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ORIGINAL ARTICLE

Intrauterine Inflammation Damages Placental Angiogenesis *via* Wnt5a-Flt1 Activation

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Abstract— Intrauterine inflammation is the main reason for neonatal adverse outcomes and normal placenta perfusion plays an important role in fetal development. However, whether inflammation will affect placental angiogenesis and the underlying mechanism are still poorly understood. To investigate lipopolysaccharide (LPS)-induced intrauterine inflammation on placenta angiogenesis and Wnt5a-Flt1 expression. LPS-induced intrauterine inflammation rat model was established. Preterm rat outcomes were analyzed and angiogenesis of placenta villi was calculated by immunohistochemistry (IHC) of CD34 staining, and placenta Wnt5a-Flt1 expression was detected by western blot and IHC. Compared to control group, neonatal rats in LPS group showed higher death rate (1.4% *vs* 10.1%, $p < 0.05$) and lower birth weight (6.36 ± 0.48 *vs* 5.70 ± 0.67, $p < 0.01$); the villi vessel area and mean diameter in the placenta were significantly reduced in the LPS group (total area %, $16.7\% \pm 0.6\%$ *vs* $8.7\% \pm 0.4\%$, $p < 0.01$, $n = 9$; mean diameter (pixel), 15.6 ± 0.5 *vs* 12.9 ± 0.3, $p < 0.01$, $n = 9$). Placenta Wnt5a-Flt1 expression was upregulated significantly (integrated optical density (IOD) in IHC: Wnt5a, 1667 ± 1204 *vs* $11{,}076 \pm 4046$, $p < 0.05$; Flt1, 2554 ± 466.2 *vs* 7998 ± 1613, $p < 0.05$; western blot: Wnt5a, 0.33 ± 0.05 *vs* 0.96 ± 0.06, $p < 0.05$; Flt1, 0.36 ± 0.15 *vs* 1.08 ± 0.08, $p < 0.05$). Intrauterine inflammation gave rise to offspring death rate and low birth weight; the mechanism might be disordered placental angiogenesis *via* Wnt5a-Flt1 activation triggered by inflammation.

KEY WORDS: intrauterine inflammation; angiogenesis; placenta; preterm; Wnt5a; Flt1.

INTRODUCTION

Intrauterine inflammation occurs in around 25% preterm neonates and it is an independent risk factor leading to adverse neonatal outcomes such as bronchopulmonary dysplasia (BPD), necrotizing enterocolitis (NEC), early-onset neonatal sepsis, and abnormal neurodevelopment [1]. Previous studies had showed co-occurrence of placental malperfusion and inflammation may be a novel disorder resulting in severe neonatal health consequences [2]. However, whether placental inflammation will affect angiogenesis, thus leading to adverse birth outcomes and the underlying mechanism, are still poorly understood.

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Wnt5a plays a critical role in fetal normal cellular processes, including placental morphogenesis and inflammatory response [3, 4]. It is induced in response to conserved bacterial structures and lipopolysaccharide (LPS), thus contributing to regulation of pro-inflammatory cytokines [5]. Flt1, an important factor involved in angiogenesis, was regulated by Wnt5a [6–8]. Wnt5a-Flt1 signaling pathway has been reported to affect dermal and retina angiogenesis [9, 10]. However, whether intrauterine inflammation affects placental angiogenesis and the role of Wnt5a-Flt1 signaling are still unclear.

Based on this evidence above, inflammation is known for infiltration of neutrophils and increased expression of Wnt5a [3, 11]. Because Flt1, regulated by Wnt5a, could affect angiogenesis, it is very likely that intrauterine inflammation is regulating placental angiogenesis *via* Wnt5a-Flt1 pathway. To investigate the effect of intrauterine inflammation on placental angiogenesis and offspring outcome, we built up an LPS-induced intrauterine inflammatory rat model and compared offspring outcome, placental angiogenesis, and placental Wnt5a-Flt1 expression between the inflammatory group and control group. Here, we showed that activation of Wnt5a-Flt1 pathway is correlated with impaired placental angiogenesis during intrauterine inflammation, which may lead to disordered placental function and a possible intrauterine hypoxia, finally reduced birth weight and even dead-birth.

METHODS

Intrauterine Inflammatory Exposure Rat Models

All animal experiments were conducted under a protocol approved by the Chinese Institutional Animal Care and Use Committee, which conforms to the Guide for the Care and Use of Laboratory Animals and in accordance with the Declaration of Helsinki principles. All rats used in this study were housed in a specific pathogen-free facility under the care of the Central Research Department of Guang Zhou Medical University. Sprague Dawley (SD) rats were purchased from Animal research center in Guangzhou Medical University certification number as (SCXK(YUE)2013-0002). LPS 0.79 mg/kg was injected intraperitoneally in 8, 10, and 12 gestational days (E 8, 10, 12) in pregnant SD rats [11–14]. The LPS preparation used was serotype O26:B6 *Escherichia coli* LPS (Sigma Chemical, St. Louis, MO). Pregnant rats were confirmed by positive finding of sperm *via* vaginal smear after mating. The day of mating was designated as day 0 of gestation. These pregnant rats were randomized into two groups and injected with LPS ($n = 6$) or equal volume of saline ($n = 6$). Preterm rats were then delivered at 21 gestational days *via* cesarean section. Placentas were collected and conserved. Survival rate was observed at birth and birth weight was measured. Results were compared in the two groups.

Microscopic Analysis and Immunohistochemistry

After birth, placentas were washed with phosphate-buffered saline (PBS) and examined. Four random full-depth samples of the placentas were then taken from each tissue. Some samples were fixed in paraformaldehyde (4%) in phosphate buffer solution (NaCl 13.7 mM, KCl 2.7 mM, Na_2HPO_4 0.9 mM, KH_2PO_4 1.8 mM, pH 7.4, 4 °C) and embedded in paraffin. Other samples were immersed in liquid nitrogen and maintained at − 80 °C until used for protein extraction. Paraffin-embedded tissue sections were cut into 5-μm slices to use for H&E staining and immunodetection of CD34 (rabbit polyclonal antibody; Thermo Scientific, Waltham, MA), Wnt5a (rabbit polyclonal antibody, Abcam, ab174963, USA), and Flt1 (rabbit monoclonal antibody, Abcam, ab32152, USA) using commercial kits (Vector Laboratories, Burlingame, USA). Antigen-antibody reaction was visualized by diaminobenzidine reaction, and image analysis was performed using Image-Pro Plus software (Media Cybernetics, Rockville, MD). CD34 is a marker of endothelial cells. Maternal vessels are constructed of trophoblasts which are negative for CD34 staining. The fetal vessels consist of endothelium cells. CD34, a marker for the endothelium in fetal capillaries, separates fetal vessels from maternal vessels [15–20]. Randomly selected high-power fields (× 200) from chorion and subchorionic region in each slide were analyzed. The percent of placental villi fetal vessel area and mean diameter in each filed were measured and expressed as vessel area percent (%) and pixel [21, 22]. The expression of Wnt5a and Flt1 was determined by counting integrated optical density (IOD). Analysis of the section was blinded.

Western Blot

Western blot was performed to evaluate gene expression at the protein level. Total protein was extracted from placenta tissue using M-PER reagent (Pierce, Rockford, IL, USA) and quantified using the BCA protein assay kit (Pierce, Rockford, IL, USA). Homogenates were centrifuged at 14,000*g* at 4 °C for 10 min. The supernatant was used for western blot analysis, where protein extracts (50 mg) were separated by

SDS-PAGE (12%), transferred to nitrocellulose membranes, and probed with primary anti-Wnt5a, anti-Flt1, and anti-GAPDH (Abcam, USA) antibodies for 12 h at 4 °C after being blocked with 5% fat-free milk for 1 h. The membrane was further incubated for 1.5 h with appropriate horseradish peroxidase–conjugated secondary antibodies (1:1000, goat anti-mouse/rabbit IgG-HRP, Abcam) at room temperature. The proteins were detected by enhanced chemiluminescence (Beyotime, China). GAPDH was employed as an internal control.

Statistical Analyses

All experimental groups were carried out at least three times in triplicates. Results are expressed as mean ± SEM if the data were normally distributed. Statistical analyses were performed using an unpaired two-tailed Student's *t* test. A value of $p < 0.05$ was considered statistically significant. All statistical analyses were done using SPSS 21.0 (IBM).

RESULTS

Placenta Characteristics and Preterm Rat Outcomes

Acute chorioamnionitis in the LPS group was confirmed by abundant infiltration of neutrophil cells in chorion and subchorionic fibrin of the placenta detected by H&E under a microscope (Fig. 1). Compared to the control group, neonatal rats in the LPS group showed higher death rate (1.4% *vs* 10.1%, $p < 0.05$) and lower birth weight (6.36 ± 0.48 *vs* 5.70 ± 0.67, $p < 0.01$). Data are shown in Table 1.

Angiogenesis in Placenta Villi

To assess angiogenesis in placenta villi, CD34 staining was used to identify the fetal vascular endothelial cells. Compared to the control group, the total area and mean diameter of fetal vessels were decreased obviously in the LPS group (vessel total area %, 16.7% ± 0.6% *vs* 8.7% ± 0.4%, $p < 0.01$, $n = 9$; mean diameter, 15.6 ± 0.5 *vs* 12.9 ± 0.3, $p < 0.01$, $n = 9$). Furthermore, the mean diameter of vessels was larger in the control group (Fig. 2).

Protein Expression of Wnt5a-Flt1 Signal in the Placenta

The total protein expression of Wnt5a and Flt1 was both upregulated significantly during LPS-induced inflammatory response in placentas, which were testified *via* IHC and western blot (integrated optical density in IHC: Wnt5a, 1667 ± 1204 *vs* 11,076 ± 4046, $p < 0.05$; Flt1, 2554 ± 466.2 *vs* 7998 ± 1613, $p < 0.05$; western blot: Wnt5a, 0.33 ± 0.05 *vs* 0.96 ± 0.06, $p < 0.05$; Flt1, 0.36 ± 0.15 *vs* 1.08 ± 0.08, $p < 0.05$) (Figs. 3 and 4).

Correlation Analysis on Flt1 and Wnt5a with Placental Villi Vessel Area

In the LPS group, there was a significantly positive relationship between Wnt5a protein level and Flt1 protein level ($r = 0.914$, $p < 0.001$; Fig. 5a). Very interesting, it was also revealed that both Wnt5a protein level and Flt1 protein level correlated with placental villi vessel area negatively (IOD of Wnt5a and vascular area%: $r = -0.888$, $p < 0.001$; IOD of Flt1 and vascular area: $r = -0.862$, $p < 0.003$, Fig. 5b, c). However, none of these relationships was observed in the control group (Fig. S1).

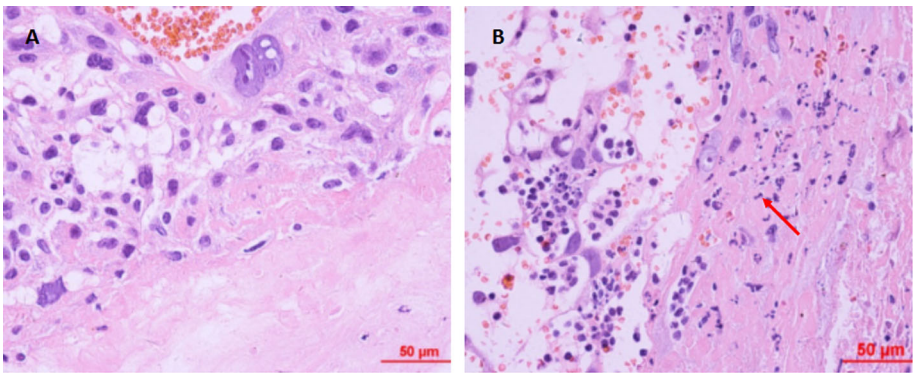

Fig. 1. Representative placental inflammation images detected by H&E staining. **a** Control group—few neutrophils are infiltrated in all levels of placental membranes. **b** LPS group—acute chorioamnionitis. Abundant neutrophils are present in chorion and subchorionic fibrin at bottom. Arrow indicates neutrophils (hematoxylin and eosin stain; scale bars represent 50 μm).

Table 1. Outcomes of Preterm Rats in Control and LPS Group

	Control group ($n = 72$)	LPS group ($n = 59$)	p
Gestation (days)	21	21	
Stillbirth (%)	1/72 (1.4%)	6/59 (10.1%)	0.032
Birth weight (g)	6.36 ± 0.48	5.70 ± 0.67	$p < 0.01$

DISCUSSION

This study was undertaken to understand the possible role of intrauterine inflammation and placental angiogenic factors in adverse outcomes of neonatal rats of preterm pregnancies. The key findings of this study are (1) placental inflammation could lead to intrauterine growth retardation and even neonatal death. (2) Placentas of the LPS-induced intrauterine inflammation group had more infiltration of neutrophil cells and higher Wnt5a and Flt1 expression; and lower vessel area and diameter. (3) A significantly positive correlation between Wnt5a and Flt1 protein level, and a negative correlation between Wnt5a or Flt1 protein level and placental villi vessel area was observed in the inflammation group, but not in the control group.

Placental malperfusion caused by impaired angiogenesis led to reduced fetal growth and intrauterine hypoxia [15, 23]. Previous studies have confirmed that intrauterine inflammation is associated with neonatal complications and increased mortality [2, 15]. Janet M. Catov. et al. [2] revealed that co-occurrence of malperfusion and inflammation/infection, especially among spontaneous preterm births, may be a novel placental disorder resulting in severe adverse outcomes [2]. However, whether the placental inflammation will affect its angiogenesis and the underlying mechanism still remained to be explored.

Different from vasculogenesis (*de novo* synthesis of blood vessels accounting for the major part of new blood vessel formation in the placenta during the first trimester),

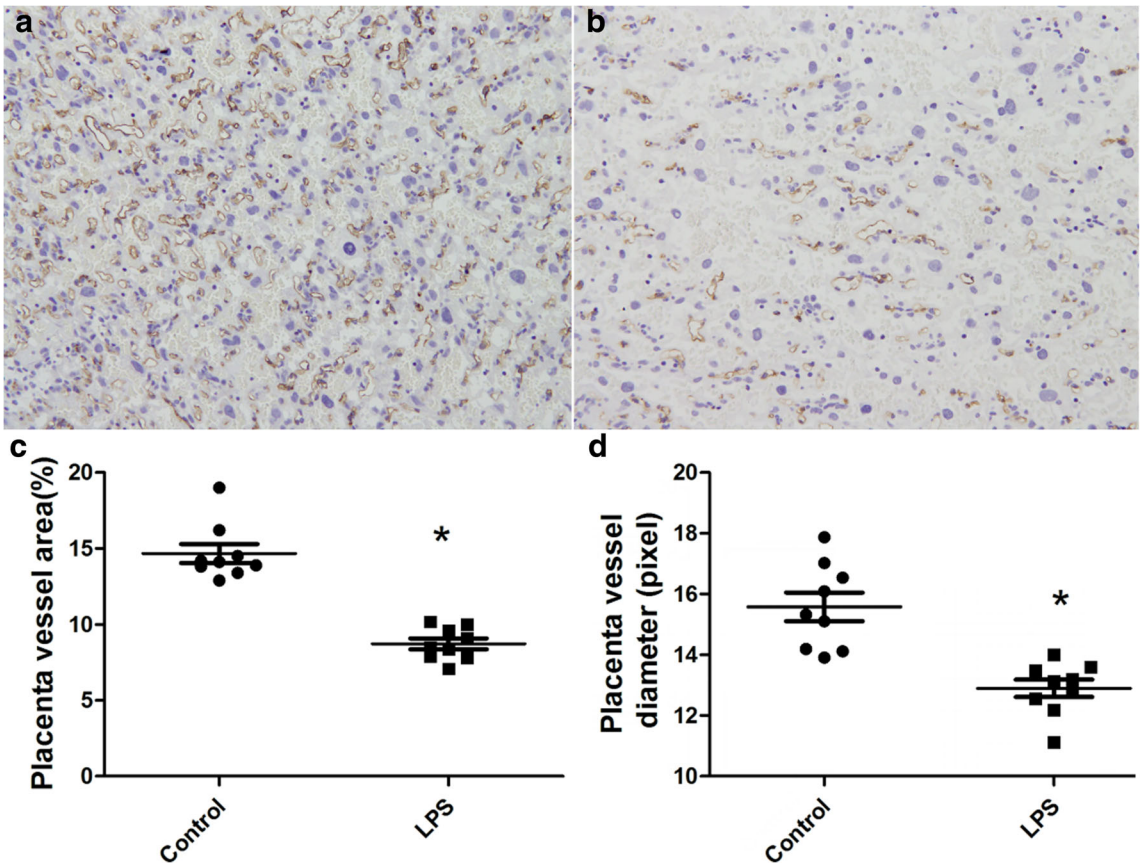

Fig. 2. Vessel total area and mean diameter in placental villi were calculated by immunohistochemistry for CD34. Representative slides are shown. **a** Control group. **b** LPS group. **c** and **d** Quantification of vessel area and diameter (*$p < 0.01$). Significant differences are indicated with respective p value. $N = 9$ in each group. Values are mean ± SEM.

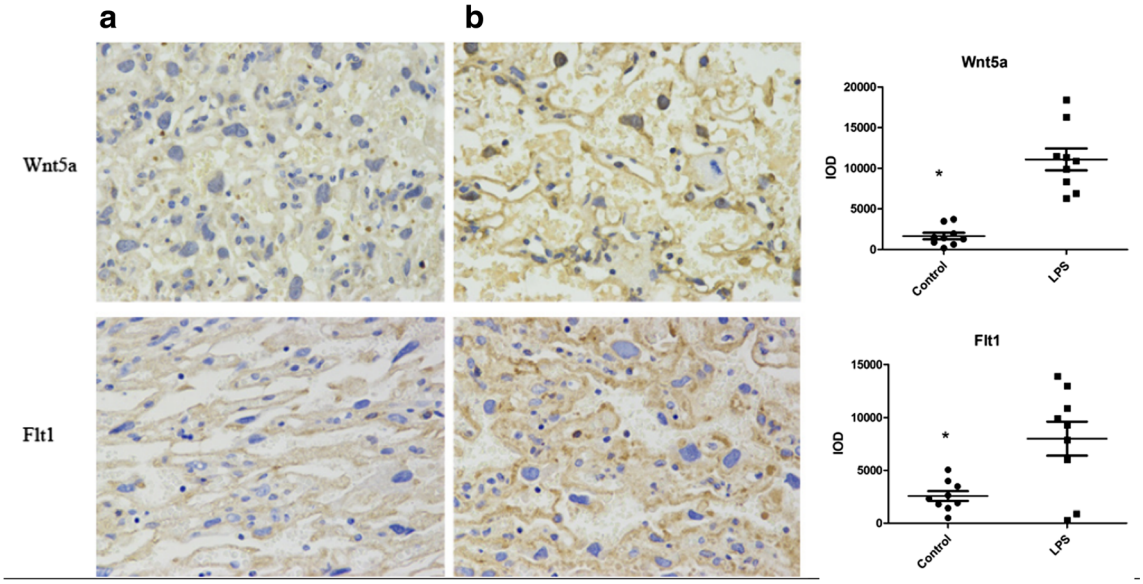

Fig. 3. Wnt5a-Flt1 protein expression in placenta by IHC. Representative slides are shown. **a** Control. **b** LPS (original magnification × 200). Quantification of the integrated optical density (IOD) is presented in the corresponding graph (*$p < 0.05$). $N = 9$ in each group. Values are mean ± SEM.

angiogenesis is growth of existing vessels which is following vasculogenesis in the following trimesters [12]. The ramification or pruning of intervillous vessels is a main character of angiogenesis [15]. Here, in our study, we built a rat model of intrauterine inflammation by multiple injections of LPS during pregnancy, and the success of the animal model was confirmed by placental neutrophil infiltration. We used CD34 immunostaining (to quantify vessel area and size) as a measure of angiogenesis [21, 22] and found that in the LPS group, villi vessel area and diameter declined obviously compared with the control group. Therefore, we indicated that placental malperfusion, symbolized by decreased vessel area, was

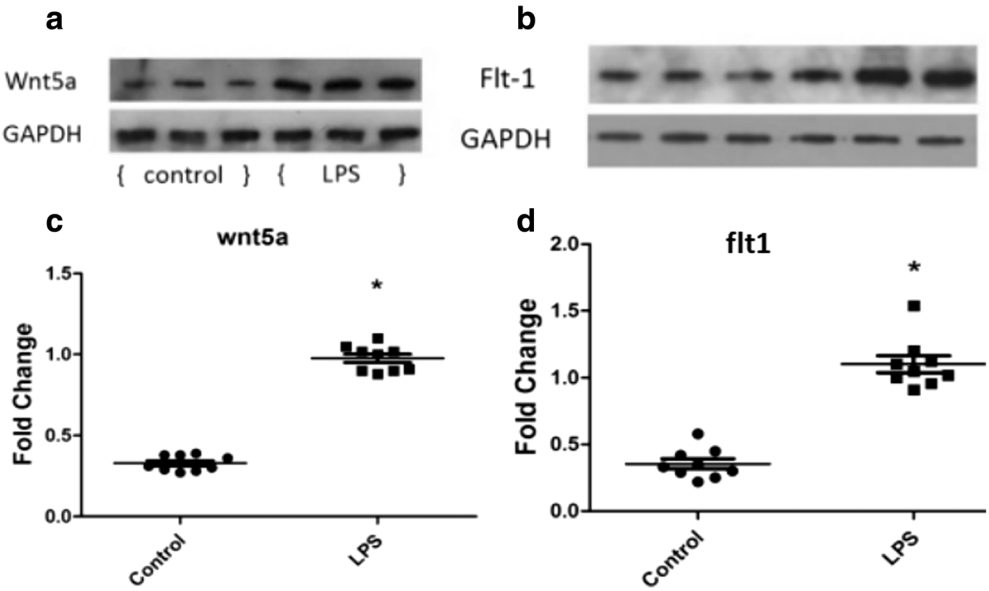

Fig. 4. Wnt5a-Flt1 signal protein expression in placenta by western blot. **a** Wnt5a. **b** Flt1. Representative image of western blot for Wnt5a (42 kD) and Flt1 (151 kDa). GAPDH (36 kDa) was used as loading control. **c** and **d** Densitometric analysis is presented in the corresponding bottom graph. Significant differences are indicated with respective p value (*$p < 0.05$). $N = 9$ in each group. Values are mean ± SEM.

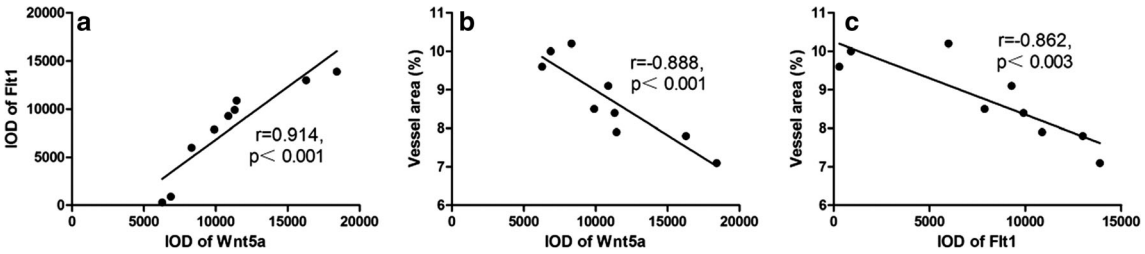

Fig. 5. Correlations between Wnt5a-Flt1 protein level (by IHC) and placenta vascular area in LPS group. $N = 9$. **a** A positive correlation between Wnt5a and Flt1 protein levels. $r = 0.914$, $p < 0.001$. **b** Wnt5a protein level correlated negatively with placental vascular area. $r = -0.888$, $p < 0.001$. **c** Flt1 protein level correlated negatively with placental vascular area. $r = -0.862$, $p < 0.003$.

induced by placental inflammation. As we know, angiogenesis of chorionic villi is very important for maintaining normal placental perfusion, which is the key factor to assure adequate maternal-fetal exchanges therefore providing enough nutrition and oxygen to fetus [15, 23, 24]. The direct result of imbalanced angiogenesis of the placenta is insufficient maternal-fetal exchanges and lack of oxygen, then causing intrauterine fetal distress; sustained hypoperfusion and hypoxia will lead to fetal growth retardation and even death [2, 25]. This is the first time to reveal the possible relationship between inflammation and abnormal placenta angiogenesis, thus partly illuminating the underlying mechanisms of adverse neonatal outcomes caused by intrauterine inflammation.

In the next step, we explored the molecular pathway involved in the inflammation-vascular pathology. Wnt5a belongs to a family of secreted, lipid-modified glycoproteins. It was expressed abundantly in the placenta and played a pivotal role in early fetal development by regulating cell proliferation and survival. Recently, it was noticed that Wnt5a expression was upregulated in bacterial infection and acted as an effector that triggered inflammation [26–28]. Our research also demonstrated that Wnt5a was activated in placental inflammation. *In vitro* studies have showed that Wnt5a plays a role in regulating angiogenesis. James A. Stefater III et al. [10] showed that a noncanonical Wnt5a-Flt1 pathway in myeloid cells could suppress angiogenic branching [10]. Flt1 was an inhibitor of vascular endothelial growth factor–mediated angiogenesis [6]. It was demonstrated that Flt1 was regulated by Wnt5a, and the Wnt5a-Flt1 signaling pathway could affect repairing *via* inhibiting dermal angiogenesis and regulating neovascularization [8]. Our results revealed that LPS injection during pregnancy led to an inflammatory response in the placenta, thus triggering upregulation of Wnt5a-Flt1 signal, which was demonstrated through both IHC and western blot. Increased Flt1 expression is correlated with decreased placental angiogenesis. A positive correlation between Wnt5a and Flt1 in our study was consistent with previous study and demonstrated the Wnt5a's regulatory function of Flt1 [10]. It was very interesting that in the LPS group, expression of Wnt5a-Flt1 was negatively correlated with placenta villi vessel area, which suggested that Wnt5a-Flt1 activation may damage angiogenesis of the placenta and thus resulted in adversed neonatal outcomes mediated by malperfusion. However, there is lack of correlation of Wnt5a or Flt1 protein with vessel area in the control group. This may be attributed to comparative low expression of Wnt5a without inflammatory stimulation. This low level of Wnt5a may not be able to activate Flt1 expression as well as affect vessel area. Here, we indicated that Wnt5a-Flt1 activation may mediate inflammation-induced disordered angiogenesis. These results are consistent with the published link between intrauterine inflammation and poor offspring outcomes.

However, our study also has some limitations. First, except for Wnt5a-Flt1 signal, there may be other pathways playing a role in this pathological condition. A Wnt5a knockdown or knockout animal model may be helpful to demonstrate the specific function of Wnt5a-Flt1 signal in intrauterine inflammation–induced placental malperfusion. Second, observational clinical trials in patients with intrauterine inflammation and their offspring are needed to further confirm the relationship between intrauterine inflammation and placental angiogenesis clinically.

In conclusion, we showed that intrauterine inflammation may use the Wnt5a-Flt1 pathway to damage placental angiogenesis. This study provides an insight into possible novel therapeutic approaches for treatment of intrauterine inflammation–induced adverse neonatal complications.

AVAILABILITY OF DATA AND MATERIAL

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

AUTHORS' CONTRIBUTIONS

Xu F, Zhong XM, and Zhang JY performed the experiments; Zhang Q analyzed data and collected the references; and Ren ZX was a major contributor in writing the manuscript. Yang J designed this study and edited the manuscript. All authors read and approved the final manuscript.

FUNDING INFORMATION

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest. The authors declare that they have no conflicts of interest.

Ethics Approval. Applicable

Name of the Ethics Committee That Approved the Study. Guangdong Women and Children Hospital Ethics committee

The Committee's Reference Number. 201701079

Consent for Publication. Not applicable.

REFERENCES

1. Erdemir, G., N. Kultursay, S. Calkavur, O. Zekioğlu, O.A. Koroglu, B. Cakmak, M. Yalaz, M. Akisu, and S. Sagol. 2013. Histological chorioamnionitis: effects on premature delivery and neonatal prognosis. *Pediatrics & Neonatology.* 54 (4): 267–274.
2. Catov, J.M., C.M. Scifres, S.N. Caritis, M. Bertolet, J. Larkin, and W.T. Parks. 2017. Neonatal outcomes following preterm birth classified according to placental features. *American Journal of Obstetrics and Gynecology* 216 (4): 411.
3. Bhatt, P.M., and R. Malgor. 2014. Wnt5a: a player in the pathogenesis of atherosclerosis and other inflammatory disorders. *Atherosclerosis* 237 (1): 155–162.
4. Meinhardt G, Saleh L, Otti GR, et al. Wingless ligand 5a is a critical regulator of placental growth and survival. SCI REP-UK. 2016;6(1).
5. Schaale, K., J. Neumann, D. Schneider, S. Ehlers, and N. Reiling. 2011. Wnt signaling in macrophages: augmenting and inhibiting mycobacteria-induced inflammatory responses. *European Journal of Cell Biology* 90 (6–7): 553–559.
6. Khankin EV, Mandala M, Colton I, Karumanchi SA, Osol G. Hemodynamic, vascular, and reproductive impact of FMS-like tyrosine kinase 1 (Flt1) blockade on the uteroplacental circulation during normal mouse pregnancy 1. BIOL REPROD. 2012 2012-02-01;86(2).
7. Weissgerber, T.L., A. Rajakumar, A.C. Myerski, L.R. Edmunds, R.W. Powers, J.M. Roberts, R.E. Gandley, and C.A. Hubel. 2014. Vascular Pool of releasable soluble VEGF receptor-1 (sFlt1) in women with previous preeclampsia and uncomplicated pregnancy. *The Journal of Clinical Endocrinology & Metabolism.* 99 (3): 978–987.
8. Murdoch, C.E., M.M. Bachschmid, and R. Matsui. 2014. Regulation of neovascularization by S-glutathionylation via the Wnt5a/sFlt-1 pathway. *Biochemical Society Transactions* 42 (6): 1665–1670.
9. Stefater JR, Rao S, Bezold K, et al. Macrophage Wnt-Calcineurin-Flt1 signaling regulates mouse wound angiogenesis and repair. BLOOD. 2013 2013-03-28;121(13):2574–8.
10. Stefater III JA, Lewkowich I, Rao S, et al. Regulation of angiogenesis by a non-canonical Wnt–Flt1 pathway in myeloid cells. NATURE. 2011 2011-05-29;474(7352):511–5.
11. Kim, C.J., R. Romero, P. Chaemsaithong, N. Chaiyasit, B.H. Yoon, and Y.M. Kim. 2015. Acute chorioamnionitis and funisitis: definition, pathologic features, and clinical significance. *American Journal of Obstetrics and Gynecology* 213 (4 Suppl): S29–S52.
12. Demir, R.E.A., et al. 1989. Fetal vasculogenesis and angiogenesis in human placental villi. *Acta Anatomica (Basel)* 136 (3): 190–203.
13. Wei, Y., W. du, X. Xiong, X. He, N. Ping Yi, Y. Deng, D. Chen, and X. Li. 2013. Prenatal exposure to lipopolysaccharide results in myocardial remodelling in adult murine offspring. *Journal of Inflammation (Lond)* 10: 35.
14. Wei, Y.L., X.H. Li, and J.Z. Zhou. 2007. Prenatal exposure to lipopolysaccharide results in increases in blood pressure and body weight in rats. *Acta Pharmacologica Sinica* 28 (5): 651–656.
15. Resta, L., C. Capobianco, A. Marzullo, D. Piscitelli, F. Sanguedolce, F.P. Schena, and L. Gesualdo. 2006. Confocal laser scanning microscope study of terminal villi vessels in normal term and preeclamptic placentas. *Placenta* 27 (6–7): 735–739.
16. Escudero, C., C. Celis, T. Saez, S. San Martin, F.J. Valenzuela, C. Aguayo, P. Bertoglia, J.M. Roberts, and J. Acurio. 2014. Increased placental angiogenesis in late and early onset pre-eclampsia is associated with differential activation of vascular endothelial growth factor receptor 2. *Placenta* 35 (3): 207–215.
17. Troncoso, F., J. Acurio, K. Herlitz, C. Aguayo, P. Bertoglia, E. Guzman-Gutierrez, M. Loyola, M. Gonzalez, M. Rezgaoui, G. Desoye, and C. Escudero. 2017. Gestational diabetes mellitus is associated with increased pro-migratory activation of vascular endothelial growth factor receptor 2 and reduced expression of vascular endothelial growth factor receptor 1. *PLoS One* 12 (8): e0182509.
18. Szabova, L., M.Y. Son, J. Shi, M. Sramko, S.S. Yamada, W.D. Swaim, P. Zerfas, S. Kahan, and K. Holmbeck. 2010. Membrane-type MMPs are indispensable for placental labyrinth formation and development. *Blood* 116 (25): 5752–5761.
19. Cornelis, G., C. Vernochet, Q. Carradec, S. Souquere, B. Mulot, F. Catzeflis, M.A. Nilsson, B.R. Menzies, M.B. Renfree, G. Pierron, U. Zeller, O. Heidmann, A. Dupressoir, and T. Heidmann. 2015. Retroviral envelope gene captures and syncytin exaptation for placentation in marsupials. *Proceedings of the National Academy of Sciences of the United States of America* 112 (5): E487–E496.
20. Miner, J.J., B. Cao, J. Govero, A.M. Smith, E. Fernandez, O.H. Cabrera, C. Garber, M. Noll, R.S. Klein, K.K. Noguchi, I.U.

Mysorekar, and M.S. Diamond. 2016. Zika virus infection during pregnancy in mice causes placental damage and fetal demise. *Cell* 165 (5): 1081–1091.

21. Higashisaka, K., et al. 2018. Neutrophil depletion exacerbates pregnancy complications, including placental damage, induced by silica nanoparticles in mice. *Frontiers in Immunology* 9: 1850.
22. Jones, H.N., S.K. Olbrych, K.L. Smith, J.F. Cnota, M. Habli, O. Ramos-Gonzales, K.J. Owens, A.C. Hinton, W.J. Polzin, L.J. Muglia, and R.B. Hinton. 2015. Hypoplastic left heart syndrome is associated with structural and vascular placental abnormalities and leptin dysregulation. *Placenta* 36 (10): 1078–1086.
23. Lockwood, C., S.J. Huang, G. Krikun, R. Caze, M. Rahman, L. Buchwalder, and F. Schatz. 2011. Decidual hemostasis, inflammation, and angiogenesis in pre-eclampsia. *Seminars in Thrombosis and Hemostasis* 37 (02): 158–164.
24. Seliga-Siwecka, J.P., and M.K. Kornacka. 2013. Neonatal outcome of preterm infants born to mothers with abnormal genital tract colonisation and chorioamnionitis: a cohort study. *Early Human Development* 89 (5): 271–275.
25. Gram, A., B. Hoffmann, A. Boos, and M.P. Kowalewski. 2015. Expression and localization of vascular endothelial growth factor A (VEGFA) and its two receptors (VEGFR1/Flt1 and VEGFR2/FLK1/KDR) in the canine corpus luteum and utero-placental compartments during pregnancy and at normal and induced parturition. *GEN COMP ENDOCR.* 223: 54–65.
26. Kumawat, K., and R. Gosens. 2016. WNT-5A: signaling and functions in health and disease. *Cellular and Molecular Life Sciences* 73 (3): 567–587.
27. Blumenthal A. The Wingless homolog Wnt5a and its receptor Frizzled-5 regulate inflammatory responses of human mononuclear cells induced by microbial stimulation. BLOOD. 2006 2006-08-01;108(3):965–73.
28. Kikuchi, A., H. Yamamoto, A. Sato, and S. Matsumoto. 2012. Wnt5a: its signalling, functions and implication in diseases. *Acta Physiologica* 204 (1): 17–33.