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Placental endothelin-converting enzyme-1 is decreased in preeclampsia

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Abstract

Endothelin-converting enzyme-1(ECE-1) is a key regulatory enzyme in the processing of endothelin-1 (ET-1). We quantified and localized ECE-1 in normal and preeclamptic placentas. Normal (n=6) and preeclamptic (n=6) placentas were serially sectioned for immunofluorescence (IF). Cell type specific markers identified endothelial, trophoblast, macrophage, smooth muscle, and fibroblast cells. Quantitative analyses were performed by western blot and ELISA. IF identified ECE-1 expression within the stroma and villous space. Cellular localization of ECE-1 was limited to endothelial membranes. There was significantly less ECE-1 in preeclamptic placentas, suggesting ECE-1 is important for proper regulation of ET-1 within the placenta.

Keywords

Endothelial cells; Endothelin; Endothelin-converting enzyme 1(ECE-1); Placenta; Preeclampsia; Trophoblast

1. Introduction

The endothelin system is thought to play a central role in the pathogenesis of preeclampsia [1,2]. Endothelin-converting enzyme-1 (ECE-1) is a membrane bound, predominantly extracellular, proteolytic processing enzyme that cleaves big endothelin-1 to its active form, endothelin-1 (ET-1) [3]. ET-1 is considered the most potent vasoconstrictor and has been isolated in endothelial and trophoblast cells in the placenta [4–6]. Unregulated production of ET-1 may be involved in disease states associated with excessive vasoconstriction, including preeclampsia [1,7,8].

ECE-1 exists primarily as a plasma membrane-bound ectoenzyme [9–12]. ET-1 signals via ET Receptors A and B. ETR_A are dominant in smooth muscle cells, while ETR_B are found within the endothelial, and to a lesser extent smooth muscle cells [6,13]. ET-1 promotes

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Declaration of Competing Interest

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vasoconstriction when acting on smooth muscle cells, but vasodilation when acting via endothelial ETR_B [5,14,15]. Global ECE-1 knock out mouse studies have a lethal phenotype despite the presence of ET-1, suggesting the importance of ECE-1 as a local regulator of ET-1. The goal of the present study was to characterize the expression of ECE-1 in normal and preeclamptic placentas.

2. Methods

2.1. Recruitment

Written informed consent was obtained (February 2016-January 2018). Preeclampsia was defined according to International Society for the Study of Hypertension in Pregnancy (ISSHP). Exclusion criteria included fetal anomalies, multiple gestations, diabetes, other major medical or obstetrical complications. The study was approved by the IRB.

2.2. Tissue preparation

Within 30 min of delivery, a placental biopsy was taken two centimeters from the cord insertion including all three layers: maternal, fetal and basal. Samples were snap frozen and stored at −80 °C.

2.2.1. Histology—Full thickness 4m frozen sections were stained using standard methods (H&E, IF). Antibodies against cell-specific markers were used: rabbit polyclonal IgG against ECE-1 (ab71829, Abcam), mouse monoclonal IgG to CD31 (ab24590, Abcam), mouse monoclonal IgG to cytokeratin 18 (ab668, Abcam), mouse monoclonal IgG to CD68 (ab955, Abcam), mouse monoclonal IgG to TE-7 (CBL271, Sigma Aldrich), mouse monoclonal IgG to alpha smooth muscle actin (ab7817, Abcam). Sections were blocked with 10% goat serum. Biotinylated goat anti-rabbit IgG Alexa Fluor 488 (A11034) (Invitrogen, ThermoFisher Scientific) was used as the secondary antibody for ECE-1 and goat anti-Mouse IgG (H&L) Alexa Fluro594 (R37121, Invitrogen, ThermoFisher Scientific) for the remaining. Counterstaining with 4′,6-diamidino-2-phenylindole (DAPI) was performed. Negative controls omitted primary antibody. Imaging was performed using Carl Zeiss Axioimager Z1 Axiocam and Axiovision™.

2.2.2. Western blot—Protein concentrations were determined with Bradford Assay (BioRad) and total protein (37μg) and negative control were loaded in SDS-PAGE (10% polyacrylamide) gel, electrophoretically separated, transferred onto nitrocellulose membranes and blocked overnight. Primary and secondary antibodies were added. Immunolabeled bands were visualized using chemiluminescence reaction (RPN2232 GE Healthcare Life Sciences). Densitometry analysis was performed with ImageJ 1.49i. The intensity of signal was normalized to β-Actin (Fig. 1).

2.2.3. Elisa—ECE-1 was measured (total protein 100 μg) using a sandwich ELISA kit (catalogue number abx151373) (Abbexa Ltd). The standard curve was generated from serial dilutions of recombinant ECE-1 (2000–31.25 pg/mL).

2.2.4. Statistics—Relative band densities were compared between groups (student's *t*-test Excel 2016). Differences were considered significant when $p < 0.05$.

3. Results and discussion

Baseline characteristics are shown in Table 1. IF of normal (n = 6) and preeclamptic placentas (n = 6) revealed positive staining for ECE-1within the main stem villi. Cell type specific staining revealed ECE-1 occasionally localized within endothelial cells in both groups and ECE-1 was not found within other cell types evaluated, although close proximity of ECE-1 to these cell types was suggested (Fig. 1).

Immunoblotting produced bands at the expected 130 kDa, confirming the presence of ECE-1 in both normal and preeclamptic placentas (Fig. 1). Densitometry indicated ~ 60% less ECE-1 present in the preeclamptic compared to control group (0.55 ± 0.17 and 1.36 ± 0.21 ECE-1/β-Actin, respectively, $p = 0.008$, Fig. 1) and confirmed with ELISA (59.9 ± 23.9 and 119.0 ± 37.8 ng/mg, respectively, $p = 0.004$, Fig. 1).

Only one prior study of ECE-1 expression in human placentas was performed, which reported ECE-1 presence in five distinct cell populations [16]. Our work showing ECE-1 localization occasionally within endothelial cells is consistent with other investigations showing ECE-1 within endothelial cells in the lung [3,4,17,18]. One possibility that could account for this finding is if a portion of the placental ECE-1 is located extracellularly, suggesting a secreted isoform exists, which has been proposed previously [19–22].

Previous studies of ET-1 expression in normal and preeclamptic placentas have been conflicting. One reported decreased ET-1 in preeclamptic placentas, while another found increased expression [23,24]. Importantly, a recent study showed ECE-1 expression in both preeclamptic and control placentas but found no difference in mRNA levels between the groups. They evaluated message of ECE-1, not protein levels and did not specify a cell population [24].

Others reported higher plasma levels of ECE-1 and ET-1 in preeclamptic patients when compared to controls [25]. However, they did not identify the source of ET-1 or ECE-1.

A mid-late gestation lethal phenotype results from a mouse knock out model of ECE-1, demonstrating the importance of ECE-1 in normal vascular development [3]. This occurs in spite of the presence of ET-1, suggesting the need for highly localized ET-1 signaling [3].

Our work has several limitations. First, the current study included small numbers and was not matched for route of delivery. We did not address whether ET-1 levels differ between normal and preeclamptic pregnancies nor did we address the source of circulating ET-1. Moreover, our findings do not exclude the possibility that enzymes other than ECE-1 catalyze ET-1 activation in preeclampsia. Our work focused on localization and quantification of ECE-1 expression in preeclamptic and control placentas and should be considered preliminary.

In conclusion, ECE-1 is expressed within human placentas. Significantly less ECE-1 is present in preeclamptic placentas when compared to controls. Future work focused on functions of specific cell types within the placenta is needed to determine whether local auto-crine signaling, dependent on ECE-1 plays a role in preeclampsia pathogenesis.

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References

[1]. Paradis A, Zhang L, Role of endothelin in uteroplacental circulation and fetal vascular function, Curr. Vasc. Pharmacol 11 (5) (2013) 594–605. [PubMed: 24063378]

[2]. Ajne G, Ahlborg G, Wolff K, Nisell H, Contribution of endogenous endothelin-1 to basal vascular tone during normal pregnancy and preeclampsia, Am. J. Obstet. Gynecol 193 (1) (2005) 234–240. [PubMed: 16021085]

[3]. Yanagisawa H, Yanagisawa M, Kapur RP, Richardson JA, Williams SC, Clouthier DE, de Wit D, Emoto N, Hammer RE, Dual genetic pathways of endothelin-mediated intercellular signaling revealed by targeted disruption of endothelin converting enzyme-1 gene, Development (Cambridge, England) 125 (5) (1998) 825–836.

[4]. Yanagisawa M, Inoue A, Ishikawa T, Kasuya Y, Kimura S, Kumagaye S, Nakajima K, Watanabe TX, Sakakibara S, Goto K, et al., Primary structure, synthesis, and biological activity of rat endothelin, an endothelium-derived vasoconstrictor peptide, PNAS 85 (18) (1988) 6964–6967. [PubMed: 3045827]

[5]. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T, A novel potent vasoconstrictor peptide produced by vascular endothelial cells, Nature 332 (6163) (1988) 411–415. [PubMed: 2451132]

[6]. Kawanabe Y, Nauli SM, Endothelin, Cellular and molecular life sciences, CMLS 68 (2) (2011) 195–203. [PubMed: 20848158]

[7]. Wilkes BM, Susin M, Mento PF, Localization of endothelin-1-like immunoreactivity in human placenta, J. Histochem. Cytochem 41 (4) (1993) 535–541. [PubMed: 8450193]

[8]. George EM, Palei AC, Granger JP, Endothelin as a final common pathway in the pathophysiology of preeclampsia: therapeutic implications, Curr. Opin. Nephrol. Hypertens 21 (2) (2012) 157–162. [PubMed: 22257796]

[9]. Corder R, Harrison VJ, Khan N, Anggard EE, Vane JR, Effects of phosphor-amidon in endothelial cell cultures on the endogenous synthesis of endothelin-1 and on conversion of exogenous big endothelin-1 to endothelin-1, J. Cardiovasc. Pharmacol 22 (Suppl 8) (1993) S73–S76. [PubMed: 7510004]

[10]. Gui G, Xu D, Emoto N, Yanagisawa M, Intracellular localization of membrane-bound endothelin-converting enzyme from rat lung, J. Cardiovasc. Pharmacol 22 (Suppl 8) (1993) S53–S56.

[11]. Waxman L, Doshi KP, Gaul SL, Wang S, Bednar RA, Stern AM, Identification and characterization of endothelin converting activity from EAHY 926 cells: evidence for the physiologically relevant human enzyme, Arch. Biochem. Biophys 308 (1) (1994) 240–253. [PubMed: 8311460]

[12]. Xu D, Emoto N, Giaid A, Slaughter C, Kaw S, deWit D, Yanagisawa M, ECE-1: a membrane-bound metalloprotease that catalyzes the proteolytic activation of big endothelin-1, Cell 78(3) (1994) 473–85. [PubMed: 8062389]

[13]. Thaete LG, Neerhof MG, Silver RK, Differential effects of endothelin A and B receptor antagonism on fetal growth in normal and nitric oxide-deficient rats, J. Soc. Gynecol. Investig 8 (1) (2001) 18–23.

[14]. White DG, Cannon TR, Garratt H, Mundin JW, Sumner MJ, Watts IS, Endothelin ETA and ETB receptors mediate vascular smooth-muscle contraction, J. Cardiovasc. Pharmacol 22 (Suppl 8) (1993) S144–S148. [PubMed: 7509927]

[15]. Sumner MJ, Cannon TR, Mundin JW, White DG, Watts IS, Endothelin ETA and ETB receptors mediate vascular smooth muscle contraction, Br. J. Pharmacol 107 (3) (1992) 858–860. [PubMed: 1472978]

[16]. Ahmad Z, Reznik SE, Immunohistochemical localization of ECE-1 in the human placenta, Placenta 21 (2–3) (2000) 226–233. [PubMed: 10736246]

[17]. Takahashi M, Fukuda K, Shimada K, Barnes K, Turner AJ, Ikeda M, Koike H, Yamamoto Y, Tanzawa K, Localization of rat endothelin-converting enzyme to vascular endothelial cells and some secretory cells, Biochem. J 311 (Pt 2) (1995) 657–665. [PubMed: 7487910]

[18]. Naomi S, Iwaoka T, Disashi T, Inoue J, Kanesaka Y, Tokunaga H, Tomita K, Endothelin-1 inhibits endothelin-converting enzyme-1 expression in cultured rat pulmonary endothelial cells, Circulation 97 (3) (1998) 234–236. [PubMed: 9462522]

[19]. MacLeod KJ, Husain RD, Gage DA, Ahn K, Constitutive phosphorylation of human endothelin-converting enzyme-1 isoforms, J. Biol. Chem 277 (48) (2002) 46355–46363. [PubMed: 12244060]

[20]. Ikeda K, Emoto N, Raharjo SB, Nurhantari Y, Saiki K, Yokoyama M, Matsuo M, Molecular identification and characterization of novel membrane-bound metalloprotease, the soluble secreted form of which hydrolyzes a variety of vasoactive peptides, J. Biol. Chem 274 (45) (1999) 32469–32477. [PubMed: 10542292]

[21]. Schweizer A, Valdenaire O, Nelbock P, Deuschle U, Dumas Milne Edwards JB, Stumpf JG, Loffler BM, Human endothelin-converting enzyme (ECE-1): three isoforms with distinct subcellular localizations, Biochem. J 328 (Pt 3) (1997) 871–877. [PubMed: 9396733]

[22]. Klipper E, Levy N, Gilboa T, Muller L, Meidan R, Identification of a novel alternatively spliced variant endothelin converting enzyme-1 lacking a transmembrane domain, Exp. Biol. Med (Maywood, N.J.) 231 (6) (2006) 723–728.

[23]. Barros JS, Bairos VA, Baptista MG, Fagulha JO, Immunocytochemical localization of endothelin-1 in human placenta from normal and preeclamptic pregnancies, Hypertens. Pregnancy 20 (1) (2001) 125–137. [PubMed: 12044321]

[24]. Hitzerd E, Neuman RI, Broekhuizen M, Simons SHP, Schoenmakers S, Reiss IKM, Koch BCP, van den Meiracker AH, Versmissen J, Visser W, Danser AHJ, Transfer and Vascular Effect of Endothelin Receptor Antagonists in the Human Placenta, Hypertension (Dallas, Tex : 1979) (2019) Hypertensionaha11914183.

[25]. Ajne G, Wolff K, Fyhrquist F, Carlstrom K, Hemsen-Mortberg A, Nisell H, Endothelin converting enzyme (ECE) activity in normal pregnancy and preeclampsia, Hypertension in pregnancy 22 (3) (2003) 215–224. [PubMed: 14572358]

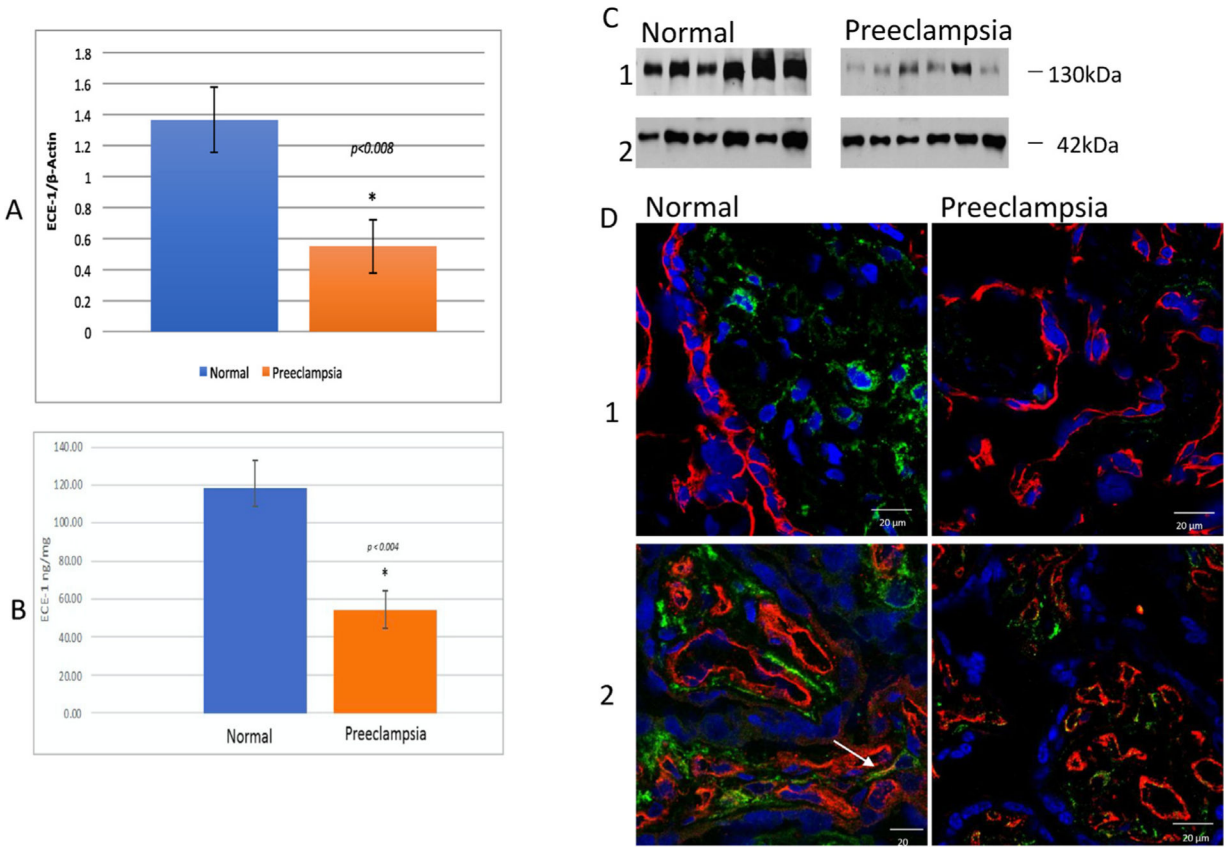

Fig. 1.

A: Western blot analysis comparing ECE-1 expression in normal and preeclamptic placentas. Densitometric analysis of western blot analyses of normal placentas (n = 6, blue) compared with preeclamptic samples (n = 6, orange) showing significantly less ECE-1 expressed in the preeclamptic placentas ($p < 0.008$). Histograms are presented as mean ± standard error of the mean. Statistical analysis using Student's *t*-test comparing normal to preeclamptic placentas was performed in Excel 2016. B: ELISA statistical analysis of ECE-1 expression in normal (n = 6, blue) compared to preeclamptic (n = 6, orange) placenta samples showing significantly less expression of ECE-1 in the preeclamptic placentas ($p < 0.004$). Histograms are presented as mean ± standard error of the mean. Statistical analysis using Student's *t*-test comparing normal to preeclamptic placentas was performed in Excel 2016. C: Panel 1: Western blot image of ECE-1 protein in normal (n = 6, left) and preeclamptic placentas (n = 6, right) shown at the 130 kDa region. Panel 2: Western blot image of β-actin shown at 42 kDa for the same. Panels 1 and 2: Lanes 1–6: Normal placenta samples at 35w5d, 37w2d, 37w6d, 38w3d, 38w6d placenta and 39w6d, respectively. Lanes 7–12: Preeclamptic placenta samples at 37w0d, 37w6d, 38w0d, 38w2d, 38w3d and 39w1d, respectively. D: ECE-1 with Trophoblast and Endothelial Cells in normal and preeclamptic placentas. Immunofluorescent studies at 63X magnification. Panels A and B: left: normal placenta at 37w2d, right: preeclamptic placenta at 37w6d. Panel A: ECE-1 (green), trophoblast cells (red) and cell nuclei (blue). Panel B: ECE-1 (green), endothelial cells (red), cell nuclei (blue). Arrows indicate areas of ECE-1.

Table 1

Maternal clinical characteristics: Mean ± Standard Deviation.

	Normal n = 6	Preeclampsia n = 6	P value
Maternal Age	32.5 ± 4.7	34.6 ± 4.1	0.46
BMI, kg/m^2	32.6 ± 8.7	33.8 ± 5.8	0.80
Ethnicity			
White	5	3	NS
Black	1	2	
Hispanic	0	1	
Nulliparous	1	3	0.54
Gestational age at delivery, (weeks)	38.0 ± 1.3	37.6 ± 1.2	0.70
Preeclampsia		3	
Preeclampsia with Severe Features		3	
Highest Systolic Pressure (range)	112 (90–122)	166 (142–186)	<0.005
Highest Diastolic Pressure (range)	76 (68–82)	98 (92–107)	<0.005
Protein/Creatinine Ratio	0.14	1.16	<0.005
Sex of Infant			
Males	2	3	NS
Females	4	3	NS
5 min Apgar	9 ± 0	8.3 ± 0.75	0.07
Vaginal delivery	4	3	NS
Cesarean Section	2	3	NS
Fetal Weight (g)	3145 ± 472.6	3238.5 ± 678.0	0.81
Placental Weight (g)	431.3 ± 142.8	457.8 ± 75.1	0.05
Placental Efficiency	7.4 ± 1.1	6.5 ± 0.2	0.29

BMI: Body Mass Index.