Pregnancy Hypertension 18 (2019) 21–28

Contents lists available at ScienceDirect

Pregnancy Hypertension

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

The potentially protective role of ATP-binding cassette transporters in preeclampsia via Nrf2

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ARTICLE INFO

Keywords:
ATP-binding cassette transporter
Placenta
Preeclampsia
Nrf2

ABSTRACT

Objective: Preeclampsia (PE) is a severe placental syndrome that likely results from placental oxidative stress and inflammation, and can lead to maternal hypertension and premature delivery. Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) activates several genes involved in antioxidant defense in the placentae, along with the ATP-binding cassette (ABC) transporters which regulate substrate flow between maternal and fetal circulation. Although several ABC transporters are down-regulated in PE, their exact mechanistic role is poorly understood.
Methods & Results: In this study, we compared the levels of major ABC transporters and NRF2 in placentae of healthy full-term pregnant women and those with early and term onset PE. We found a significant decrease in the levels of Nrf2 and several ABC transporters in the placentae of early onset compared to term onset PE. In addition, women with term onset PE showed improved post-partum parameters (lower blood pressure, and greater placental and neonatal weights) compared to those with early onset PE. Mechanistically, Nrf2 knockdown/knockout downregulated the genes for ABC transporters and antioxidant enzymes, and upregulated pro-inflammatory factors, whereas Nrf2 upregulation had the opposite effects.
Conclusions: Nrf2 protects the placenta against PE by activating the ABC transporter-mediated efflux, indicating a novel target in PE therapy.

1. Introduction

Preeclampsia (PE) is a severe complication of pregnancy that affects approximately 3–5% of all pregnant women, and a major cause of feto-maternal mortality and morbidity worldwide [1,2]. It is characterized by maternal hypertension and/or proteinuria, which resolves after placental separation [2,3]. Although the pathogenesis of PE is not completely clear, recent studies indicate that inflammation caused by oxidative stress in the placenta is the pathogenic basis of PE development [4,5].

Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a transcription factor that protects cells against oxidative stress [6–8] by inducing the expression of antioxidant enzymes like heme oxygenases (HO-1, HO-2), copper zinc superoxide dismutase (SOD), and glutathione peroxidase (GPx) [8]. Giovanni et al showed that Nrf2 upregulated the expression of antioxidant genes in the endothelial cells involved in PE [9]. However, preeclamptic patients show decreased nuclear accumulation of Nrf2 and lower levels of heme oxygenase-1 (HO-1) in the placenta, which can potentially lead to oxidative damage [10]. Nrf2 also attenuates inflammation by inhibiting the production of inflammatory cytokines [11,12]. Therefore, the potential therapeutic utility of Nrf2 in PE has garnered increased attention in recent years [13].

In addition to the antioxidant enzymes, the ATP-binding cassette (ABC) transporters also protect cells against oxidative stress by pumping out toxic oxidative metabolites, which are then detoxified by conjugation to GSH, glucuronide, and sulfates [14,15]. The placenta is the protective barrier between maternal and fetal circulation, and protects the fetus from maternal toxins and xenobiotics. The protective function of the placenta depends largely on the ABC transporters [16,17], which allow the efflux of exogenous and endogenous substances across a concentration gradient, depending on their composition, structure and specificity [18,19]. The placenta primarily expresses the family A (ABCA1), family B (ABCB1/P-gp),

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https://doi.org/10.1016/j.preghy.2019.08.002
Received 15 January 2019; Received in revised form 25 July 2019; Accepted 9 August 2019
Available online 09 August 2019

family C (ABCC1/MRP1 and ABCC2/MRP2) and family G (ABCG1 and G2/BCRP) transporters [16,20]. However, an inflammatory response in the placenta downregulates ABC transporter expression. For example, the pro-inflammatory cytokines IL-1β and TNF significantly downregulated both the expression and function of ABCB1 and ABCG2 in human placental trophoblasts *in vitro* [21]. Furthermore, lipopolysaccharide (LPS) significantly down-regulated *Abcb1a*, *Abcb1b*, *Abcc1*, *Abcc2* and *Abcg2* mRNA levels in the rat placenta [22]. Studies also show that Nrf2 binds to the antioxidant responsive elements (AREs) of the *Abcg2*, *Abcc1* and *Abcc2* genes, and regulates their expression in some cell lines [23–25].

The precise role of ABC transporters in PE has not been elucidated so far. We analyzed and compared the expression levels of several ABC transporters (P-gp, BCRP, MRP1, and MRP2) in placenta samples obtained from women with normal pregnancy and those with PE. Furthermore, the regulatory role of Nrf2 was determined using suitable *in vitro* and *in vivo* models simulating PE.

2. Materials and methods

2.1. Placental sample collection

All clinical samples were collected at the First People's Hospital of Chengdu, Sichuan, China. Placental biopsies were obtained from full term (37 weeks or later) healthy pregnancies (NT group, n = 6), early onset (earlier than 36 weeks) PE (PEE group, n = 11), and late onset (37 + weeks) PE (PET group, n = 5). PE was defined as hypertension above 140/90 mmHg manifesting after 20 weeks of gestation and concurrent proteinuria ≥0.3 g over 24 h in the absence of underlying renal disease or chronic hypertension [1,26]. Informed consent was obtained from each patient, and approved by The Ethical Committees of the First People's Hospital of Chengdu. Human placental biopsies were obtained immediately after cesarean sections, and full-depth placental tissue samples were collected within 20 min and immediately rinsed in ice-cold PBS. All samples were stored in liquid nitrogen until used.

2.2. Cell culture and transient transfection

The human trophoblast-like choriocarcinoma cell line JEG-3 was obtained from the Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (SIBS, CAS, Shanghai, China), and maintained in DMEM-H complete medium (Gibco, New York, NY, USA) supplemented with 10% fetal bovine serum (HyClone, Logan, UT, USA), 1% glutamine, and 1% penicillin-streptomycin, at 37 °C under 5% CO_2. The cells were seeded into 6-well plates at the density of 1×10^5 cells/well, and after overnight culture were transfected with pcDNA-NRF2, pcDNA-shRNA-NRF2 or a scrambled shRNA control (provided by Johns Hopkins University, USA) using Lipofectamine 3000 according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). The cells were incubated at 37 °C for 5 h and harvested for the subsequent experiments.

2.3. Establishment of the cellular inflammation model

JEG-3 cells were seeded at the density of 1×10^4 cells per well and incubated for 12 h, followed by 4 days of culture with different concentrations (0–100 μg/ml) of LPS (Sigma, St. Louis, MO, USA) or DMSO (control). The viability of the cells was assessed by MTT assay [27] in order to determine the non-toxic doses of LPS. To determine the optimum LPS dose and duration for inducing the inflammation model, the JEG-3 cells were treated with 0, 1, 10 and 20 μg/ml LPS for 0–4 days. The culture medium was harvested, and concentration of IL-6, IL-8 and TNF were determined by ELISA. The optimal LPS exposure conditions were used for subsequent experiments.

2.4. Establishment of the murine PE model and tissue collection

Nrf2 −/− mice (ICR/129SVJ chimeric mice) [28] and their wild-type littermates were obtained from the Shuo Da Animal Center Animal Center (Shuo Da, Chengdu, China), and their genotypes were verified by PCR and DNA sequencing. All animal experiments were performed according to the Guidelines for the Care and Use of Laboratory Animals, and the Institutional Animal Care and Use Committee of Sichuan University approved all protocols. Virgin female mice aged 7–10 weeks were mated with 9 to 14-week-old males at the ratio of 2:1, and the females were inspected daily for vaginal plugs. The appearance of the plug was taken as pregnancy day 1. The pregnant mice were randomly divided into the following treatment groups (n = 12 each): placebo control – saline injected, PE – one i.p. injection of 1.4 μg/kg body weight LPS on day 14 of pregnancy, and PE + CUR – LPS injection followed by 40 mg/kg curcumin (a Nrf2 activator) p.o. from day 16 of pregnancy till day 20. All groups included the wild-type and Nrf2 −/− subgroups (n = 6 each).

The mean arterial pressure (MAP) of the pregnant mice was measured once every 2 days in the mornings from day 6 through 20 of pregnancy, using a tail-cuff acquisition system (Kent Scientific Corp., USA). The mice were placed in standard metabolic cages on days 12 and 19 of pregnancy, and urine was collected over 24 h. The pooled urine samples were centrifuged at 2000 rpm for 15 min at room temperature, and the protein content of the supernatants was measured using a urine protein assay kit (Cheng Jian Institute of Biological Engineering, Nanjing, China). All mice were anesthetized with 10% chloral hydrate (3 mL/kg) on day 20 of pregnancy, and the fetuses were collected by cesarean sections, rinsed immediately in ice-cold PBS and weighed. The samples were stored at −70 °C for subsequent analyses.

Table 1
Clinical characteristics of the women in NT, PEE and PET groups.

	NT (n = 6)	PEE (n = 5)	PET (n = 6)
Maternal age (y)	27 ± 3	29 ± 4	28.5 ± 4
Maternal BMI	25.7 ± 2.3	28.3 ± 2.1	27.5 ± 1.3
Gestational age (wk)	40 ± 1	32 ± 3	38 ± 1.5
Systolic pressure (mmHg)	121 ± 11	177 ± 12[a]	164 ± 8[b, c]
Diastolic pressure (mmHg)	73 ± 8	109 ± 10[a]	102 ± 7[b, c]
Urinary protein (g/L)	ND	2.0 ~ 4.0 [aa]	2.0 ~ 4.0 [bb]
Urinary protein (mg/24 h)	ND	642 ~ 3910[aa]	527 ~ 3269[bb]
Cesarean delivery	6	5	6
Placental weight (g)	613.2 ± 51.5	411 ± 72.8[a]	463.7 ± 66.1[b, c]
Newborn weight (kg)	3.4 ± 0.3	1.8 ± 0.7[a]	2.7 ± 0.5[b, c]

The data indicate the means ± SDs.
[a, b] < 0.05 and [aa, bb] < 0.01 indicate statistical significance compared with the women in NT group.
[c] < 0.05 indicates statistical significance compared with the women in PEE group.

2.5. RNA isolation and RT-PCR

Total RNA was isolated from the frozen fetuses using TRIzol reagent (Invitrogen, Grand Island, NY, USA) according to the manufacturer's instructions, and 2 μg RNA per sample was reverse transcribed with the PrimeScript™ RT reagent kit and gDNA Eraser (TaKaRa, Kyoto, Japan). Real-time PCR was performed in a reaction mix consisting of 10 μl SYBR Green Master Mix, 1 μl cDNA, 1 μl each of 10 mM forward/reverse primer and 7 μl RNase-free water in a total reaction volume of 20 μl. The qPCR was performed on a Real-Time Fluorescence Quantitative PCR System (Hangzhou Boji Technology Co., Ltd., China). The $2^{-\triangle\triangle ct}$ method was used to calculate the relative gene expression levels. The primer sequences are shown in Supplementary Table 1.

2.6. Western blotting

Total protein was extracted from the tissue samples using RIPA Lysis Buffer (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 1% PMSF (Sigma-Aldrich, St. Louis, MO, USA). Equal amount of protein (40 μg) per sample were separated using SDS-PAGE (10%

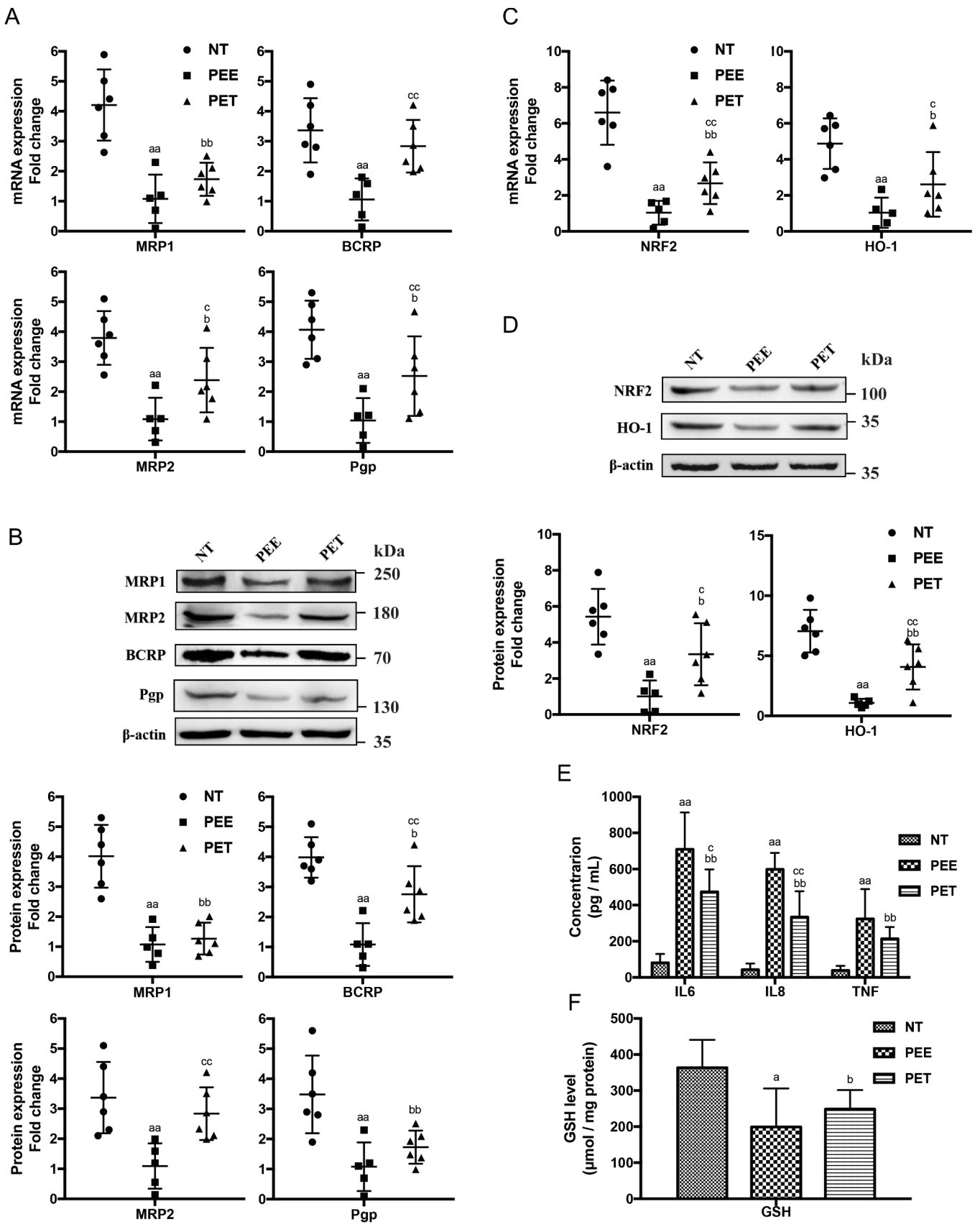

Fig. 1. Molecular differences between placental biopsies of the NT, PEE and PET groups. Expression levels of MRP1, MRP2, BCRP and Pgp mRNAs (A), MRP1, MRP2, BCRP and Pgp proteins (B), NRF2 and HO-1 mRNAs (C), NRF2 and HO-1 proteins (D), IL-6, IL-8 and TNF (E), and GSH (F). Data indicate the means ± SD. The lower-case letters above the columns indicate $P < 0.05$, and double letters $P < 0.01$. a: NT vs PEE; b: NT vs PET; c: PEE vs PET.

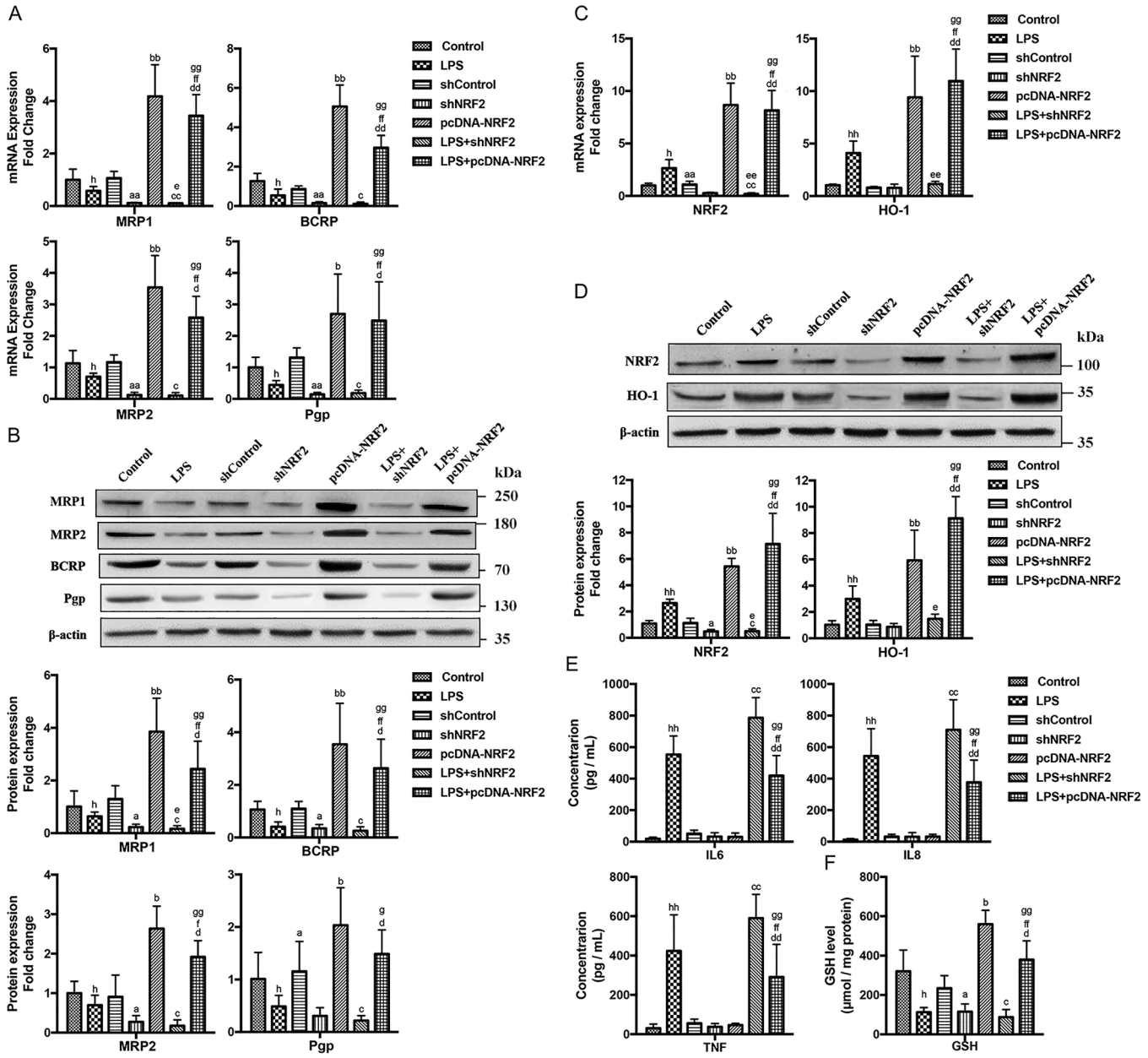

Fig. 2. NRF2 overexpression up-regulates antioxidant and ABC transporters genes *in vitro*. Expression levels of MRP1, MRP2, BCRP and Pgp mRNA (A) and protein (B), NRF2 and HO-1 mRNA (C) and protein (D), IL-6, IL-8 and TNF (E), and GSH (F) in the Control, LPS, shControl, shNRF2, pcDNA-NRF2, LPS + shNRF2 and LPS + pcDNA-NRF2 groups. The data indicate means ± SD. The lower-case letters above the columns indicate $P < 0.05$, and double letters $P < 0.01$. a: Control vs shNRF2; b: Control vs pcDNA-NRF2; c: Control vs shNRF2 + LPS d: Control vs pcDNA-NRF2 + LPS. e: LPS vs shNRF2 + LPS; f: LPS vs pcDNA-NRF2 + LPS; g: shNRF2 + LPS vs pcDNA-NRF2 + LPS; h: Control vs LPS.

Table 2
Characteristics of the wild-type and Nrf2−/− pregnant ICR mice in the control, PE and PE + CUR groups.

Group	Control		PE		PE + CUR	
Subgroup	Nrf2+/+	Nrf2−/−	Nrf2+/+	Nrf2−/−	Nrf2+/+	Nrf2−/−
Body weight (g)	59.75 ± 2.703	56.11 ± 4.559	52.84 ± 4.579[a]	51.925 ± 4.598[bb]	55.12 ± 3.277[c]	52.325 ± 4.015[d]
Placental weight (g)	0.124 ± 0.030	0.113 ± 0.019	0.093 ± 0.012[a]	0.085 ± 0.013[bb, e]	0.099 ± 0.020[c]	0.092 ± 0.019[d,f]
Fetal weight (g)	1.168 ± 0.210	1.116 ± 0.171	1.016 ± 0.185[a]	0.959 ± 0.147[b,ee]	1.087 ± 0.196[c]	1.006 ± 0.155[d,f]

The data indicate the means ± SDs.
[a, b, c, d, e] < 0.05 and [bb] < 0.01 indicate statistical significance compared with the control group (corresponding subgroups).
[e, f] < 0.05 and [ee] < 0.01 indicate statistical significance compared with the Nrf2+/+ mice in corresponding subgroups.

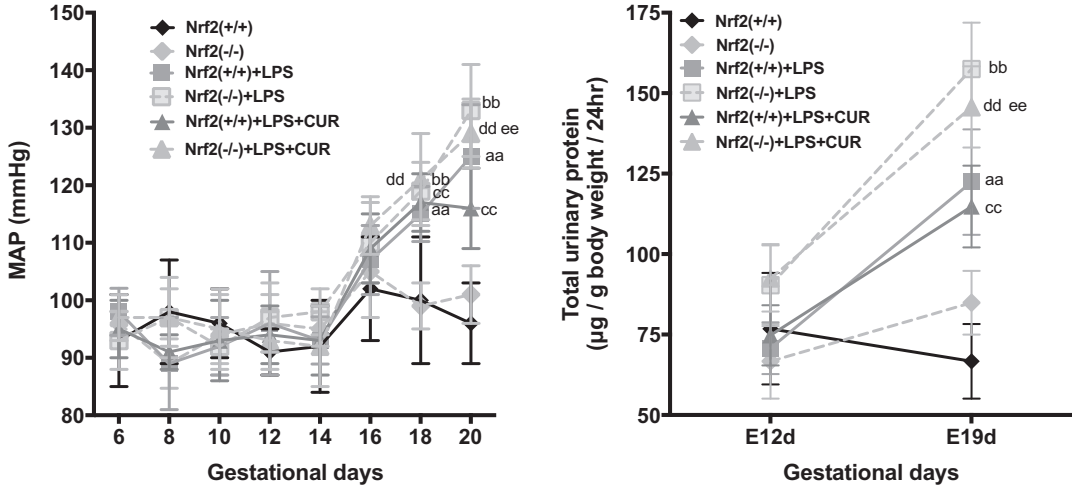

Fig. 3. Evaluation of the *in vivo* model of PE. Mean arterial blood pressure (MAP) (A) and 24-hr urinary protein levels (B) in the Control, PE, and PE + CUR groups (with wild type and Nrf2 −/− subgroups) throughout pregnancy. The data indicate the means ± SD. The lower-case letters above the columns indicate $P < 0.05$, and the double letters $P < 0.01$. a: Nrf2(+/+) vs Nrf2(+/+) + LPS; b: Nrf2(−/−) vs Nrf2(−/−) + LPS; c: Nrf2(+/+) vs Nrf2(+/+) + LPS + CUR; d: Nrf2(−/−) vs Nrf2(−/−) + LPS + CUR; e: Nrf2(+/+) + LPS + CUR vs Nrf2(−/−) + LPS + CUR.

polyacrylamide gels) and transferred to PVDF membranes. The membranes were blocked with 5% non-fat milk for 2 h at 37 °C, and incubated overnight with anti-Nrf2 (Abcam, USA), anti-BCRP (Immunoway, USA), anti-MRP1 (Santa Cruz Biotechnology, USA), anti-MRP2 (Immunoway, USA), anti-P-gp (Abcam, USA), anti-IL-6 (Immunoway, USA), anti-IL-8 (Immunoway, USA), anti-TNF (Immunoway, USA), anti-HO-1 (Abcam, USA), and anti-β-actin (Boaoweixin Inc., China) antibodies at 4 °C. After washing the membranes thrice with TBST buffer, they were incubated with the corresponding secondary antibodies (1:1000) for 1.5 h at 37 °C. The blots were rinsed thrice with TBST and twice with TBS, and developed with the Immobilon Western Chemiluminscent HRP Substrate (Millipore, Boston, MA, USA). The intensities of different protein bands were determined using WCIF ImageJ software, and their band intensities relative to that of β-actin were calculated [29].

2.7. Enzyme-linked immunosorbent assay (ELISA)

IL-6, IL-8 and TNF levels in the placental homogenates were determined using ELISA according to the manufacturer's instructions (eBioscience, CA, USA). The glutathione (GSH) levels in the cells and placental samples were quantified using the GSH ELISA kit (Cayman Chemical, MI, USA).

2.8. Statistical analysis

All data are expressed as the mean ± SD. Statistical analyses were performed using the SPSS software (version 22.0, Chicago, IL, USA). The groups were compared using two-way ANOVA, and p values < 0.05 were considered statistically significant.

3. Results

3.1. ABC transporters are highly activated in term onset PE placenta

The clinical characteristics of the women in the NT, PEE and PET groups are summarized in Table 1, and show higher blood pressure and albuminuria, and lower placental and neonatal weights in the PE women.

However, these indices were better among those with term onset PE compared to early onset PE. Consistent with this, the expression levels of ABC transporters, NRF2, and its downstream genes HO-1 and GSH were lower in the preeclampsic compared to the healthy women (Fig. 1A-D, F). In addition, IL-6, IL-8 and TNF levels were significantly higher in the placental tissues of women with PE (Fig. 1E). The PE subjects with term onset showed higher MRP2, BCRP, Pgp, NRF2, HO-1 and GSH levels, and lower IL-6, IL-8 and TNF levels in their placental biopsies compared to those with early onset PE.

3.2. NRF2 reverses the downregulation in ABC transporters and antioxidant genes induced by inflammation in vitro

The viability of JEG-3 cells was greater than 90% after exposure to up to 20 μg/ml LPS (Supplementary Fig. S1). Therefore, 10 μg/ml LPS was selected for establishing the *in vitro* inflammation model. NRF2 knockdown significantly decreased the expression levels of MRP1, MRP2, BCRP, Pgp, HO-1 and GSH, whereas Nrf2 overexpression had the opposite effects (Fig. 2A–D, F). LPS treatment significantly increased the levels of IL-6, IL-8 and TNF, which was partly attenuated by NRF2 overexpression, and augmented by NRF2 knockdown (Fig. 2E). In addition, the expression levels of ABC transporters and GSH were also reduced by LPS treatment alone. Taken together, Nrf2 reverses the pathological changes induced by LPS in human trophoblasts and PE mouse model.

3.3. NRF2 overexpression attenuates PE by upregulating ABC transporters and antioxidant genes

Administration of LPS into pregnant wild-type and Nrf2−/− mice induced both hypertension and proteinuria (Table 2).

The pre-treatment MAP of all mice was below 100 mmHg and increased to over 120 mmHg after LPS injection on the 20th day of pregnancy. In the control group, the MAP was consistent throughout pregnancy. In addition, CUR treatment decreased the MAP in the preeclampsic wild-type mice but did not significantly affect that in the Nrf2−/− subgroup (Fig. 3A). The mean albuminaria prior to LPS or saline infusion was similar between the control and PE groups on pregnancy day 12, and increased significantly in the PE mice on day 19 (Fig. 3B).

The mean weights of the fetuses and placentae from the LPS-treated groups were significantly lower than that of the control mice, especially among the Nrf2−/− mice, and were restored after CUR treatment only in the wild-type mice (Table 2). The placental Mrp1, Mpr2, Bcrp and Pgp mRNA and protein levels were significantly decreased after LPS treatment, and levels of Mpr2, Bcrp and Pgp restored by CUR in the wild-type mice (Fig. 4A–D). Similar results were observed for GSH

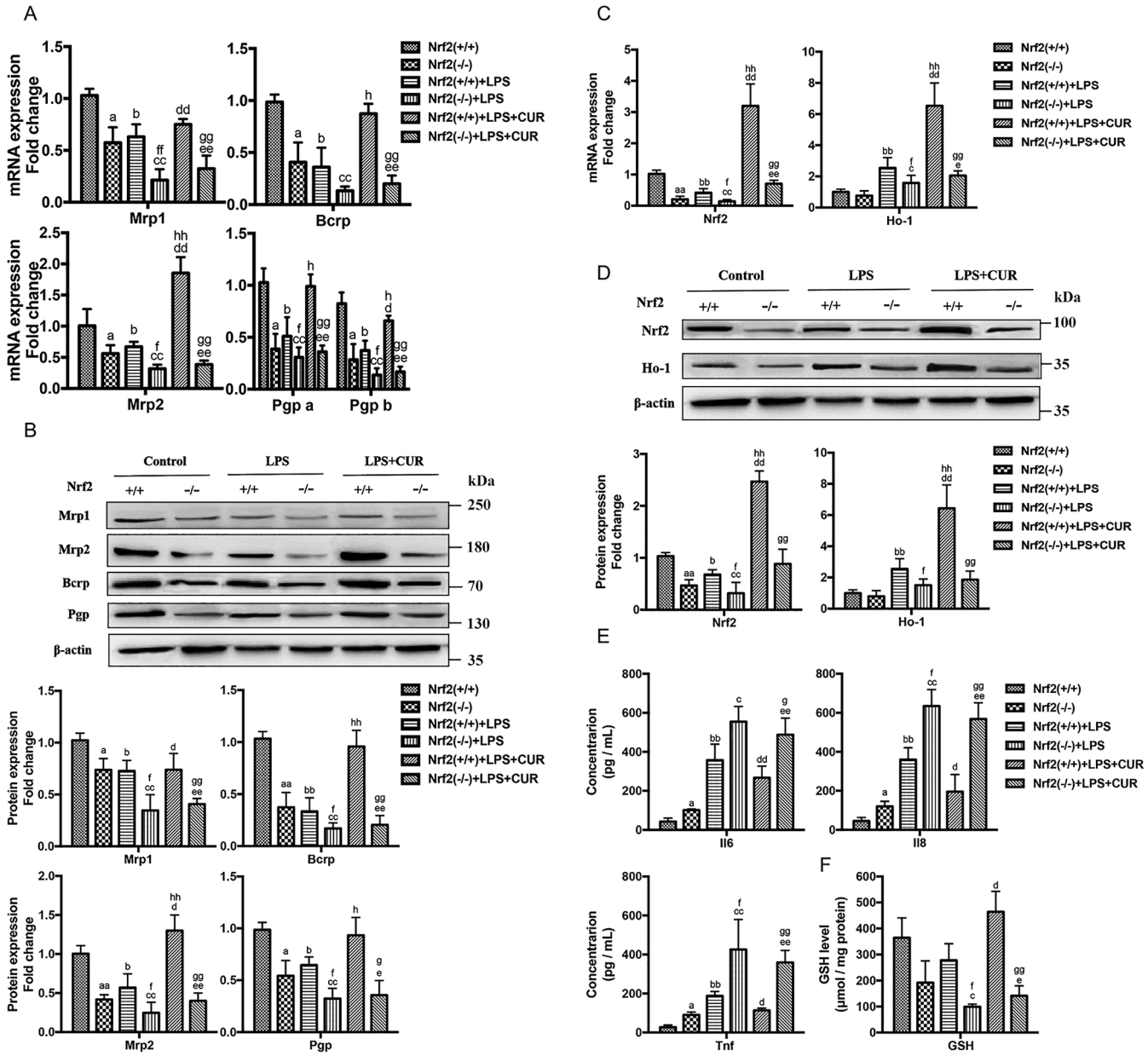

Fig. 4. NRF2 overexpression alleviates PE by up-regulating the antioxidant and ABC transporter genes *in vivo*. Expression levels of Mrp1, Mrp2, Bcrp, Pgp a and Pgp b mRNA (A) and protein (B), Nrf2 and HO-1 mRNA (C) and protein (D), IL-6, IL-8 and TNF (E), and GSH (F) in the Control, PE, and PE + CUR groups (with wild type and Nrf2 −/− subgroups). The data indicate the means ± SD. The lower-case letters above the columns indicate $P < 0.05$, and the double letters $P < 0.01$. a: Nrf2(+/+) vs Nrf2(−/−); b: Nrf2(+/+) vs Nrf2(+/+) + LPS; c: Nrf2(+/+) vs Nrf2(−/−) + LPS; d: Nrf2(+/+) vs Nrf2(+/+) + LPS + CUR; e: Nrf2(+/+) vs Nrf2(−/−) + LPS + CUR f: Nrf2(+/+) + LPS vs Nrf2(−/−) + LPS; g: Nrf2(+/+) + LPS + CUR vs Nrf2(−/−) + LPS + CUR; h: Nrf2(+/+) + LPS vs. Nrf2(+/+) + LPS + CUR.

(Fig. 4F). Finally, higher IL-6, IL-8 and TNF levels were observed in the PE mice, and were relatively greater in the Nrf2−/− subgroup. CUR treatment significantly decreased the levels of these cytokines in the wild-type mice (Fig. 4E). Taken together, Nrf2 protected the placenta against PE by upregulating the ABC transporters and antioxidant genes, and downregulating that of pro-inflammatory cytokines.

4. Discussion

The placenta is a multi-functional organ that connects the growing fetus to the uterine wall, and along with enabling fetal nutrient uptake from the maternal circulation, also removes maternally originating toxins and xenobiotics from the fetus [30]. The protective function of the placenta relies on the ABC transporters, which actively pump out toxins in an ATP-dependent manner [31,32]. The integrity of the placental barrier varies considerably depending on maternal health, and can be disrupted by several pathophysiological conditions such as PE [33]. PE is a systemic syndrome in pregnant women that results in incomplete spiral arterial remodeling in the uterus, leading to placental ischemia, structural damage [34], and high levels of oxidative stress [34,35]. Reduced GSH is arguably the most crucial intracellular antioxidant [36,37], and is used by glutathione peroxidase (GPx) to reduce peroxides. Depletion of GSH or GPx decreases antioxidant capacity, resulting in DNA peroxidation, protein degradation, and cell death. GSH is indispensable in the epithelial cells of the placenta and gastrointestinal tract [38], which are exposed to a multitude of toxic organic compounds and metal adducts. The latter are scavenged through glutathione conjugation and eliminated by the ABC transporters. MRP1 is an ABC transporter that specifically effluxes reduced and oxidized glutathione, organic anions conjugated to glutathione, glucuronide and

sulfate [14], while MRP2 targets the glutathione conjugates [15]. Furthermore, in the context of oxidative stress, placental Pgp transporter protects the cells via the efflux of oxidized cholesterol metabolites [39].

The placenta from PE patients showed a reduction in the levels of ABC transporters and Nrf2, consistent with previous studies [10,16,40,41]. Interestingly, term onset PE was associated with better feto-maternal parameters (lower blood pressure and higher placental and newborn weights) compared to early onset PE, in addition to increased expression levels of ABC transporters, NRF2, HO-1 and GSH, and decreased IL-6, IL-8 and TNF levels. Based on these findings, and the fact that several ABC transporters are regulated by Nrf2 via ARE binding, we hypothesized that Nrf2 activated ABC transporters in the preeclampsic placenta resulting in the efflux of toxic substances. Our hypothesis was validated in both the *in vitro* and *in vivo* models of PE/inflammation. Nrf2 overexpression in a human trophoblast cell line or curcumin (a Nrf2 activator) treatment in wild-type mice exposed to LPS induced the expression of some ABC transporters (MRP2, BCRP, and Pgp), HO-1 and GSH, and suppressed that of inflammatory factors. In the *in vivo* model, this lessened the PE state by decreasing MAP and increasing both fetal and placental weights. In contrast, Nrf2 deficiency aggravated the inflammatory effects in the JEG-3 cells and Nrf2−/− mice. Therefore, the Nrf2 pathway is vital in protecting the placenta and fetus from oxidative damage and inflammation. Normally, Nrf2 binds with KEAP1 (its negative regulator) in cytoplasm for ubiquitination [42,43]. When inflammation, oxidative stress, or other harmful effects increase, bindings between Nrf2 and KEAP1 become weak, decreasing the ability of KEAP1 to cause Nrf2 ubiquitination [44]. Enriched Nrf2 transfers into the nucleus and binds to AREs in promoter regions of many cytoprotective genes (phase II detoxification enzymes, antioxidant proteins, and ABC transporters) with a conserved basic region-leucine zipper domain. Then, the transcription of the target genes is increased [45]. Thus, under PE condition, high Nrf2 levels can alleviate this complication and enable full term gestation by upregulating the antioxidant and anti-inflammatory genes (HO-1 and GSH) to resisted oxidative damage and inflammation. Moreover, it can also induce the expression levels of some ABC transporters (MRP2, BCRP, and Pgp) to increase efflux of toxic and harmful substances.

Interestingly, MRP1 was not increased significantly by Nrf2 under an inflammatory/PE state, indicating that it might be vulnerable to toxic factors like LPS. Since Pgp, BCRP and MRP2 are maternal-facing apical membrane proteins, their up-regulation can prevent fetal accumulation of xenobiotics and toxins by limiting their entry into the placenta [40]. MRP1 on the other hand is located on the basolateral membrane of the placenta and transports xenobiotics from the matrix to the fetus; therefore, lower levels of MRP1 during PE is more protective. Changes in ABC transporter expression during pathophysiological conditions may also alter drug pharmacokinetics and influence treatment outcomes. Several studies have demonstrated that *trans*-placental administration of digoxin, a Pgp substrate, is an effective therapy for fetuses with supraventricular tachycardia [46–48]. Therefore, any change in Pgp levels, which is expressed on the syncytiotrophoblast apical membrane of the placenta, may impact PE therapy by pumping out digoxin [49].

In conclusion, our study demonstrates that increased expression of some ABC transporters in the placentae could be one of the mechanisms underlying the protective effects of Nrf2 in PE. Functional studies, for e.g. tracking dynamic uptake or efflux, can further validate these findings.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank President Ronghua Xu, Director of Obstetrics and Gynecology Xia Gu, the Minister of Science and Education He Ai for their help, and the patients at the First People's Hospital of Chengdu for providing placental tissue samples. The shRNA-Nrf2 and pcDNA-NRF2 constructs were kindly provided by Johns Hopkins University.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.preghy.2019.08.002.

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