Inhibiting PDGF-D alleviates the symptoms of HELLP by suppressing NF-κB activation

Haiqin Wang¹, Li Nian², Zhonghua Li³ and Changhui Lu⁴

¹Department of Obstetrics and Gynecology, the Fifth People’s Hospital of Jinan, Jinan, Shandong, China
²Department of Intensive Care Unit, the Fourth People’s Hospital of Jinan, Jinan, Shandong, China
³Department of Burn and Plastic Surgery, the Fourth People’s Hospital of Jinan, Jinan, Shandong, China
⁴Department of Nursing, the Fourth People’s Hospital of Jinan, Jinan, Shandong, China

Correspondence should be addressed to C Lu: jnsylch@163.com

Abstract

Hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome is a life-threatening pregnancy complication. Though there are several medications widely used to treat HELLP syndrome, delivery is the only efficient treatment. The goal of the present study was to investigate the effects of platelet-derived growth factor-D (PDGF-D), a newly identified PDGF, in a rat model of HELLP syndrome which was accomplished by sFlt-1 and sEng injection. The expression levels of PDGF-D in pregnant women diagnosed with HELLP syndrome was determined. A HELLP rat model was established and the PDGF-D expression level in the plasma and the placenta tissue was evaluated. To evaluate the effects of PDGF-D in HELLP syndrome model, siPDGF-D was injected into the rats and the HELLP syndrome-related parameters were measured. The levels of inflammatory cytokines and PDGF-D were determined by ELISA. The oxidative stress activities in the plasma were also determined. Furthermore, the expression of PDGF-D/PDGFR-β/nuclear factor κB (NF-κB) p65 in placenta tissues was evaluated by Western blotting. Compared to the normal pregnant (NP) group, the levels of PDGF-D were augmented regardless of species. Knockdown of PDGF-D can result in the alleviation of HELLP syndrome development and progression in the HELLP rat model. Importantly, as a result of PDGF-D knockdown, the serum levels of inflammatory cytokines and oxidative stress activities were modulated, and the phosphorylation of PDGFR-β and NF-κB p65 in placenta tissue was inhibited. Taking together, our findings indicate that targeting PDGF-D could be used as a novel strategy to treat patients with HELLP syndrome.

Key Words
→ HELLP
→ PDGF-D
→ inflammatory cytokine
→ oxidative stress
→ NF-κB p65

Introduction

Hemolysis (H), elevated liver enzymes (EL), and low platelet count (LP) were termed as the HELLP syndrome (Weinstein 1982). The HELLP syndrome is diagnosed in about 0.5–1% of pregnancy patients, and the majority of the patients have hypertension (Weinstein 1982). The Tennessee classification system diagnostic criteria are widely used for HELLP syndrome diagnosis, including hemolysis (total serum lactate dehydrogenase (LDH) activity >600 U/L), hepatic injury or dysfunction (alanine aminotransferase (ALT) activity >50 U/L, and thrombocytopenia (platelet count ≤ 100 x 10⁹ cells/L)) (Isler et al. 2001). Studies from other investigators have shown that HELLP syndrome may occur in a circulatory inflammatory milieu, and the host inflammatory responses are involved in the development
and progression of HELLP syndrome (Stojanovska & Zenclussen 2020). Increased expression of tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and IL-17 are associated with HELLP syndrome (Wallace et al. 2014).

Once HELLP syndrome is diagnosed, delivery is the best way to stop the progression of the disease. However, there is a high chance that the baby is born prematurely. The treatments for HELLP syndrome depend on the gestation of the pregnancy and the severity of the symptoms. Corticosteroids are widely used for HELLP syndrome treatment in early pregnancy to promote the baby’s lung development, while improved platelet count is the only clear beneficial effect observed in the mother’s outcome (Woudstra et al. 2010). Using magnesium sulfate is another common treatment for alleviating HELLP syndrome; however, the application of magnesium sulfate does not have a significant improvement on HELLP syndrome progression in the women with mild preeclampsia (Livingston et al. 2003). Several clinical case reports reveal that the administration of anti-hypertensive medication is associated with more unpromising outcomes (Roelofsen et al. 2003). These findings attract a growing interest in developing more potential effective treatments for HELLP syndrome.

Platelet-derived growth factors (PDGFs) are directly and/or indirectly involved in various cellular functions and numerous human disease development and progression (LaRochelle et al. 2001, Wang et al. 2009, Yang et al. 2016). Currently, four PDGF family members, PDGF A-D, have been identified and they can mediate cell functions via the selective interaction with two PDGF receptors (PDGFR-α and PDGFR-β) (LaRochelle et al. 2001, Wang et al. 2009). Interestingly, PDGF-D only selectively binds to and activates with PDGFR-β, while other PDGFs bind to PDGFR-α and/or -β (Li et al. 2003). Growing studies show that PDGF-D can regulate cell proliferation, transformation, invasion, and inflammatory cell activities through the PDGFR-β activation (Wang et al. 2009, Yang et al. 2016). The dysfunction of PDGF-D/PDGFR-β signaling and the overexpression of PDGF-D are observed in human malignancies (Wang et al. 2009). Studies have shown that the interaction between PDGF-D and PDGFR-β can activate phosphatidylinositol 3-kinase/protein kinase B signaling pathway to promote the proliferation and migration of human adipose-derived Stromal cells (ASCs), tumor development and progression (Wang et al. 2009, Hye Kim et al. 2015). Previous study also reveals that the overexpression of PDGF-D is associated with the augment of TNF-α secretion and the infiltration of macrophages in the intracerebral hemorrhage mouse model (Yang et al. 2016). Additionally, reactive oxygen species (ROS) and nuclear factor-κB (NF-κB) are also believed to crosstalk with PDGFR-D signaling pathway (Wang et al. 2010, Hye Kim et al. 2015). However, the detailed functions of PDGF-D/PDGFR-β in HELLP syndrome development and progression are largely elusive.

The effects of PDGF-D have been investigated in various diseases, but the functional roles of PDGF-D in HELLP syndrome are not fully understood. Thus, the current targeted work is necessary to identify the potential regulation of PDGF-D in HELLP syndrome. This study evaluated the expression of PDGF-D in human with HELLP syndrome. To evaluate the potential effects and mechanisms of PDGF-D in HELLP syndrome, the rat model with induced HELLP syndrome was used and the expression of PDGF-D was identified. In the previous studies, 4.7 µg/kg soluble fms-like tyrosine kinase (sFlt-1) and 7 µg/kg soluble endoglin (sEng) were injected to induce HELLP syndrome in rats (Morris et al. 2016). By using this rat model, we investigated the effects of PDGF-D knockdown on HELLP syndrome, inflammatory cytokine (TNF-α, IL-6, IL-17, and IL-10) secretion, and the oxidative stress activity. We further investigate the expression of NF-κB p65 to determine whether PDGF-D regulates HELLP syndrome and corresponding inflammatory responses through PDGF-D/PDGFR-β/NF-κB signaling pathway.

**Materials and methods**

**Ethics statement**

Human samples used for the present study were procured from the Fourth People’s Hospital of Jinan. The collection and use of tissues were approved by the Fourth People’s Hospital of Jinan. All animal experimental procedures in the present study were approved by the Fourth People’s Hospital of Jinan. In this study, 230–250 g timed-pregnant Sprague–Dawley rats were purchased from Shanghai Experimental Animal Center.

**Patients**

The study group comprised 35 women: 20 women with HELLP syndrome and 15 healthy pregnant control subjects. The patients were assessed for blood pressure and urinary output measurements on gestational age (GA) 25 weeks. Whole blood was harvested and used for LDH and platelet counts on gestational age (GA) 25 weeks. To evaluate the levels of PDGF-D and ALT in the plasma, the blood was centrifuged (600 g, 10 min, 4°C) and the
plasma was harvested on gestational age (GA) 25 weeks. The expression of PDGF-D in the plasma was quantified with the commercial ELISA (R&D systems, Minneapolis, MN; Cat. No. MAB1159). The mean arterial blood pressure was measured by an automatic oscillometric method with Dinamap device. Finally, placentas were collected and used for Western blot detection.

**Rats with HELLP syndrome**

To induce HELLP syndrome in rats, mini-osmotic pumps were placed into normal pregnant (NP) rats on gestational day (GD) 12 and sFlt-1 and sEng (R&D systems) were infused with a rate of 4.7 and 12 µg/kg, respectively. The NP rats without sFlt-1 and sEng infusion were used as controls (n = 6). Compared to the vehicle group (saline buffer), the rats received sFlt-1 and sEng infusion showed typical HELLP syndrome characters: (1) high LDH and ALT, (2) low platelets, and (3) high mean arterial pressure. Therefore, in our current study, 4.7 µg/kg sFlt-1 and 7 µg/kg sEng were perfused into rats to induce HELLP syndrome.

To study the effects of PDGF-D on HELLP rats, the PDGF-D siRNA mixture (8 mg/kg) was administered from a tail vein on GD12 (n = 6/group). The rats subjected scramble 8 mg/kg siRNA were used as control. The whole blood was collected in ethylenediaminetetraacetic acid (EDTA) tube for further evaluations.

**Mean arterial pressure (MAP), LDH, ALT, urinary protein, and platelet measurement**

The blood and tissues were harvested, and MAP was measured according to the previous reports on GD19 (Wallace et al. 2014, Luo et al. 2016). To brief, the rats were anesthetized with isoflurane on GD18, and catheters were inserted for MAP measurements. The inserted catheters were tunneled to the back of the neck and exteriorized. On GD19, the rats were placed in individual restraining cages and the arterial blood pressure was recorded and analyzed. The urinary protein levels were determined by Coomassie blue staining according to the previous report (Luo et al. 2016). Briefly, Coomassie brilliant blue G-250 (Qcbio Science & Technologies Co., Ltd, Shanghai, China; Cat. No. G-250) was dissolved in 95% ethanol and the urinary sample was incubated with Coomassie brilliant blue solution for 5 min at room temperature. The optical density was measured at 595 nm. The urinary protein concentrations were calculated based on the standard curve. The levels of LDH and platelet counts in the whole blood were automatically determined by Sysmex®XE-5000 (Sysmex America Inc., Lincolnshire, IL). To evaluate the levels of ALT in the plasma, the collected blood was centrifuged (600 g, 10 min, 4°C) and the supernatant was collected. A Roche c501 Cobas® Series (Roche Diagnostics) was used to measure the levels of ALT in the plasma.

**ELISA assay**

The plasma was harvested for the following ELISA assays to determine the levels of PDGF-D (ELK Biotechnology, Wuhan, CN; Cat. No. ELK7115), TNF-α (Abcam; Cat. No. ab236712), IL-6 (Abcam; Cat. No. ab234570), IL-17 (Abcam; Cat. No. ab214028), and IL-10 (Abcam; Cat. No. ab214566) according to the manufactures’ instructions. Briefly, the plasma samples were diluted with supplied sample diluent buffer and the diluted samples were added into the 96-well plates. The plates were incubated for 2.5 h at room temperature with gentle shaking, followed by the incubation with the corresponding detective antibody for another 1 h at room temperature. The horseradish peroxidase (HRP)-streptavidin solution was added to each well and incubated for 45 min at room temperature. After discarding the solution, TMB One-Step substrate reagent was added and incubated for an additional 30 min at room temperature in the dark. The absorbance value was measured at 450 nm after adding stop solution to each well.

**RNA isolation, cDNA synthesis, and qRT-PCR**

Total RNA from placenta tissues was extracted using Trizol (Life Technologies; Cat. No. 15596018) and the concentration of RNA was measured with UV spectrophotometry (NanoDrop Technologies, Wilmington, NC, US; Cat. No. ND-1000). The extracted RNA was reverse transcribed to synthesize cDNA with random hexamers and SuperScript III First Strand Synthesis system (Life Technologies; Cat. No. 18080051). The mRNA level of PDGF-D expression was performed by quantitative RT-PCR using 10 µL reaction mixture containing 100 ng cDNA, SYBR Green Master Mix (Life Technologies; Cat. No. 4334973), 6 µM forward primer and 6 µM reverse primer. Results were analyzed with Real-Time PCR System Software. All mRNA data were normalized against the reference gene glyceraldehyde-3 phosphate dehydrogenase.

**Oxidative stress evaluation**

To evaluate the effects of PDGF-D on oxidative stress, the oxidative stress parameters, including endothelin (ET),
nitric oxide (NO), superoxide dismutase (SOD), and reactive oxygen species (ROS) were evaluated according to the previous reports (Luo et al. 2016, Zhou et al. 2019).

The ET kit was generously provided by Institute of East Asian Immune Technology, People’s Liberation Army General Hospital (Luo et al. 2016). The plasma was mixed with the supplied reaction solution and incubated at room temperature for 15 min. The supernatant was aspirated after the mixture was centrifuged (590 g, 20 min). 125I-ET competed with the standard and sample solution to combine with the ET antibody. The ET level was determined based on the standard curve from the radioactive content in the sample solution.

To determine the level of NO, the plasma was mixed with 0.02% 4-hydroxycoumarin (Sigma-Aldrich; Cat. No. 8043300025) and the mixture was incubated for 5 min on ice. 8% sodium hyposulphite (Sigma-Aldrich; Cat. No. 563188) was added for an additional 10 min incubation. The fluorescence intensity was measured with a fluorescence spectrophotometer after the mixture was incubated with 1.5 M NaOH for 10 min.

The SOD detective kit was generously provided by the Nanjing Jiansheng Bioengineering Institute (Luo et al. 2016). Briefly, the plasma was mixed with the supplied reactive solution, and the mixture was incubated at room temperature for 10 min. A spectrophotometer was used to determine the optical density at a wavelength of 550 nm.

An OxiSelect In Vitro ROS/RNS assay (Cell Biolabs, San Diego, CA, US; Cat. No. STA-347-5) was employed to determine the ROS level in the plasma according to the manufacturer’s instruction (Zhou et al. 2019). To brief, the samples were incubated with supplied Catalyst for 5 min at room temperature, following by incubated with DCFH solution at room temperature for an additional 15 min. The fluorescence intensity was determined with a fluorescence plate reader at 480 nm (excitation) and 530 nm (emission).

Protein extraction and Western blot

The collected placenta tissues were washed with ice-cold PBS and homogenized in radioimmunoprecipitation assay buffer (RIPA buffer, Sigma-Aldrich; Cat. No. R0278) containing 1:100 diluted protease and phosphatase inhibitor cocktail (Sigma-Aldrich; Cat. No. P5726). The lysates were centrifuged at 10,000 g for 20 min at 4°C and the supernatants were collected. Total protein concentration was determined using Pierce Bicinchoninic acid (BCA) protein assay kit (Thermo Scientific; Cat. No. 23252) according to the manufacturer’s instructions. Thirty micrograms of total protein samples were separated by SDS-PAGE and transferred to the nitrocellulose membrane using the Trans-Blot Turbo system (Bio-Rad Laboratories). Membranes were blocked with 5% dry milk in TBS containing 0.1% Tween (TBST) and subsequently incubated with primary antibodies (PDGF-D (1:1000, Santa Cruz Biotechnology; Cat. No. sc-137030), p-PDGFR-β (1:1000, Santa Cruz; Cat. No. sc-365464), PDGFR-β (1:1000, Santa Cruz; Cat. No. sc-374573), p-NF-κB p65 (1:1000, Santa Cruz; Cat. No. sc-398442), NF-κB p65 (1:1000, Santa Cruz; Cat. No. sc-8008), and GAPDH (1:1000, Santa Cruz Cat. No. sc-47724) diluted in TBST containing 5% dry milk overnight at 4°C. Blotting membranes were then washed frequently in TBST and incubated for 1.5 h at room temperature probed with the appropriate HRP linked IgG (Santa Cruz). Immunoreactive bands were detected by chemiluminescence using Gel Doc XR+ System (Bio-Rad). Image J (NIH) was employed to determine the density of target protein bands, and the density was normalized to the reference protein GAPDH.

Statistical analysis

All of the data were present as mean ± s.d. Student’s t-test, or one-way ANOVA analysis with a post hoc test was used to analyze the differences. A value of P < 0.05 was considered statistically significant.

Results

PDGF-D was associated with HELLP syndrome

To assess whether the expression of PDGF-D was associated with HELLP syndrome, we evaluate MAP, platelets, the levels of LDH and ALT. The HELLP patients had significantly greater MAP, ALT, and LDH levels, but remarkably lower platelets comparing to the NP women (Fig. 1C, E and F), which met the HELLP syndrome diagnostic criteria. Furthermore, we also found the expression level of PDGF-D was higher in HELLP patients (Fig. 1A and B). Other characteristics of both study groups including laboratory data on admission were shown in Table 1. The levels of primiparity and proteinuria in the HELLP patients were significantly higher than that in NP women. Additionally, the neonatal birth weight was significantly lower in the HELLP patient group vs those from the NP group (Table 1).

In a rat model with induced HELLP syndrome, the expression of PDGF-D in plasma was significantly higher than that in NP rats (Fig. 2A). The mRNA and protein levels...
of PDGF-D in the placenta harvested from the HELLP rats were greater compared to the controls (Fig. 2B, C and D). The other characteristics of the HELLP rats and NP rats were shown in Table 2. Remarkably, the value of MAP and the levels of LDH ($P = 0.0012$), ALT ($P = 0.0045$), and urinary protein ($P = 0.0032$) were significantly augmented because of the HELLP syndrome; by contrast, the platelet counts were significantly lower. These results indicated that the augment of PDGF-D level may be associated with HELLP syndrome.

**PDGF-D knockdown rats were resistant to induced HELLP syndrome**

To address the role of PDGF-D in HELLP syndrome, we transfected short interfering RNA (siRNA) to knockdown PDGF-D in pregnant rats. A series of siPDGF-D concentrations (1, 2, 4, and 8 mg/kg) was used to evaluate the knockdown efficiency of siRNA on HELLP syndrome (Supplementary Fig. 1, see section on supplementary materials given at the end of this article), and we found that the 8 mg/kg siPDGF-D injection could achieve the promising outcome. Therefore, 8 mg/kg concentration was used for the present study (Fig. 3A). Compared to control siRNA, PDGF-D knockdown in pregnant rat plasma revealed reduced expression levels of LDH, ALT, and MAP, but higher platelet counts (Fig. 3B, C, D and E). These data showed that the knockdown of PDGF-D had a protective effect against HELLP syndrome.

**PDGF-D knockdown suppressed the inflammation response**

We further tested whether knockdown PDGF-D affected inflammatory cytokines in rats with HELLP syndrome. In response to the induced HELLP, we also observed increased expression of inflammatory cytokines (TNF-$\alpha$, IL-6, and IL-17, Fig. 4A, B and C) but decreased expression of IL-10 (Fig. 4D). By contrast, the rats with the absence of PDGF-D expression demonstrated the preservation of inflammatory responses, as evidenced by (1) lower TNF-$\alpha$, IL-6, and IL-17 secretion and (2) higher IL-10 secretion.

### Table 1  Demographic and clinical characteristics in HELLP patients.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Normal pregnant (NP)</th>
<th>HELLP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>Maternal age (years)</td>
<td>26 (22–30)</td>
<td>26.5 (21–32)</td>
</tr>
<tr>
<td>Gestational age at delivery (weeks)</td>
<td>37.0 (30.1–39.0)</td>
<td>34.8 (30.3–37.9)</td>
</tr>
<tr>
<td>Primiparity</td>
<td>26.7</td>
<td>60**</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>20</td>
<td>100**</td>
</tr>
<tr>
<td>Maternal BMI (kg/m$^2$)</td>
<td>26.1 (21.4–32.5)</td>
<td>26.4 (22.6–32.2)</td>
</tr>
<tr>
<td>Neonatal birth weight (g)</td>
<td>3068 (1253–3263)</td>
<td>1833 (1078–2643)**</td>
</tr>
<tr>
<td>Caesarean section</td>
<td>53.3</td>
<td>60</td>
</tr>
<tr>
<td>Small-for-gestational-age neonates</td>
<td>0</td>
<td>55**</td>
</tr>
</tbody>
</table>

$^{a}$Values are presented as median (interquartile (IQR) range); $^{b}$Values are presented as percentage (%). HELLP, hemolysis–elevated liver enzymes–low platelets.
in plasma in comparison to control siRNA (Fig. 4). These data revealed that PDGF-D regulated the expression of cytokines during HELLP syndrome.

**PDGF-D was associated with oxidative stress**

Prior studies show that oxidative stress is associated with HELLP syndrome (Torrance et al. 2008). In our current study, we found that the rats with HELLP syndrome had significantly greater levels of ROS and ET in the plasma, but lower levels of SOD and NO (Fig. 5). To determine whether PDGF-D was associated with oxidative stress, we evaluated the oxidative stress-specific marker expression in the rats without PDGF-D expression. In response to induced HELLP, the expression of ROS and ET was augmented in the rats with HELLP; by contrast, the levels of SOD and NO decreased (Fig. 5). However, the rats with the absence of PDGF-D revealed the protection from the oxidative stress damage, as evidenced by (1) lower levels of ROS and ET (Fig. 5A and B) and (2) higher levels of SOD and NO (Fig. 5C and D) compared to the HELLP rats treated with control siRNA. These data showed that the absence of PDGF-D had protective effects against oxidative stress in rats with HELLP.

**PDGF-D influences PDGFR-β and NF-κB p65 phosphorylation**

Critical signaling mechanisms involving NF-κB p65 (also known as RelA) have been demonstrated to play a key role to regulate various inflammatory cytokine gene expression (Ngo et al. 2020). To address whether PDGF-D mediates inflammatory cytokine expression via regulating NF-κB p65 phosphorylation, we evaluated relative levels of phosphorylation of PDGFR-β and NF-κB p65 in the placenta tissues. The NP rats had significantly lower PDGF-D and p-PDGFR-β expression compared to the HELLP rats (Fig. 6A and B). The expression of PDGF-D was inhibited in the rats treated with siPDGF-D, which confirmed the knockdown efficiency of PDGF-D. The phosphorylation of PDGFR-β was remarkably inhibited when siPDGF-D was injected to the HELLP rats (Fig. 6A and B). Similar to PDGFR-β, the p65 phosphorylation was enhanced in the rats with HELLP syndrome compared to the NP rats. The level of NF-κB p65 phosphorylation in the rats with HELLP syndrome received siPDGF-D injection was suppressed vs the rats with HELLP syndrome received control siRNA treatment (Fig. 6C and D). These results suggested the augment of the phosphorylation of PDGFR-β and NF-κB p65 may be associated with the expression of PDGF-D.

**Discussion**

PDGF and its receptors were reported to play an important role in embryonic development and neurogenesis, suggesting that they may be able to modulate the development-related events (Heldin & Westermark 1999, Funa & Sasahara 2014). However, the function...
of PDGF-D in HELLP has not been reported yet. In this study, we firstly found that PDGF-D was upregulated in HELLP patients, which prompted us to further evaluate the effects of PDGF-D in HELLP progression. Our further results showed that the upregulated expression of PDGF-D was associated with HELLP syndrome. Parallel outcomes disproved that the expression of PDGF-D was associated with the upregulation of inflammatory cytokines and ROS.
Role of PDGF-D in HELLP syndrome. We also observed that the placental trophoblasts are the central issue (Abildgaard & Heimdal 2013). The increased circulating ROS, αα, κκ, γγ was also measured (D). GAPDH was used as an internal control (A). The optical density of p-PDGFR-β and NFκB p65 was also measured (D). **P < 0.01. A full colour version of this figure is available at https://doi.org/10.1530/JME-20-0308.

Figure 6
Knockdown of PDGF-D suppresses PDGFR-β and NFκB activation in HELLP in rats. (A and B) The tissues of placenta were prepared for Western blot against PDGF-D, p-PDGFR-β and PDGFR-β. GAPDH was used as an internal control (A). The optical density of p-PDGFR-β was also measured (B). The tissues of placenta were prepared for Western blot against p-NFκB p65 and NFκB p65. GAPDH was used as an internal control (C). The optical density of p-NFκB p65 was also measured (D). **P < 0.01. A full colour version of this figure is available at https://doi.org/10.1530/JME-20-0308.

activity. Mechanistically, we showed that knocking down PDGF-D expression diminished PDGFR-β and NFκB p65 phosphorylation, which provided an important clue for further mechanical studies.

HELLP syndrome is usually associated with preeclampsia (PE). The maternal symptoms and signs often diminish after the delivery of the placenta (Abildgaard & Heimdal 2013). However, even after delivery of the placenta, it is not surprising to observe severe HELLP syndrome for a long time. Though the detailed pathogenesis of HELLP syndrome is not completely understood, the contributions of inflammatory cytokines derived from the placenta, immune maladaptation, and fetal markers for PE are important for the pathogenesis of HELLP syndrome and worth further investigation (Landi & Tranquilli 2008, James et al. 2010, Young et al. 2010). Therefore, the maternal immune responses to the invading trophoblasts are the central issue (Redman & Sargent 2010). We also observed that the placental trophoblast cells had significantly greater PDGF-D expression in HELLP rats compared to NP rats (Supplementary Fig. 2), which further confirmed the association between the upregulation of PDGF-D and HELLP syndrome.

The potential regulation mechanisms between PDGF-D and HELLP syndrome development will be discussed in our further investigations. Additionally, we determined the expression of PDGF-D in the plasma. In our current study, we found that the patients with diagnosed HELLP syndrome had enhanced PDGF-D expression in the plasma, indicating that the upregulation of PDGF-D is associated with HELLP syndrome. To address whether PDGF-D plays a critical role in the development of HELLP syndrome, we evaluated the effects of the knockdown of PDGF-D in rats with HELLP syndrome. The PDGF-D knockdown rats demonstrate an alteration HELLP syndrome evidenced by (1) relatively low LDH activity, (2) relatively low ALT activity, and (3) relatively high platelet count compared to the HELLP syndrome model rats with the control siRNA.

There is a growing body of literature demonstrating the pivotal role of PDGF family in the development and physiological process in several human diseases (Lindahl et al. 1997, Heldin & Westermark 1999). Overexpression of PDGF-D is associated with the infiltration of macrophages and induces TNF-α secretion in a ICH mouse model (Yang et al. 2016), which is consistent with our findings. In our current study, we found that the level of TNF-α in the rats with HELLP syndrome was augmented; by contrast, the augment was diminished when the expression of PDGF-D was inhibited. The high level of TNF-α contributes to the thrombotic microangiopathy and the thrombotic microangiopathy in patients with HELLP syndrome is observed (Koenig et al. 2005). The increased circulating levels of IL-6 and IL-17 are observed in HELLP rats and women with HELLP (Wallace et al. 2014, Ribeiro et al. 2017). IL-17 is secreted by T helper 17 (Th17) cells that have been implicated in PE (Wallace et al. 2014, Ribeiro et al. 2017). The study by other investigators reveals that HELLP can increase Th17 cells (Wallace et al. 2014), therefore, the expression of PDGF-D may be associated with the Th17 cell activation. Taken together, our findings reveal that the expression of PDGF-D can mediate inflammatory cytokines expression. The effects of PDGF-D on immune responses need to be investigated in the future study.

The alteration of ROS is associated with HELLP syndrome pathogenesis (Matsubara et al. 2015, Morris et al. 2018). The upregulation of ROS can cause tissue damage and the dysfunction of ET activation plays a critical role in hypertension in a rat model of HELLP syndrome (Matsubara et al. 2015, Morris et al. 2016). The blockade of the ET receptor can result in a decrease of ROS level and alleviation of the progression of HELLP syndrome in a rat model (Morris et al. 2018).
The level of SOD increased during normal pregnancy while the SOD activity is decreased in the patients with HELLP syndrome (Jauniaux et al. 2000, Wang & Walsh 2001). We found that the levels of ROS and ET increased in a rat model of HELLP syndrome; by contrast, the activities of SOD and NO decreased, which are consistent with these previous studies. The upregulation of oxidative stress may be involved in the pathophysiology of HELLP syndrome, and the mediation of oxidative stress parameters might be a new insight for HELLP syndrome investigation. In our present study, the association between PDGF-D and the oxidative stress activity has been confirmed and blocking PDGF-D function resulted in the rebalance of oxidative damage in vivo, suggesting that PDGF-D may play a key role in regulating oxidative stress. Emerging investigations have identified PDGF-D is involved in ROS alteration (Wang et al. 2010, Hye Kim et al. 2015). Therefore, PDGF-D influences the oxidative stress activities in several cell types and it could be used as a HELLP therapeutic target based on our present findings.

An association between PDGF-D and NF-κB was revealed in the present study. In the previous study, PDGF-D is considered as a selective agonist for the PDGFR-β isoform and PDGFR-D administration should primarily activate PDGFR-β which is involved in macrophage activation and inflammatory cell infiltration (Fuhrman et al. 2009, Bethel-Brown et al. 2012, Borkham-Kamphorst et al. 2015). In our study, we demonstrated that the loss of PDGF-D function can suppress PDGF-D downstream signaling via the inhibition of PDGFR-β. Interestingly, NF-κB p65 phosphorylation was inhibited when the expression of PDGF-D was inhibited. As a part of the canonical NF-κB signaling pathway, p65 has a central role in regulating T cell receptor (TCR) signaling and inflammatory responses (Oh & Ghosh 2013, Liu et al. 2017). The alternations of the levels of TNF-α and oxidative stress were observed in the rats with HELLP syndrome in the present study. However, these alternations were diminished after the expression of PDGF-D was inhibited. Taken together, PDGF-D/PDGFR-β axis in placenta tissues may mediate the inflammatory responses via the canonical NF-κB signaling pathway. In further investigations, it is necessary to demonstrate the detailed regulation mechanisms of PDGF-D in inflammatory responses.

In this study, we observed that PDGF-D is highly expressed in both human with HELLP syndrome and a HELLP rat model. Decreased expression of PDGF-D is closely associated with the alleviation of the symptoms of HELLP syndrome, and the mediation of inflammatory cytokine secretion and oxidative stress. The canonical NF-κB signaling pathway may be involved in the PDGF-D/PDGFR-β axis regulation. Since NF-κB signaling pathway regulates a large array of genes that are involved in various processes of the immune and inflammatory responses, the detailed mechanisms of how PDGF-D mediates immune responses via NF-κB are elusive based on the present findings, thus need further investigation. However, the current finding for the function of PDGF-D in the rat model of HELLP syndrome has a limitation. Overall, our current findings provide a theoretical support for the development of PDGF-D targeted therapy for HELLP syndrome. Of note, placenta has myriads of cell types, it is important to localize the specific cell type for PDGF-D receptor and understand the signaling pathways involved in PDGF-D regulation. In our further investigation, immunohistological staining assay and other corresponding evaluations will be used to address these questions, to further investigate the function of PDGF-D in HELLP syndrome. Additionally, the potential downstream target of PDGF-D/PDGFR-β axis including NF-κB family members need to be clarified.

**Conclusion**

In conclusion, expression of PDGF-D in the patients with HELLP syndrome was found elevated, knockdown of PDGF-D in the rat model can result in the alleviation of HELLP syndrome, probably through reduction of the oxidative stress damage via the inhibition of phosphorylation of NF-κB p65. Therefore, targeting PDGF-D may provide a potential novel strategy to treat HELLP syndrome.

**Supplementary materials**

This is linked to the online version of the paper at https://doi.org/10.1530/JME-20-0308.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of research reported.

**Funding**

This work did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.
References


Received in final form 18 December 2021
Accepted 22 February 2021
Accepted Manuscript published online 26 February 2021