Molecular and Cellular Endocrinology xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Differential expression of calcium transport channels in placenta primary cells and tissues derived from preeclamptic placenta

Hyun Yang [a], Tae-Hee Kim [b], Beum-Soo An [a,c], Kyung-Chul Choi [a], Hae-Hyeog Lee [b], Jong-Min Kim [d], Eui-Bae Jeung [a,*]

[a] Laboratory of Veterinary Biochemistry and Molecular Biology, College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk 361-763, Republic of Korea
[b] Department of Obstetrics and Gynecology, College of Medicine, Soonchunhyang University, Bucheon 420-767, Republic of Korea
[c] Department of Biomaterials Science, College of Natural Resources & Life Science, Pusan National University, Miryang, Republic of Korea
[d] Department of Anatomy and Cell Biology, College of Medicine, Dong-A University, Dongdaeshin-dong 3-1, Seo-gu, Busan, Republic of Korea

ARTICLE INFO

Article history:
Received 2 August 2012
Received in revised form 13 November 2012
Accepted 14 December 2012
Available online xxxx

Keywords:
Preeclampsia
Calcium-related proteins
Hypoxia

ABSTRACT

Preeclampsia is a pregnancy-specific disease characterized by hypertension, proteinuria, and oxidative stress in the placenta. During the last trimester of gestation, calcium (Ca^{2+}) transport from mother to fetus increases dramatically in response to the increased demand for Ca^{2+} caused by bone mineralization in the fetus. Ca^{2+} supplementation can significantly reduce the incidence and severity of preeclampsia or delay its onset. Ca^{2+} transport channels (CTCs) include transient receptor potential vanilloid 6 (TRPV6), plasma membrane Ca^{2+} ATPase (PMCA1), and Na^{+}/Ca^{2+} exchangers (NCKX3 or NCX1). We hypothesized that trans-placental Ca^{2+} exchange in preeclamptic trophoblasts may be compensated for successful fetal bone mineralization. The roles of cell membrane channels (TRPV6, PMCA1, NCKX3 and NCX1) were examined in placental primary cells and in normotensive and preeclamptic placentas. The biomarker gene for preeclampsia, soluble fms-like tyrosine kinase-1 (sFLT1) or marker for oxygen-sensitive gene, hypoxia-sensitive inducible factor 1α (HIF-1α), were up-regulated in the preeclamptic placentas and hypoxic cells. The detection of sFLT1 and HIF-1α genes demonstrated that our experimental conditions were suitable to verify a preeclamptic condition. In women experiencing preterm labor, CTC expressions was found to be increased in the fetal and maternal regions of the preeclamptic placenta compared to the observed in normotensive placenta. During term labor, TRPV6 and PMCA1 were highly expressed in the fetal and maternal sections of preeclamptic placenta, while the expression of NCKX3 and NCX1 was reduced. In addition, the expression of CTCs was altered in hypoxia-stressed placental cells. Taken together, our findings demonstrated that the expression of CTCs was regulated by hypoxia stress in placenta tissues and cells, suggesting that our experimental *in vitro* hypoxic conditions were similar to those of preeclampsia. Furthermore, impaired Ca^{2+} metabolism found in preeclamptic syncytiotrophoblasts was resulted from hypoxic stress, which may induce expression of Ca^{2+} transport proteins in the placenta to maintain the balance between maternal and fetal Ca^{2+} demand during pregnancy.

© 2012 Published by Elsevier Ireland Ltd.

1. Introduction

The placenta is a highly vascularized organ, and its major function is to mediate maternal–fetal exchange of required solutes, mineral (such as Ca^{2+}) and oxygen for successful development of the fetus. The placenta is also fundamental organs involved in the pathogenesis of preeclampsia. Preeclampsia, a pregnancy-specific disease, is a major contributor to maternal and perinatal morbidity and mortality (Sibai et al., 2005), and occurs 7–10% in pregnancies (Walker, 2000). This syndrome is characterized by maternal gestational hypertension, and proteinuria and fetal symptoms including reduced amniotic fluid, abnormal oxygenation, and intrauterine growth retardation (IUGR) in 30% of cases (Redman and Sargent, 2009). This disease is principally characterized by impaired cytotrophoblast invasion and abnormal remodeling of the spiral arteries (Huppertz, 2008). In preeclampsia, villous injury is related to poor perfusion/oxygenation in the inter-villous space, uteroplacental hypoxia, and oxidative stress (Cindrova-Davies, 2009; Huppertz, 2010). Hypoxic stress resulting from abnormal remodeling of spiral arteries is thought to be an important factor in preeclampsia (Nishi et al., 2004), which exposes the placenta to fluctuating oxygen concentrations (Kingdom and Kaufmann, 1999). This stress induces the placenta to release inflammatory cytokines, apoptotic waste, and anti-angiogenic factors (Redman and Sargent, 2009).

* Corresponding author. Tel.: +82 43 261 2397; fax: +82 43 267 3150.
E-mail address: ebjeung@chungbuk.ac.kr (E.-B. Jeung).

0303-7207/$ - see front matter © 2012 Published by Elsevier Ireland Ltd.
http://dx.doi.org/10.1016/j.mce.2012.12.012

Please cite this article in press as: Yang, H., et al. Differential expression of calcium transport channels in placenta primary cells and tissues derived from preeclamptic placenta. Molecular and Cellular Endocrinology (2012), http://dx.doi.org/10.1016/j.mce.2012.12.012

UNCORRECTED PROOF

A biomarker gene of oxygenation, hypoxia-sensitive inducible factor 1α (HIF-1α), is abundantly expressed in the placenta in cases of preeclampsia, and generally used to identify the hypoxia stress-mediated conditions in diverse tissues and cell lines (Hoar et al., 2004; Kumai et al., 2007; Zhao et al., xxxx). HIF-1α is a major transducers of hypoxia signaling in human placenta that modulates the expression of various genes (Rajakumar and Conrad, 2000; Semenza, 2001), and the level of HIF-1α protein is increased under hypoxia in syncytiotrophoblasts, cytotrophoblasts and the placental vasculature (Ietta et al., 2006). The expression of soluble fms-like tyrosine kinase-1 (sFLT1), a known preeclampsia biomarker is highly expressed under both preeclamptic and hypoxic condition (Podjarny et al., 2004; Maynard et al., 2003, Karumanchi and Stillman, 2006). sFLT1, an anti-angiogenic factor, binds to vascular endothelial growth factor (VEGF), pro-angiogenic proteins, and placental growth factor (PlGF), preventing prevents their interaction with endothelial cell receptors (Maynard et al., 2003). VEGF signaling inhibitors have been found to induce hypertension and proteinuria (Yang et al., 2003). These secreted soluble factors are thought to alter endothelial metabolic processes, mitochondrial integrity, and vascular function to induce preeclampsia (Robinson et al., 2008).

Preeclampsia is considered a multi-systemic disorder and it is currently unclear precisely which factors are involved in the pathogenesis (Sibai et al., 2005). The most effective treatment for preeclampsia is delivery itself. However, several randomized trials have reported the effective use of various methods to reduce the rate or severity of preeclampsia (Sibai et al., 2005), including Ca^{2+} supplementation (Hofmeyr et al., 2007; Belizan et al., 1991). Although two clinical trials evaluating the ability of dietary Ca^{2+} supplementation to prevent preeclampsia produced disparate results (Belizan et al., 1991; Levine et al., 1997), increased intracellular Ca^{2+} levels have been implicated in the development of cell injury and hypoxic stress (Seta et al., 2004). Furthermore, Ca^{2+} entry blockers have been reported to protect against cellular necrosis caused by experimental ischemia in the liver, kidney and other tissues (Peck and Lefer, 1981; Lee and Lum, 1986). Epidemiologic data suggest an inverse correlation between dietary Ca^{2+} uptake and the incidence of hypertensive disorders during pregnancy in diverse populations (Hofmeyr et al., 2007).

Ca^{2+} is a universal intracellular second messenger involved in many processes such as signal transduction, neurotransmission, enzyme and hormone secretion, cell cycle regulation, and programmed cell death (Levine et al., 1997; Lee and Lum, 1986; Pitkin, 1983; Abramovich et al., 1987). Several alterations in maternal Ca^{2+} homeostasis have been identified in cases of preeclampsia (including low urinary Ca^{2+} excretion along with low circulating levels of 1,25-dihydroxyvitamin D3, parathyroid hormone-related peptide, and calcitonin gene-related peptide) (Halhali et al., 2000; Seely et al., 1992). Ca^{2+} is actively transported across the placenta at a rate of 140 mg/kg/d to achieve a total of 25–30 mg Ca^{2+} in a full-term fetus (Salle et al., 1987).

During pregnancy, stable materno-fetal Ca^{2+} transfer via the placental blood pool is required for adequate fetal skeletal mineralization (Pitkin, 1983). In the placenta, Ca^{2+} diffuses from the maternal blood into the cytoplasm of the syncytioblasts through specific transient receptor potential vanilloid (TRPV) channels via an electrochemical gradient (Stumpf et al., 2008; Lee et al., 2009). In the cytoplasm of syncytioblasts, Ca^{2+} binding proteins (CaBPs) binds to Ca^{2+} and shuttles it to the basolateral side of the cells. Subsequently Ca^{2+} is actively exported to the fetal blood circulation by plasma membrane Ca^{2+} ATPase (PMCA) (Belkacemi et al., 2005; Belkacemi et al., 2002) or Na^+/Ca^{2+} exchangers (Altimimi and Schnetkamp, 2007). Mammalian plasma membrane Na^+/Ca^{2+} exchange proteins are divided into two families: one for which Ca^{2+} flux is dependent only on Na^+ [Na^+–Ca^{2+} exchanger (NCX) 1–3], and another for which Ca^{2+} flux is also dependent on potassium [Na^+/Ca^{2+}–K^+ exchangers (NCKX) NCKX1–4; Dong et al., 2006; Kraev et al., 2001; Tsoi et al., 1998). The mechanism of Ca^{2+} transport in the placenta is still an area of active investigation and not well understood.

Preeclampsia has been associated with impaired maternal Ca^{2+} homeostasis. We hypothesized that trans-placental Ca^{2+} exchange and/or transport from mother to fetus through trophoblasts may be modulated by preeclampsia. Therefore, the objectives of our were to: (1) characterize placental Ca^{2+} channel activities in the preeclamptic placenta; and (2) examine the expressions of proteins involved in preeclampsia such as CTCs (TRPV6, PMCA1, NCKX3, and NCX1).

2. Materials and methods

2.1. Placental tissue

Tissue was obtained from pregnancies monitored at SCH Medical Center, Bucheon. Approval of the institutional ethical committee (#SCHBC-IRB-2010-43) and written patient consent were obtained before initiating the study. Tissues were recovered from the placentas of women 28–45 years of age who underwent vaginal or cesarean delivery. Human placental tissues ($n = 75$) were divided into four groups: Normotensive (PTL) or preeclamptic preterm labor (PE-PTL), and normotensive (TL) or preeclamptic term labor (PE-TL). After delivery, placental tissues were dissected into three sections: fetal (chorionic plate), central (floating villi), and maternal (basal plate). The tissues were transported to the laboratory on ice in 10% neutral buffered formalin for immunohistochemistry. For RNA and protein analysis, tissues were snap-frozen in liquid nitrogen and stored at −70 °C until further use. Identification of three placental tissue sections was confirmed by the expression transcriptional markers according to standard criteria (Byrne et al., 2007; Huppertz, 2006).

2.2. Human placental cell (hPC) culture

Primary cultures of hPCs were established from first-trimester human placentas obtained from elective pregnancy terminations (Caniggia et al., 1997). Placental tissues ($n = 7$) were obtained during the basal placentation period (7 weeks of gestation) when preeclampsia is caused by a shallowly implanted placenta that is consequently subjected to hypoxic stress (Caniggia et al., 1997). The tissues were transferred to 150-mL of warmed calcium- and magnesium-free Hank's solution (CMF Hank's) containing 25 mM HEPES and 0.125% trypsin (Sigma Chemical Co., St. Louis, MO), and then incubated in a shaking water bath at 37 °C for 20 min. The flask was centrifuged and the pellets were resuspended in 4 mL of Dulbecco's modified eagle medium (high-glucose DMEM; Gibco-BRL, Grand Island, NY). This suspension was layered over a preformed Percoll gradient in Hank's solution, containing 70–5% Percoll (vol/vol). After centrifugation for 20 min at 1200*g*, three regions were formed. The middle layer containing placenta cells was harvested, and the cells were cultured in high-glucose DMEM supplemented with 10% fetal bovine serum (FBS), 100 μg/ml streptomycin, 100 U/mL penicillin, and 5 μg/ml plasmocin (pH 7.4). The cells were plated at 37 °C under either standard cell culture conditions (5% CO_2 in 95% air) or hypoxic condition (2% O_2/93% N_2/5%CO_2) for 24 h.

2.3. Total RNA extraction and quantitative real-time PCR

Placental tissues were rapidly excised and washed in cold, sterile saline (NaCl 0.9%). Total RNA was prepared using TRIzol reagent

UNCORRECTED PROOF

Please cite this article in press as: Yang, H., et al. Differential expression of calcium transport channels in placenta primary cells and tissues derived from preeclamptic placenta. Molecular and Cellular Endocrinology (2012), http://dx.doi.org/10.1016/j.mce.2012.12.012

(Invitrogen, Carlsbad, CA) and RNA concentration was determined by measuring absorbance at 260 nm. Total RNA (1 μg) was reverse transcribed into first-strand cDNA using Moloney murine leukemia virus (MMLV) reverse transcriptase (iNtRON Bio, Sungnam, Kyunggi, South Korea) and random primers (9-mers; TaKaRa Bio. Inc., Otsu, Shiga, Japan). cDNA template (2 μl) was added to 10 μl of 2× SYBR Premix Ex Taq (TaKaRa Bio) containing 10 pmol of specific primer pairs as shown in Table 1. PCR were carried out for 40 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C (for *TRPV6, PMCA1, NCKX3* and *NCX1*) or 58 °C (for *HPRT1*) for 30 s, and extension at 72 °C for 45 s. Fluorescence intensity was measured at the end of the extension phase of each cycle. The threshold value of fluorescence intensity for all samples was set manually. The reaction cycle at which the PCR products exceeded this threshold was identified as the threshold cycle (CT) of the exponential phase of PCR amplification (Tatsumi et al., 1999). The expressions of *TRPV6, PMCA1, NCKX3* and *NCX1* were quantified relative to that of *HPRT1*. Relative quantification was obtained by comparing CT values at constant fluorescence intensity (Lee and Jeung, 2007). The expression of each gene relative to that of *HPRT1* was calculated using the equation: $R = 2^{-[\Delta CT\ \text{sample} - \Delta CT\ \text{control}]}$ (Lee and Jeung, 2007). All experiments were performed more than three times.

2.4. Immunoblot analysis

Placental tissues proteins were extracted using Pro-prep (In-Tron Inc., Seoul, South Korea) according to the manufacturer's instructions. Whole-cell lysates were prepared by 20 strokes of a Dounce homogenizer, followed by centrifugation at 13,000*g* for 20 min at 4 °C. Total protein (50 μg per lane) were separated in a 10% SDS–PAGE gel and transferred to a polyvinylidene fluoride transfer membrane (PerkinElmer Co., Wellesley, MA) in a TransBlot Cell (TE-22, Hoefer Co.) according to the manufacturer's instructions. The blots were blocked with TBS-T containing 5% skimmed milk for 60 min, then incubated with primary antibodie against the following factors: TRPV6, NCKX3, NCX1 (goat polyclonal, 1:500; Santa Cruz, CA), PMCA1 (rabbit polyclonal, 1:1000; SWANT, Bellinzona, Switzerland), or β-actin (rabbit polyclonal, 1:2000; Santa Cruz). After washing with TBS-T, membranes were incubated with an appropriate horseradish peroxidase-conjugated secondary antibody (anti-goat, 1:2000; Santa Cruz, or anti-rabbit, 1:5000; Santa Cruz) for 1 h at room temperature. After washing, the blots were developed by incubation in ECL chemiluminescence reagent (Santa Cruz) and subsequently exposed to Biomax™ Light film (Kodak) for 1–5 min. Signal specificity was confirmed by blotting without primary antibody. Bands densities were normalized to β-actin immunoreactive bands visualized on the same membrane after stripping the blot and reprobing. The densitry of each band was measured using ImageJ software (National Institutes of Health, Bethesda, MD).

2.5. Immunohistochemistry

CTC localization was observed with immunohistochemistry. Placental tissues were embedded in paraffin before sections (5 μm) were cut, deparaffinized in xylene, and hydrated in descending grades of ethanol. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in TBS-T for 30 min. Non-specific reactions were blocked by incubating the sections in 10% normal goat serum for 2 h at room temperature. Sections were subsequently incubated at room temperature for 4 h with antibodies against TRPV6, NCKX3, NCX1 (goat polyclonal, 1:300; Santa Cruz), or PMCA1 (rabbit polyclonal, 1:500; SWANT, Bellinzona, Switzerland), diluted in 10% normal goat serum. After washing with TBS-T, the sections were incubated with a biotinylated secondary antibody (goat IgG or rabbit IgG, Vector Laboratories, Burlingame, CA) for 30 min at 37 °C and then incubated with ABC Elite for 30 min at 37 °C. Diaminobenzidine (DAB; Sigma Chemical Co.) was used as a chromogen, and sections were counterstained with Harris hematoxylin before being mounted in Canada balsam.

2.6. Data analysis

Data are presented as means ± SEM and analyzed with a one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. Statistical analysis was performed using Prism Graph Pad (v4.0; GraphPad Software Inc., San Diego, CA). *P*-values < 0.05 were considered to be statistically significant.

3. Results

3.1. Characterization of normotensive and preeclamptic pregnancies

To compare the clinical characteristics between normotensive- and preeclamptic pregnant women, we assessed the gestational- and maternal age, placental- and fetal weight, systolic- and diastolic blood pressure of the examined subjects as shown in Table 2. Although there appeared to be no differences in maternal age, gestational age, or placental weight between normotensive and preeclamptic women, high levels of maternal systolic and diastolic blood pressure were observed with PE-PTL compared to PTL. For the cases of PE-PTL, fetal birth weight was not significantly altered compared to PTL patients. Additionally, the weight of the fetus was not different between TL and PE-TL patients. Maternal systolic- and diastolic blood pressures were raised with PE-PTL compared to PTL. For PE-TL patients, maternal systolic blood pressure was also elevated; however, diastolic blood pressure was not

Table 1
Oligonucleotide sequences for quantitative real-time PCR.

Gene	Primer sequences (5′ → 3′)	Product size (bp)	Annealing temperature (°C)	Accession number
TRPV6	F: ggagctaggccacttctacg R: atggcaatgaggaggttgag	177	60	NM_018646
PMCA1	F: gtggccaaatcttgtggttt R: tcggcatcagtgtcatcaat	353	60	NM_001001323
NCKX3	F: tcctacatccggctgaatag R: gcacaggaacacaccataca	156	60	NM_020689
NCX1	F: ggttgggactaacagctgga R: aatctttcaggccaatggtg	287	60	NM_001112800
HPRT1	F: tcgagatgtgatgaaggaga R: tatgtcccctgttgactggt	193	58	NM_000194

F, forward; R, reverse.

Please cite this article in press as: Yang, H., et al. Differential expression of calcium transport channels in placenta primary cells and tissues derived from preeclamptic placenta. Molecular and Cellular Endocrinology (2012), http://dx.doi.org/10.1016/j.mce.2012.12.012

UNCORRECTED PROOF

Table 2
Characteristics of normotensive- and preeclamptic pregnancies used for placental analysis.

	Maternal age (year)	Gestational age (week)	Mode of delivery	Placenta weight (g)	Systolic BP (mm Hg)	Diastolic BP (mm Hg)	Fetus weight (g)
Preterm							
Normotensive (n = 20)	32 ± 4	31.5 ± 4.5	v:17, c:3	343 ± 75	115 ± 10	73 ± 7	2205 ± 550
Preeclampsia (n = 20)	30 ± 3	30.5 ± 3.5	v:6, c:14	356 ± 86	**168 ± 10**[a]	**108 ± 11**[a]	1765 ± 606
Term							
Normotensive (n = 20)	30 ± 3	38.5 ± 1.5	v:16, c:4	491 ± 53	113 ± 8	73 ± 9	3152 ± 447
Preeclampsia (n = 15)	30 ± 4	38.5 ± 1.5	v:5, c:10	462 ± 28	**150 ± 14**[a]	74 ± 5	3020 ± 217

BP, blood pressure; v, vaginal delivery; c, Caesarean section.
[a] $P < 0.05$ versus NPP, non-preeclamptic placenta.

affected. These results suggest that the symptoms of preeclampsia during preterm labor are associated with more serious problem compared to those arising during term labor.

3.2. Expression of sFLT1 and HIF-1α in placental tissues and cells

We next examined sFLT1 expression in the fetal, central and maternal sections of preeclamptic placentas during preterm and term labors to characterize our experimental samples (Fig. 1A). The expression of sFLT1 was highly enhanced in all sections of preeclamptic placentas. The greatest increase was observed in fetal placenta from cases of both preterm and term labors. Expression of a marker for hypoxic stress, HIF-1α, was also measured in the placenta tissues. HIF-1α levels were increased in both preterm- and term-labor preeclampsia placenta compared to normotensive placenta. However, the expression was not significantly altered when comparing term and preterm labor (Fig. 1B). To verify *in vitro* hypoxic condition, we isolated primary placental cells that were incubated in the presence or absence of hypoxic stress (Fig. 1C). As we expected, hypoxic stress significantly induced sFLT1 and HIF-1α expressions in the placental cells. These findings suggest that our *in vivo* and *in vitro* subjects are suitable to study preeclamptia-related mechanism.

3.3. Expression of placental CTCs during preterm and term labor

For the next experiment, we evaluated the expression of CTCs in the placenta tissues. As shown in Fig. 2, TRPV6, NCKX3 and NCX1

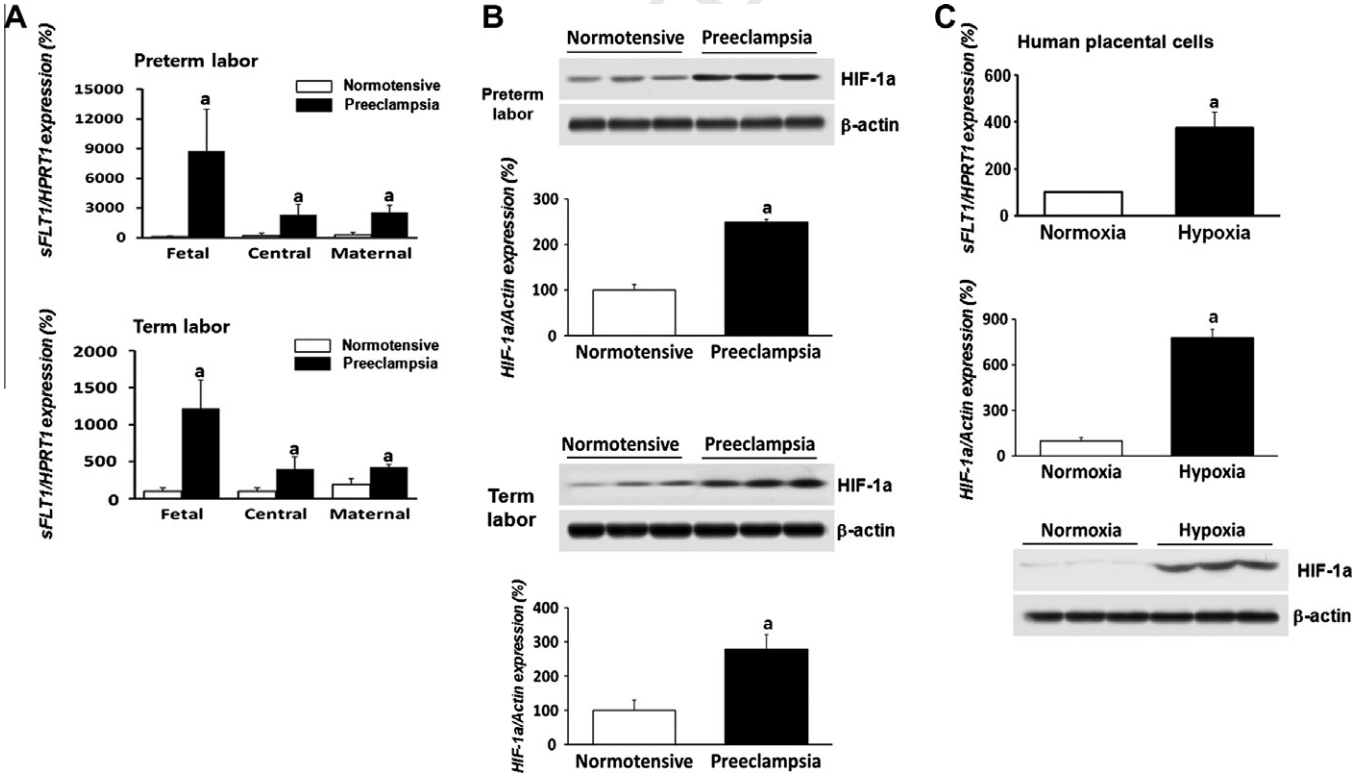


Fig. 1. The expression of sFLT1 and HIF-1α in normotensive and preeclamptic placenta and placental primary cells. Placental tissues were divided into six groups: normotensive (fetal, central and maternal tissue; n = 20) and preeclampsia (fetal, central and maternal tissue; n = 20 [PTL] or 15 [TL]). Placental expression of sFLT1 mRNA and protein were measured by real-time PCR and Western blotting, respectively. (A) sFLT1 mRNA expression in sections (fetal-, central-, maternal-) of placental tissues, (B) HIF-1α protein expression in whole placenta, and (C) sFLT1 (mRNA) and HIF1 (protein) expression in hypoxic placenta primary cells. Placental primary cells were incubated at 37 °C under either standard cell culture conditions (5% CO_2 in 95% air) or hypoxic conditions (2% O_2/93% N_2/5% CO_2). [a]$P < 0.05$ versus the normotensive controls. [b]$P < 0.05$ versus the normotensive controls (fetal-, central-, maternal) or normoxic condition.

Please cite this article in press as: Yang, H., et al. Differential expression of calcium transport channels in placenta primary cells and tissues derived from preeclamptic placenta. Molecular and Cellular Endocrinology (2012), http://dx.doi.org/10.1016/j.mce.2012.12.012

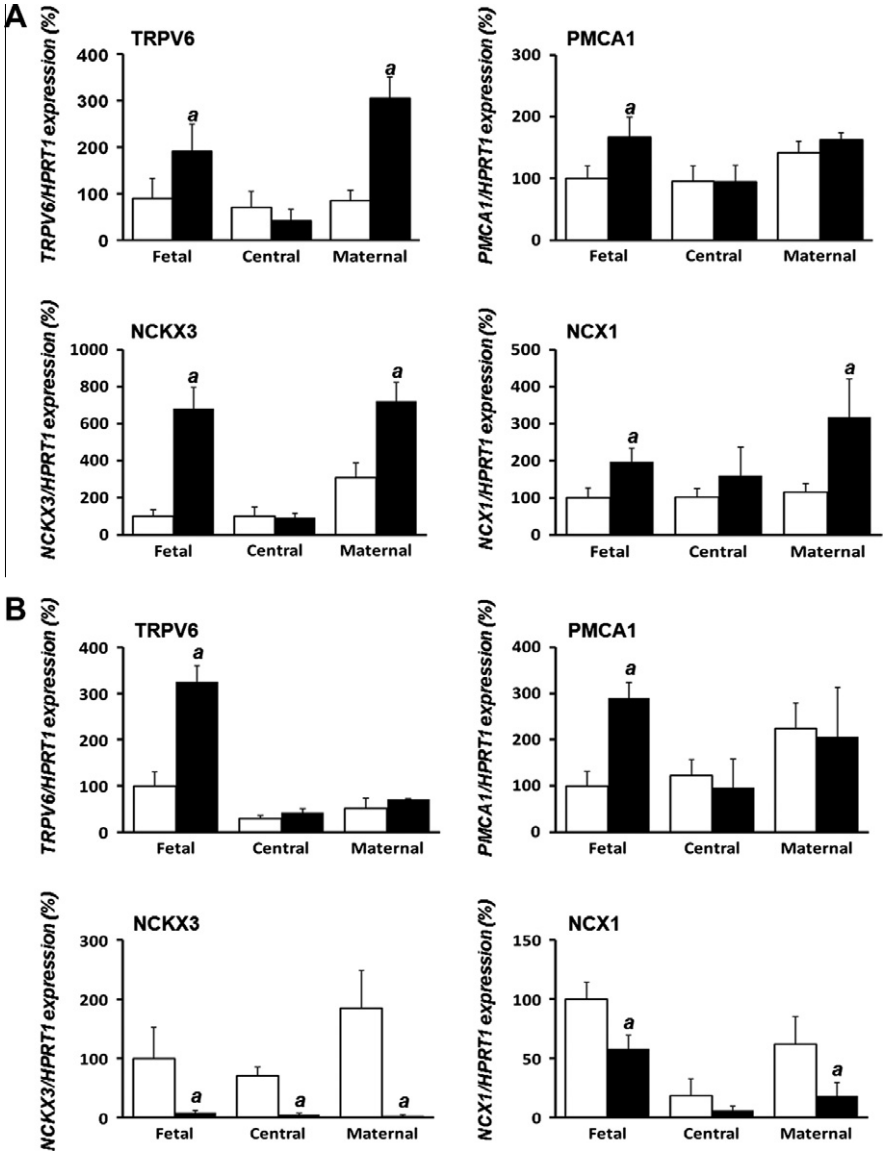


Fig. 2. The expression of CTC mRNA in normotensive and preeclamptic placenta. Placental tissues were divided into six groups: normotensive (fetal, central and maternal tissue; $n = 20$) and preeclampsia (fetal, central and maternal tissue; $n = 20$ [PTL] or 15 [TL]). The expression of CTC mRNA was measured by real-time PCR. (A) Preterm labor, and (B) term labor. White bars indicate normotensive controls while black bars represent preeclamptic placenta. $^{a}P < 0.05$ versus the normotensive controls (fetal-, central-, and maternal).

mRNA levels were significantly increased in the fetal and maternal preeclamptic placenta compared to normotensive fetal- and maternal placenta during preterm labor, while central TRPV6, NCKX3 and NCX1 expression levels were unchanged. PMCA1 mRNA expression was up-regulated in fetal preeclamptic placenta but not in other sections (central, maternal). Fig. 2B shows the CTCs expression in normotensive and preeclamptic placentas during term labor. Similar to that found during preterm labor, PMCA1 mRNA expression was highest in preeclamptic placenta compared to normal placenta in only the fetal section. In parallel with the PMCA1 results, TRPV6 mRNA expression in the fetal sections was increased in preeclamptic placenta compared to normal placenta. Additionally, NCKX3 and NCX1 expression was down-regulated in all sections of preeclamptic placenta compare to normotensive placenta. CTCs protein levels were also altered in the three placental sections as shown in Fig. 3, which were consistent with changes observed in mRNA expression. These findings suggest that the expression of CTCs was perturbed in preeclamptic placenta.

3.4. Localization of placental CTC during preterm and term labor

To investigate the spatial expression of CTCs in normotensive and preeclamptic placentas, CTC localization was analyzed by immunohistochemistry using antibodies against TRPV6, PMCA1, NCKX3 and NCX1. TRPV6 was abundantly expressed in the cytoplasm of extravillous syncytiotrophoblasts, but not in extravillous cytotrophoblasts (EVCTs) or endothelial cells in both vein and arteries (Fig. 4). TRPV6 protein was more strongly expressed in the fetal and maternal sections of preeclamptic placenta than normal placenta. Expression of PMCA1, NCKX3 and NCX1 was observed in syncytiotrophoblasts cytoplasm, and was more highly greater in preeclamptic placenta than normotensive placenta. During term labor, TRPV6 protein was abundantly expressed in the cytoplasm of syncytiotrophoblasts cytoplasm in the fetal preeclamptic placenta. PMCA1 protein expression was limited in the syncytiotrophoblasts cytoplasm. The expression of Ca^{2+} homeostasis proteins, NCKX3 and NCX1, was high in the cytoplasm of both

Please cite this article in press as: Yang, H., et al. Differential expression of calcium transport channels in placenta primary cells and tissues derived from preeclamptic placenta. Molecular and Cellular Endocrinology (2012), http://dx.doi.org/10.1016/j.mce.2012.12.012

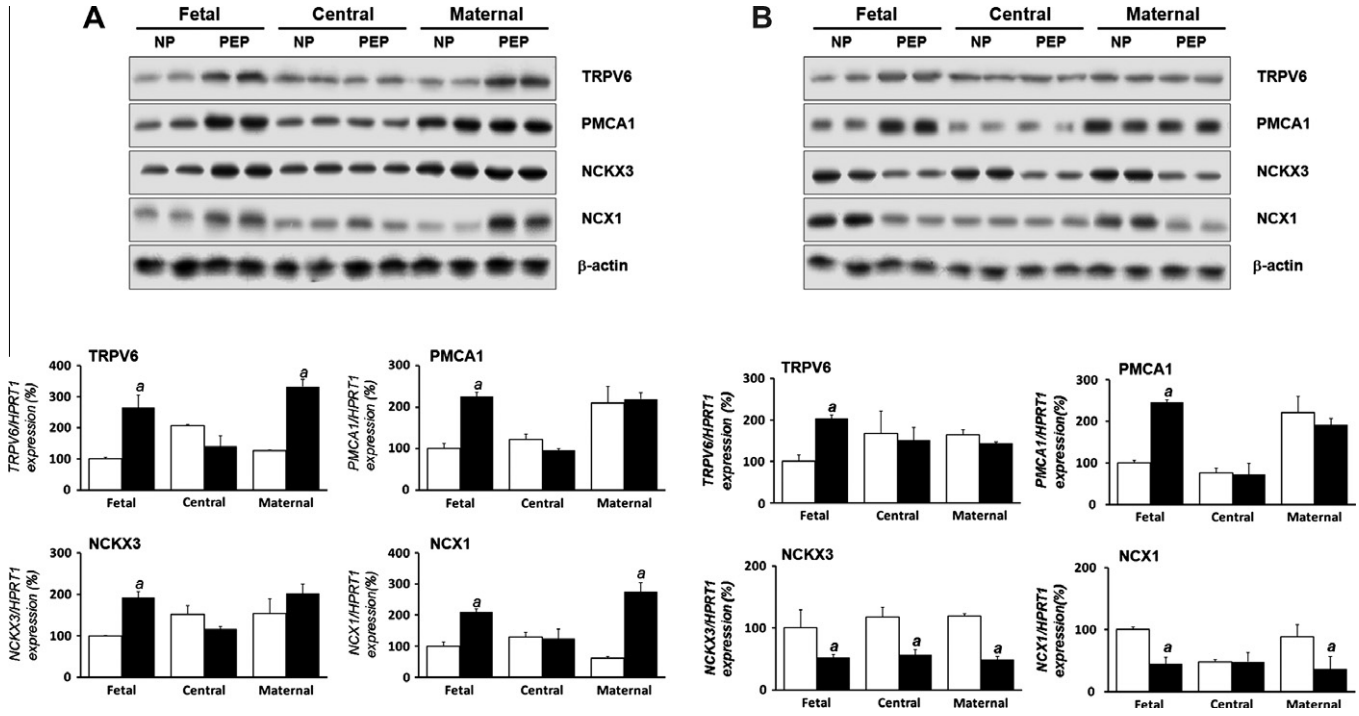


Fig. 3. The expression of CTC proteins in normotensive and preeclamptic placenta. Placental tissues were divided into six groups: normotensive (fetal, central and maternal tissue; $n = 20$) and preeclampsia (fetal, central and maternal tissue; $n = 20$ [PTL] or 15 [TL]). CTC protein expression was measured by Western blot analysis and quantified relative to that of β-actin (left panel: preterm labor, right panel: term labor); (A) preterm labor; and (B) term labor. White bars indicate normotensive controls, while black bars represent preeclamptic placenta. $^{a}P < 0.05$ versus the normotensive controls (fetal-, central-, and maternal).

syncytiotrophoblasts and giant cells in maternal normotensive placenta, and was greater in normal placenta than preeclamptic placenta during term labor. In general, CTC protein expression was limited to the cytoplasm of the villous syncytiotrophoblast layers in both preterm and term placenta.

3.5. Expression of CTCs in the hypoxia-stressed placental cells

To assess whether the expression of Ca^{2+} transport and homeostasis genes are affected by *in vitro* hypoxic conditions, CTC expression in placental primary cell was examined. We isolated the human placental cells during the first trimester (7 weeks of gestation) when studies of placenta-related disease during pregnancy are commonly conducted (Rong-Hao et al., 1996; Abrahams et al., 2004; Hiden et al., 2008). In the present study, the expression of TRPV6, PMCA1, NCKX3 and NCX1 was increased by hypoxia (Fig. 5). CTC protein levels were altered by hypoxia in a manner to similar to that of mRNAs (Fig. 5B). These results indicate that the placental CTCs expression was regulated by hypoxia. Although it is still not clear how hypoxic stress affects the regulation of CTC gene expression, gene regulation mechanisms may be involved in the hypoxic stress-mediated phenomenon we observed under preeclamptic-like conditions.

4. Discussion

Preeclampsia is a pregnancy-specific disease characterized by the malformation of placental villous due to pseudo-vasculogenesis associated with poor exchanging efficiency in the inter-villous space (Cindrova-Davies, 2009; Huppertz, 2010). Placental CTCs gene expression profiles in the three sections of normotensive or preeclamptic placentas during preterm and term labor were evaluated in the current study. To verify our experimental conditions, we first analyzed the expression of genes that serve as preeclampsia biomarkers. The expression of sFLT1 and HIF-1α was highly increased in preeclamptic placenta tissues and placental cells subjected to hypoxic stress. In addition, high levels of maternal systolic and diastolic blood pressure were observed in PE-PTL patients compared to PTL individuals. Significant differences in placental weight were not observed. Fetal weight for PE-PTL subjects was moderately decreased with preeclampsia compared to PTL patients. Intrauterine growth retardation (IUGR) refers to poor fetal growth during pregnancy, which is observed in 30% of preeclampsia cases. Results from our study indicated that our *in vivo* and *in vitro* experimental models (that induced hypoxic conditions) are suitable for studying preeclampsia or preeclamptia-like condition.

Hypoxia stress occurs during the development preeclamptic placenta in the first trimester and is involved in trophoblast differentiation (Walker, 2000). Increases of HIF-1α protein expression observed in the first trimester are consistent with the low intervillous blood flow and physiological hypoxia that have been reported during the early stages of placental development (Rodesch et al., 1992; Carter, 1997). The expression of sFLT1 reported to be a preeclampsia-mediated factor is highly expressed in the preeclamptic placenta (Podjarny et al., 2004; Maynard et al., 2003). sFLT1 is also known as an anti-angiogenic protein that binds to the pro-angiogenic proteins, VEGF and PIGF, and mediates severe preeclampsia-symptoms during pregnancy. In cases of preeclampsia, increased production of these biomarkers is associated with gestational imbalances and contributes to the development of preeclamptic symptoms, such as proteinuria, endotheliosis and edema (Maynard et al., 2003; Cunningham et al., 2011).

Since Ca^{2+} metabolism and supplementation are related to preeclampsia, we analyzed the expression of CTCs in fetal, central, and maternal sections of preeclamptic placentas and hypoxia-stressed placental cells. Our results showed that CTCs were strongly expressed on the fetal and/or maternal side of the placenta in normotensive placenta, and this expression was increased in pre-

UNCORRECTED

Please cite this article in press as: Yang, H., et al. Differential expression of calcium transport channels in placenta primary cells and tissues derived from preeclamptic placenta. Molecular and Cellular Endocrinology (2012), http://dx.doi.org/10.1016/j.mce.2012.12.012

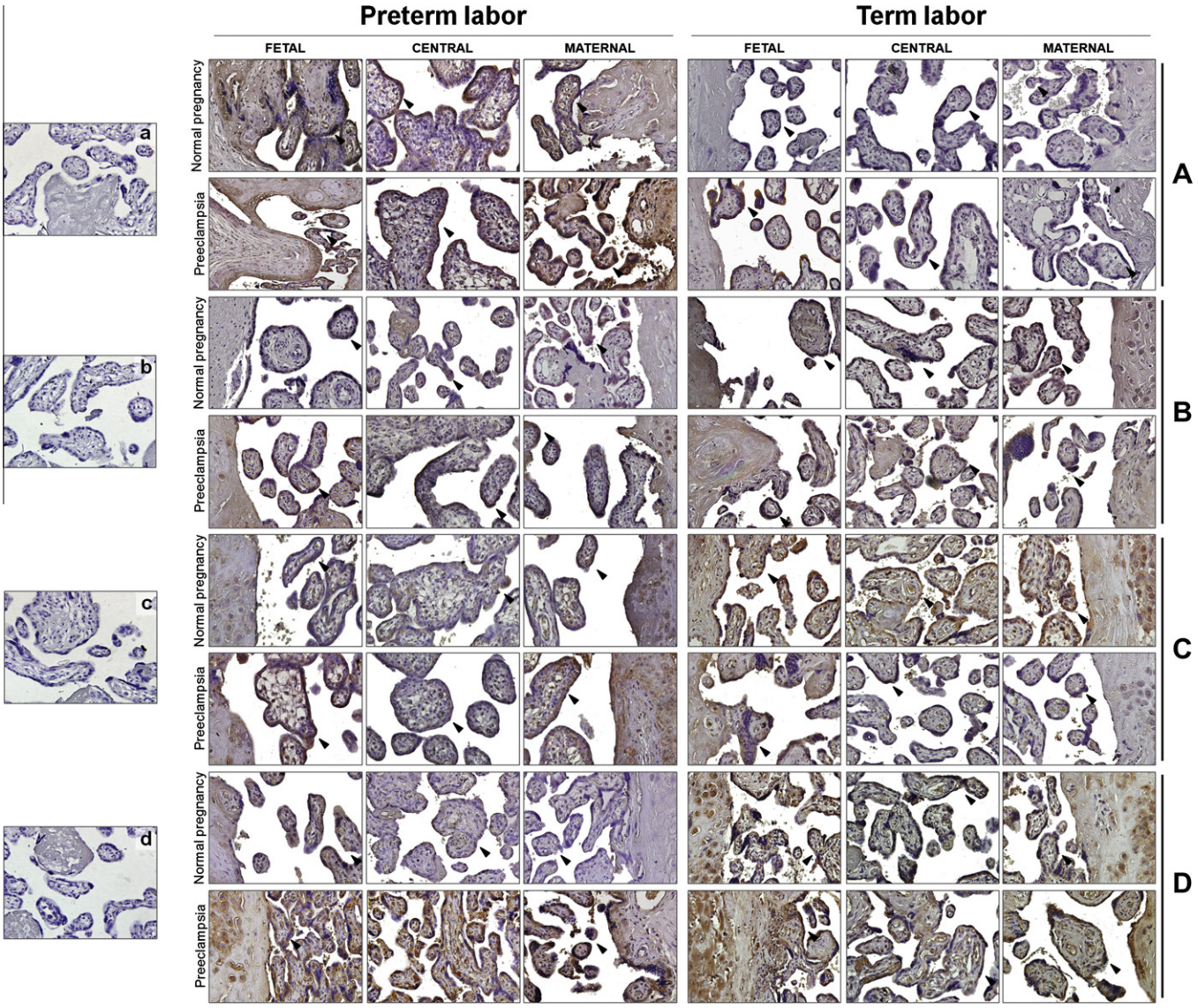


Fig. 4. Localization of placental CTC during preterm and term labor. Immuno-histochemistry was performed to observe CTCs in placental sections recovered during preterm labor (left panel) and term labor (right panel). (A) TRPV6; (B) PMCA1; (C) NCKX3; and (D) NCX1. Arrowheads indicate CTC-positive cells (400×, magnification). Negative controls are indicated for each antibody (blotting without primary antibodies).

eclamptic placenta. We further demonstrated that the expression of CTCs was regulated hypoxic placental cells. These findings suggest that augmented expression of CTCs during preeclampsia may be involved in the induction of IUGR during pregnancy, and is associated with increased fetal Ca^{2+} demand. In previous studies, Ca^{2+} ATPase activity was found to be increased with IUGR (Strid et al., 2003), while the permeability of Ca^{2+} ions decreases in the placental cell layer from IUGR patients (Mughal et al., 1989).

In our study, TRPV6 mRNA and protein were strongly expressed on the fetal side of the placenta during normal pregnancy. Other CTCs also showed increased expression on either the fetal or maternal side of the placenta. These results indicate that differential expression of CTCs in the three sections of the placenta influences maternal–fetal Ca^{2+} transport. Although increases in the expression of CTCs in the preeclamptic placentas were consistent, mRNA and proteins expression of these factors in normotensive placenta fluctuated. It is possible that genes encoding CTCs could be modulated by post-translational modification since multiple enzymatic signaling pathways are active in the placenta and enzymatic activities could vary depending on the part of placenta. It has been previously reported that CTC proteins are regulated by post-translational modification mechanisms such as phosphorylation (Hoenderop et al., 2003; Lee et al., 2006).

In a previous study, Ca^{2+} concentration was discovered to be significantly lower in murine TRPV6 KO fetal blood and amniotic fluid than in wild-type animals (Suzuki et al., 2008). In this study, transport of radioactive Ca^{2+} ($^{45}Ca^{2+}$) from mother to fetus was approximately 40% lower in TRPV6 KO fetuses than in the wild-type control. TRPV6 and CaBP-9k were found to co-localize in the yolk sac, and CaBP-9k mRNA expression is decreased in TRPV6 KO placentas (Suzuki et al., 2008). Spatial variations of gene expression in the fetal versus maternal side of the placenta have been reported in previous studies (Suzuki et al., 2008; Koo et al., 2011). For example, placental expression of TRPV5, TRPV6 and CaBP-9k are increased on the fetal side of the placenta compared with to the central- and maternal portions in mice.

In the present study, we found alterations of CTC expression in normotensive and preeclamptic placentas from premature and full-term births. Earlier studies showed that approximately 80% of total fetal Ca^{2+} accumulates during the last trimester of preg-

Please cite this article in press as: Yang, H., et al. Differential expression of calcium transport channels in placenta primary cells and tissues derived from preeclamptic placenta. Molecular and Cellular Endocrinology (2012), http://dx.doi.org/10.1016/j.mce.2012.12.012

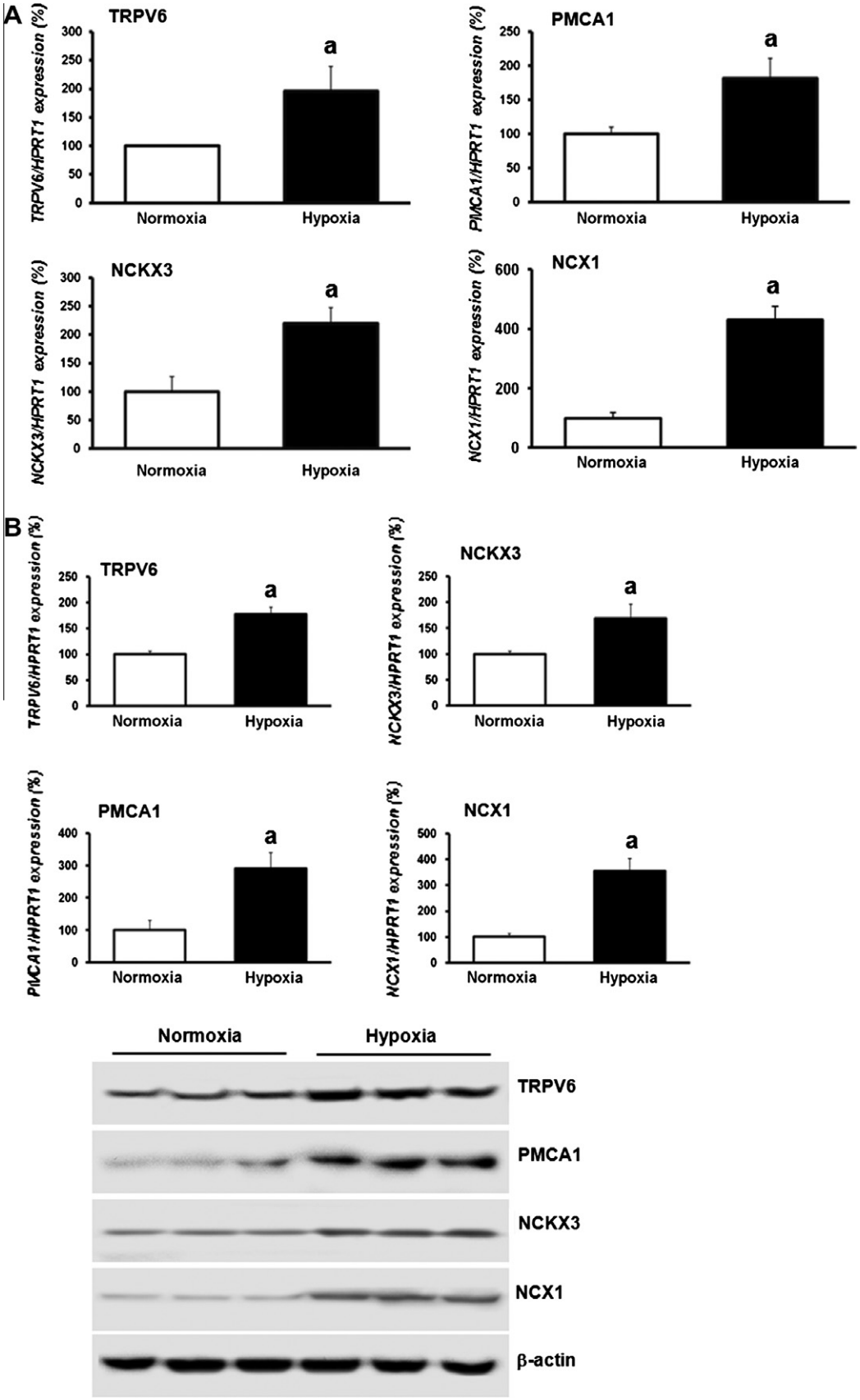


Fig. 5. The expression of CTC in placental primary cell subjected to hypoxic stress. Graphs represent the results of real-time PCR and Western blot analysis. mRNA or protein expressions was quantified relative to that of HPRT1 or β-actin, respectively. (A) mRNA expression measured by real-time PCR, and (B) protein expression assessed by Western bloting). [a]$P < 0.05$ versus normoxic conditions, indicating a significant difference in CTC expression compared to the negative control.

Please cite this article in press as: Yang, H., et al. Differential expression of calcium transport channels in placenta primary cells and tissues derived from preeclamptic placenta. Molecular and Cellular Endocrinology (2012), http://dx.doi.org/10.1016/j.mce.2012.12.012

nancy (Pitkin, 1983). This Ca^{2+} transfer enables adequate fetal skeleton mineralization and various cellular functions (Pitkin, 1983). Previous studies have suggested an important role for CTCs in Ca^{2+} homeostasis and placental transfer, and have speculated that TRPV6, PMCA1, NCKX3 and NCX1 are cooperatively involved in maternal–fetal Ca^{2+} transport in endometrial cells (Lee and Jeung, 2007; Luu et al., 2004; Yang et al., 2009). Although Bernucci et al. (2006) demonstrated the functional expression of Ca^{2+} channels in human placental syncytiotrophoblasts, the actual role of these channels has not been accurately determined.

In our study, the localization of CTCs was assessed in three sections of human placenta during preterm and term labor. CTCs were abundantly expressed in the cytoplasm of the syncytiotrophoblast layer. Maternal–fetal Ca^{2+} transport occurs via two pathways; paracellular and transcellular. In addition, human placenta permits highly passive Ca^{2+} permeability, leading to the extinction of a Ca^{2+} gradient in the absence of any other transport mechanisms (Abramovich et al., 1987; Illsley et al., 1984). In the transcellular pathway, Ca^{2+} is transported via CTCs such as TRPV, CaBP, and PMCA (Belkacemi et al., 2005; Belkacemi et al., 2002).

In conclusion, our study showed significant alterations of CTC expression in cells and tissues derived from preeclamptic placenta. In cases of preeclampsia, dynamic CTC expression may be involved in compensated maternal–fetal Ca^{2+} transfer. In preeclamptic syncytiotrophoblasts, impaired Ca^{2+} metabolism resulted from hypoxic stress, which may induce the expression of Ca^{2+} transport proteins in the placenta to maintain a balance between maternal and fetal Ca^{2+} demand during pregnancy.

Acknowledgment

This work was supported by the National Research Foundation of Korea (NRF) grant funded (No. 2010-0011433) by the South Korean government (MEST).

References

Abrahams, V.M., Straszewski-Chavez, S.L., Guller, S., Mor, G., 2004. First trimester trophoblast cells secrete Fas ligand which induces immune cell apoptosis. Mol. Hum. Reprod. 10, 55–63.
Abramovich, D.R., Dacke, C.G., Elcock, C., Page, K.R., 1987. Calcium transport across the isolated dually perfused human placental lobule. J. Physiol. 382, 397–410.
Altimimi, H.F., Schnetkamp, P.P., 2007. Na^+/Ca^{2+}–K^+ exchangers (NCKX): functional properties and physiological roles. Channels (Austin) 1, 62–69.
Belizan, J.M., Villar, J., Gonzalez, L., Campodonico, L., Bergel, E., 1991. Calcium supplementation to prevent hypertensive disorders of pregnancy. N. Engl. J. Med. 325, 1399–1405.
Belkacemi, L., Simoneau, L., Lafond, J., 2002. Calcium-binding proteins: distribution and implication in mammalian placenta. Endocrine 19, 57–64.
Belkacemi, L., Bedard, I., Simoneau, L., Lafond, J., 2005. Calcium channels, transporters and exchangers in placenta: a review. Cell Calcium 37, 1–8.
Bernucci, L., Henriquez, M., Diaz, P., Riquelme, G., 2006. Diverse calcium channel types are present in the human placental syncytiotrophoblast basal membrane. Placenta 27, 1082–1095.
Byrne, S., Ahenkorah, J., Hottor, B., Lockwood, C., Ockleford, C.D., 2007. Immunoelectron microscopic localisation of caveolin 1 in human placenta. Immunobiology 212, 39–46.
Caniggia, I., Taylor, C.V., Ritchie, J.W., Lye, S.J., Letarte, M., 1997. Endoglin regulates trophoblast differentiation along the invasive pathway in human placental villous explants. Endocrinology 138, 4977–4988.
Carter, A.M., 1997. When is the maternal placental circulation established in man? 1941. Placenta 18, 83–87.
Cindrova-Davies, T., 2009. Gabor than award lecture 2008: pre-eclampsia – from placental oxidative stress to maternal endothelial dysfunction. Placenta Suppl. A 30, S55–S65.
Q3 Cunningham, C., Rivera, J., Spence, D. 2011. Severe preeclampsia, pulmonary edema, and peripartum cardiomyopathy in a primigravida patient, AANA J. 79, 249–55.
Dong, H., Jiang, Y., Triggle, C.R., Li, X., Lytton, J., 2006. Novel role for K^+-dependent Na^+/Ca^{2+} exchangers in regulation of cytoplasmic free Ca^{2+} and contractility in arterial smooth muscle. Am. J. Physiol. Heart Circ. Physiol. 291, H1226–H1235.
Halhali, A., Tovar, A.R., Torres, N., Bourges, H., Garabedian, M., Larrea, F., 2000. Preeclampsia is associated with low circulating levels of insulin-like growth factor I and 1,25-dihydroxyvitamin D in maternal and umbilical cord compartments. J. Clin. Endocrinol. Metab. 85, 1828–1833.
Hiden, U., Glitzner, E., Ivanisevic, M., Djelmis, J., Wadsack, C., Lang, U., Desoye, G., 2008. MT1-MMP expression in first-trimester placental tissue is upregulated in type 1 diabetes as a result of elevated insulin and tumor necrosis factor-alpha levels. Diabetes 57, 150–157.
Hoar, F.J., Lip, G.Y., Belgore, F., Stonelake, P.S., 2004. Circulating levels of VEGF-A, VEGF-D and soluble VEGF-A receptor (sFlt-1) in human breast cancer. Int. J. Biol. Markers 19, 229–235.
Hoenderop, J.G., Voets, T., Hoefs, S., Weidema, F., Prenen, J., Nilius, B., Bindels, R.J., 2003. Homo- and heterotetrameric architecture of the epithelial Ca^{2+} channels TRPV5 and TRPV6. EMBO J. 22, 776–785.
Hofmeyr, G.J., Duley, L., Atallah, A., 2007. Dietary calcium supplementation for prevention of pre-eclampsia and related problems: a systematic review and commentary. BJOG 114, 933–943.
Huppertz, B., 2006. Molecular markers for human placental investigation. Methods Mol. Med. 121, 337–350.
Huppertz, B., 2008. Placental origins of preeclampsia: challenging the current hypothesis. Hypertension 51, 970–975.
Huppertz, B., 2010. IFPA award in placentology lecture: biology of the placental syncytiotrophoblast – myths and facts. Placenta 31 (Suppl.), S75–S81.
Ietta, F., Wu, Y., Winter, J., Xu, J., Wang, J., Post, M., Caniggia, I., 2006. Dynamic HIF1A regulation during human placental development. Biol. Reprod. 75, 112–121.
Illsley, N.P., Aarnoudse, J.G., Penfold, P., Bardsley, S.E., Coade, S.B., Stacey, T.E., Hytten, F.E., 1984. Mechanical and metabolic viability of a placental perfusion system in vitro under oxygenated and anoxic conditions. Placenta 5, 213–225.
Karumanchi, S.A., Stillman, I.E., 2006. In vivo rat model of preeclampsia. Methods Mol. Med. 122, 393–399.
Kingdom, J.C., Kaufmann, P., 1999. Oxygen and placental vascular development. Adv. Exp. Med. Biol. 474, 259–275.
Koo, T.H., Yang, H., An, B.S., Choi, K.C., Hyun, S.H., Jeung, E.B., 2011. Calcium transport genes are differently regulated in maternal and fetal placenta in the knockout mice of calbindin-D(9k) and -D(28k). Mol. Reprod. Dev. 79, 346–355.
Kraev, A., Quednau, B.D., Leach, S., Li, X.F., Dong, H., Winkfein, R., Perizzolo, M., Cai, X., Yang, R., Philipson, K.D., Lytton, J., 2001. Molecular cloning of a third member of the potassium-dependent sodium–calcium exchanger gene family, NCKX3. J. Biol. Chem. 276, 23161–23172.
Kumai, Y., Ooboshi, H., Ibayashi, S., Ishikawa, E., Sugimori, H., Kamouchi, M., Kitazono, T., Egashira, K., Iida, M., 2007. Postischemic gene transfer of soluble Flt-1 protects against brain ischemia with marked attenuation of blood–brain barrier permeability. J. Cereb. Blood Flow Metab. 27, 1152–1160.
Lee, G.S., Jeung, E.B., 2007. Uterine TRPV6 expression during the estrous cycle and pregnancy in a mouse model. Am. J. Physiol. Endocrinol. Metab. 293, E132–E138.
Lee, H.C., Lum, B.K., 1986. Protective action of calcium entry blockers in endotoxin shock. Circ. Shock 18, 193–203.
Lee, J.Y., Visser, F., Lee, J.S., Lee, K.H., Soh, J.W., Ho, W.K., Lytton, J., Lee, S.H., 2006. Protein kinase C-dependent enhancement of activity of rat brain NCKX2 heterologously expressed in HEK293 cells. J. Biol. Chem. 281, 39205–39216.
Lee, B.M., Lee, G.S., Jung, E.M., Choi, K.C., Jeung, E.B., 2009. Uterine and placental expression of TRPV6 gene is regulated via progesterone receptor- or estrogen receptor-mediated pathways during pregnancy in rodents. Reprod. Biol. Endocrinol. 7, 49.
Levine, R.J., Hauth, J.C., Curet, L.B., Sibai, B.M., Catalano, P.M., Morris, C.D., DerSimonian, R., Esterlitz, J.R., Raymond, E.G., Bild, D.E., Clemens, J.D., Cutler, J.A., 1997. Trial of calcium to prevent preeclampsia. N. Engl. J. Med. 337, 69–76.
Luu, K.C., Nie, G.Y., Hampton, A., Fu, G.Q., Liu, Y.X., Salamonsen, L.A., 2004. Endometrial expression of calbindin (CaBP)-d28k but not CaBP-d9k in primates implies evolutionary changes and functional redundancy of calbindins at implantation. Reproduction 128, 433–441.
Maynard, S.E., Min, J.Y., Merchan, J., Lim, K.H., Li, J., Mondal, S., Libermann, T.A., Morgan, J.P., Sellke, F.W., Stillman, I.E., Epstein, F.H., Sukhatme, V.P., Karumanchi, S.A., 2003. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J. Clin. Invest. 111, 649–658.
Mughal, M.Z., Ross, R., Tsang, R.C., 1989. Clearance of calcium across in situ perfused placentas of intrauterine growth-retarded rat fetuses. Pediatr. Res. 25, 420–422.
Nishi, H., Nakada, T., Hokamura, M., Osakabe, Y., Itokazu, O., Huang, L.E., Isaka, K., 2004. Hypoxia-inducible factor-1 transactivates transforming growth factor-beta3 in trophoblast. Endocrinology 145, 4113–4118.
Peck, R.C., Lefer, A.M., 1981. Protective effect of nifedipine in the hypoxic perfused cat liver. Agents Actions 11, 421–424.
Pitkin, R.M., 1983. Endocrine regulation of calcium homeostasis during pregnancy. Clin. Perinatol. 10, 575–592.
Podjarny, E., Losonczy, G., Baylis, C., 2004. Animal models of preeclampsia. Semin. Nephrol. 24, 596–606.
Rajakumar, A., Conrad, K.P., 2000. Expression, ontogeny, and regulation of hypoxia-inducible transcription factors in the human placenta. Biol. Reprod. 63, 559–569.
Redman, C.W., Sargent, I.L., 2009. Placental stress and pre-eclampsia: a revised view. Placenta Suppl. A 30, S38–S42.
Robinson, N.J., Wareing, M., Hudson, N.K., Blankley, R.T., Baker, P.N., Aplin, J.D., Crocker, I.P., 2008. Oxygen and the liberation of placental factors responsible for vascular compromise. Lab Invest. 88, 293–305.
Rodesch, F., Simon, P., Donner, C., Jauniaux, E., 1992. Oxygen measurements in endometrial and trophoblastic tissues during early pregnancy. Obstet. Gynecol. 80, 283–285.

UNCORRECTED PROOF

Rong-Hao, L., Luo, S., Zhuang, L.Z., 1996. Establishment and characterization of a cytotrophoblast cell line from normal placenta of human origin. Hum. Reprod. 11, 1328–1333.

Salle, B.L., Senterre, J., Glorieux, F.H., Delvin, E.E., Putet, G., 1987. Vitamin-D metabolism in preterm infants. Biol. Neonate. 52 (Suppl. 1), 119–130.

Seely, E.W., Wood, R.J., Brown, E.M., Graves, S.W., 1992. Lower serum ionized calcium and abnormal calciotropic hormone levels in preeclampsia. J. Clin. Endocrinol. Metab. 74, 1436–1440.

Semenza, G.L., 2001. HIF-1 and mechanisms of hypoxia sensing. Curr. Opin. Cell Biol. 13, 167–171.

Seta, K.A., Yuan, Y., Spicer, Z., Lu, G., Bedard, J., Ferguson, T.K., Pathrose, P., Cole-Strauss, A., Kaufhold, A., Millhorn, D.E., 2004. The role of calcium in hypoxia-induced signal transduction and gene expression. Cell Calcium 36, 331–340.

Sibai, B., Dekker, G., Kupferminc, M., 2005. Pre-eclampsia. Lancet 365, 785–799.

Strid, H., Bucht, E., Jansson, T., Wennergren, M., Powell, T.L., 2003. ATP dependent Ca^{2+} transport across basal membrane of human syncytiotrophoblast in pregnancies complicated by intrauterine growth restriction or diabetes. Placenta 24, 445–452.

Stumpf, T., Zhang, Q., Hirnet, D., Lewandrowski, U., Sickmann, A., Wissenbach, U., Dorr, J., Lohr, C., Deitmer, J.W., Fecher-Trost, C., 2008. The human TRPV6 channel protein is associated with cyclophilin B in human placenta. J. Biol. Chem. 283, 18086–18098.

Suzuki, Y., Kovacs, C.S., Takanaga, H., Peng, J.B., Landowski, C.P., Hediger, M.A., 2008. Calcium channel TRPV6 is involved in murine maternal–fetal calcium transport. J. Bone Miner. Res. 23, 1249–1256.

Tatsumi, K., Higuchi, T., Fujiwara, H., Nakayama, T., Itoh, K., Mori, T., Fujii, S., Fujita, J., 1999. Expression of calcium binding protein D-9k messenger RNA in the mouse uterine endometrium during implantation. Mol. Hum. Reprod. 5, 153–161.

Tsoi, M., Rhee, K.H., Bungard, D., Li, X.F., Lee, S.L., Auer, R.N., Lytton, J., 1998. Molecular cloning of a novel potassium-dependent sodium–calcium exchanger from rat brain. J. Biol. Chem. 273, 4155–4162.

Walker, J.J., 2000. Pre-eclampsia. Lancet 356, 1260–1265.

Yang, J.C., Haworth, L., Sherry, R.M., Hwu, P., Schwartzentruber, D.J., Topalian, S.L., Steinberg, S.M., Chen, H.X., Rosenberg, S.A., 2003. A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. N. Engl. J. Med. 349, 427–434.

Yang, H., Lee, G.S., Yoo, Y.M., Choi, K.C., Jeung, E.B., 2009. Sodium/potassium/calcium exchanger 3 is regulated by the steroid hormones estrogen and progesterone in the uterus of mice during the estrous cycle. Biochem. Biophys. Res. Commun. 385, 279–283.

Zhao, S., Gu, Y., Fan, R., Groome, L.J., Cooper, D. and Wang, Y. Proteases and sFlt-1 release in the human placenta, Placenta 31, 512–8.

UNCORRECTED PROOF

Please cite this article in press as: Yang, H., et al. Differential expression of calcium transport channels in placenta primary cells and tissues derived from preeclamptic placenta. Molecular and Cellular Endocrinology (2012), http://dx.doi.org/10.1016/j.mce.2012.12.012