figure 2D).

Discussion

This is the first report on expression of CRMP-1 in placenta of both normal and preeclamptic pregnancies. We show that CRMP-1, at both mRNA and protein levels, is expressed in trophoblasts of both normal individuals and patients with PE. The expression is high in the first trimester and decreases as pregnancy progresses. Importantly, we detect a significant increase in expression of CRMP-1 in the placenta of ePE but not lPE, when compared to gestational-matched controls. The expression of CRMP-1 in the trophoblasts suggests that CRMP-1 may play an important role in regulating implantation and that its upregulation may contribute to the pathogenesis of ePE.

The extravillous cytotrophoblasts leave the basal membrane, invade the decidua, penetrate the spiral arteries, and replace the smooth muscle and endothelium.[7] These conversions transform the low-flow and high-resistant spiral artery to high-flow and low-resistance vessels that meet the requirements of the fetus.[20] This transformation is necessary for a successful pregnancy. Inadequate and incomplete trophoblast invasion of spiral arteries is believed to correlate with ePE.

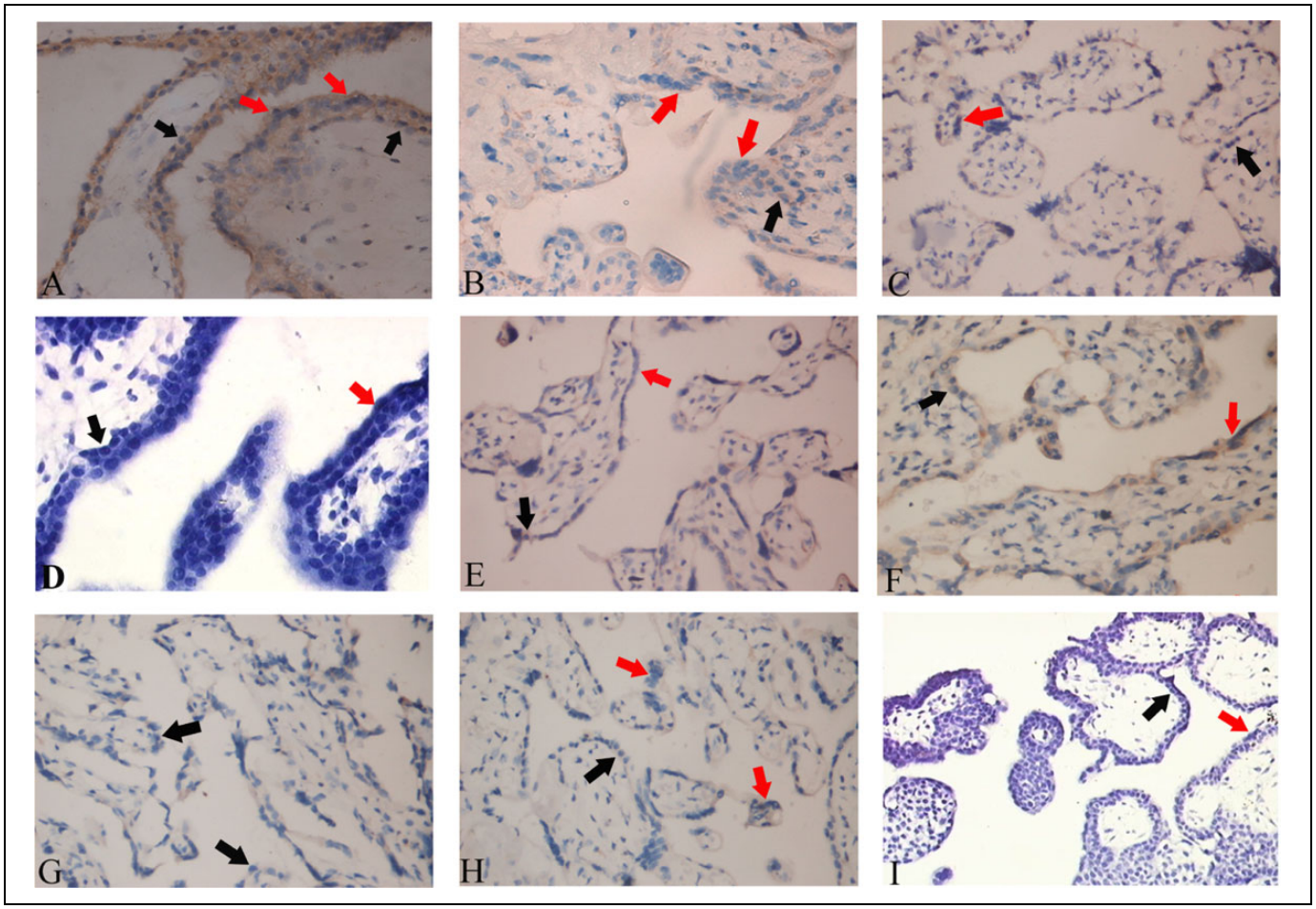

Figure 3. Immunohistochemistry (IHC) of expression of collapsin response mediator protein 1 (CRMP-1) in the chorionic villi or placentas of first trimester, ×400 (A), second trimester (B), third trimester (C), negative control (D), control A (E), early-onset preeclampsia (ePE; F), control B (G), late-onset preeclampsia (lPE; H), and isotype control (I). The CRMP-1 staining is present in both syncytiotrophoblasts and cytotrophoblasts from (A-C, E-H). Trophoblasts from the first trimester had higher expression of CRMP-1 when compared to the other 2 groups (A-C). Trophoblasts from ePE had higher expression of CRMP-1 when compared to control A (E and F). lPE had the same level of expression of CRMP-1 as compared with control B (G and H). ePE had significantly higher expression of CRMP-1 as compared with control A and lPE (F and H). Red arrows indicate the syncytiotrophoblast; black arrows indicate the cytotrophoblast.

Members of the CRMP family of phosphoproteins may mediate semaphorin/collapsin-induced growth cone collapse and are involved in axonal guidance and neuronal differentiation.[12,14] Five members of the CRMP gene family (CRMP-1, CRMP-2, CRMP-3, CRMP-4, and CRMP-5), encoding closely related 60 to 66 kD proteins, have been cloned.[12,14] However, each CRMP has a unique transcript and is expressed in a distinct pattern during development. Transcription of CRMP genes is differentially regulated.[21,22] Previous studies demonstrated that members of the semaphorin/collapsin families might control the movement of cells.[23] EST profiling showed expression of CRMP-1 in placental tissues, but its localization in human placenta and expression pattern during pregnancy are unknown until now. In this study, we detected expression of CRMP-1 at both syncytiotrophoblasts and cytotrophoblasts at different stages of normal pregnancy. In the first trimester, CRMP-1 was also detected in the intermediate trophoblasts. Expression of CRMP-1 peaks in the first trimester and declines sharply as pregnancy progresses. We propose that CRMP-1 may contribute to regulation of embryo implantation and placental development.

Most studies have been performed using samples from lPE or mixed PE. Only a limited number of studies were focused on ePE versus lPE. In addition, some studies define ePE as clinical manifestations occurring before 32[24] or 37 weeks of gestation.[25] As previously described,[8] in this study, we separated PE into ePE and lPE groups by 34 weeks of gestation.

Evidence exists that CRMP-1, as an invasion-suppressor gene, plays an important role in cancer invasion and metastasis. Previous study showed that reduced expression of CRMP-1 was statistically significantly associated with advanced stage of human lung cancer, lymph node metastasis, early postoperative relapse, and shorter survival.[18] The mechanisms by which CRMP-1 regulates tumor cell invasion have been investigated. Shih et al found that expression of CRMP-1 was inversely associated with the invasive activity of lung cancer cells.[18] Overexpression of CRMP-1 reduced the invasiveness of lung cancer

cells in vitro.[18] It was proposed that CRMP-1 may participate in cell cycle regulation by acting as a component of the mitotic machinery or interacting with other proteins.[18] We found expression of CRMP-1 in trophoblasts that had fused and become noninvasive as gestation progressed (refer to Figure 1A, B, and Figure 3A-C). This would be consistent with a role of CRMP-1 in inhibiting trophoblast invasion.

In the present studies, we show that expression of CRMP-1 was significantly increased at both RNA and protein levels in ePE, compared to either lPE or gestational age-matched controls. Importantly, there was no significant difference in CRMP-1 expression levels between lPE and the gestational age-matched controls.

Collapsin response mediator protein 1 inhibits the migration and invasion of lung cancer cells.[18] The expression of CRMP-1 decreases with pregnancy (this report). The ePE is associated with trophoblast shallow invasion.[7] We therefore postulate that upregulation of CRMP-1 may contribute to the underlying pathogenesis of ePE but not lPE.

The limitations of our study include lack of maternal serum data and being a retrospective in nature. Future prospective studies will include testing serum CRMP-1 levels of pregnant women for possible prediction of development of PE. Finally, the possibility that the observed change in the expression of CRMP-1 in ePE might be a consequence rather than cause of the disease cannot be excluded. It is difficult to rule in or rule out this possibility due to ethical constraints imposed by the time of sample collection at the onset of illness. However, we will further explore the mechanism of CRMP-1 in ePE using in vitro trophoblast cell migration and invasion assays as well as animal models with CRMP-1 deficiency and placental tissue samples from humans with mutant CRMP-1 genes.

In summary, we demonstrate for the first time that CRMP-1 is expressed in trophoblasts during normal pregnancy with decreasing expression as pregnancy progresses. We also show that expression of CRMP-1 is upregulated in ePE, relative to lPE and gestational age-matched normal controls. Our findings suggest that elevated expression of CRMP-1 may contribute to the underlying mechanisms of ePE and also support the concept that ePE and lPE are likely 2 different entities. Further studies might provide clues leading to prevention, early diagnosis, and treatment of PE.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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