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Original Article

Maternal protein restriction alters VEGF signaling and decreases pulmonary alveolar in fetal rats

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Abstract: Epidemiological studies have demonstrated that intrauterine growth restriction (IUGR) increases the risk for respiratory morbidity from infancy, throughout childhood and into adulthood. Chronic restriction of nutrients causes abnormalities in the airways and lungs of offspring, but whether IUGR adversely impacts fetal pulmonary vascular development and underlying mechanisms remain under investigation. In this study, we investigated the effects of protein malnutrition *in utero* on pulmonary alveolarization and vascular growth of the fetal lung and placentae. Pregnant rats were feed with an isocaloric low-protein diet (8% protein) until delivery. Placenta and fetal lungs were harvested on 20th day of gestation (term 21 days of gestation). Lung index (lung weight as a percentage of body weight), total DNA and protein, radial alveolar count, arteriolar wall thickness, lung maturity and angiogenic factor VEGF were assessed. The lung was hypoplastic in IUGR fetus, evidenced by reduction in lung weight, DNA and protein content. Protein restriction *in utero* led to higher glycogen levels, but reduced number of alveoli as confirmed by the measurement of radial alveolar counts. IUGR fetus had significantly reduced VEGF, Flk-1 levels in lung but no changes in Flt-1 mRNA. Furthermore, IUGR was associated with increased lung miR-126-3p levels, which modulated the expression of angiogenic factor. In contrast, with regard to the placenta, IUGR fetus presented with decreased expression of VEGF, with no changes in VEGF receptors and expression-regulating miRNAs. This work suggested that VEGF signaling defect plays an important role in the defective lung development, which may explain the increased incidence of respiratory infections in IUGR patients.

Keywords: IUGR, VEGF, pulmonary, miRNA, placenta

Introduction

Intrauterine growth restriction (IUGR), which occurs in about 3-10% of pregnancies, is one of the most important causes for perinatal morbidity and mortality [1, 2]. Decreased fetal growth rates reflect an adaptation to the adverse in utero environment, and may lead to long-lasting modifications in metabolism, growth, and organ development [3, 4]. Concern over the programming effects of IUGR on lung has gained momentum in recent years. Various epidemiological studies have demonstrated a significant association between birth weight and postnatal lung function [5-9]. Preterm infants who are small-for-gestational-age are at greater risk for chronic respiratory illnesses [3, 10]. Even infants with IUGR born at term can experience adverse pulmonary sequelae such as bronchopulmonary dysplasia (BPD) [11]. These infants are more vulnerable to wheezing and other respiratory disorders during the growth process [12]. IUGR has been undoubtedly considered as an independent risk factor for the development of pulmonary morbidity, but the underlying mechanisms of programming effect on the fetal lung remains unknown.

Mammal lung development is divided into five stages: embryonic, pseudoglandular, canalicular, saccular and alveolar stages [13]. Impaired fetal nutrient and oxygen availability, as frequently occurs in association with IUGR, can affect any of these stages, potentially impacting permanently upon lung structure, lung function and respiratory morbidity. Recent animal studies have shown that IUGR has long-term impact on lung structure, including reduction in lung weight, protein and DNA content, reduced alveolarization, decreased surfactant content,

impaired Type II alveolar cell maturation, thickened air-blood barrier as well as decreased density of the pulmonary vessels [14-18].

Genetic program of fetal lung development is a tightly regulated and complex process, and can be influenced by epigenetic and environmental factors during the period of development. The cooperation of multiple pathways across different cellular compartments is essential for comprehensive development of lung. Vascular endothelial growth factor (VEGF, also designated VEGF-A) has been shown to play multiple critical roles in pre- and postnatal lung development. Alternative splicing of the single mouse Vegf gene gives rise to three protein isoforms: Vegf 120, VEGF 164, and VEGF 188 [19]. VEGF 164 is the major endothelial mitogenic factor among the 3 different VEGF isoforms [20]. The key receptors mediating VEGF signaling in lung development are two related tyrosine kinase receptors, receptor-1 (fins-like tyrosine kinase [Flt]-1) and receptor-2 (fetal liver kinase [Flk]-1, or the kinase domain-containing receptor [KDR]) [21]. Targeted deletion of VEGF-A, Flk-1, or Flt-1 has demonstrated the importance of this signaling pathway in lung development [22-24]. Disturbance of the VEGF- receptor signaling with VEGFR blockade in the developing rat not only inhibited angiogenesis but also reduced alveolarization, subsequently led to emphysema, and pulmonary hypertension [25-27].

MicroRNAs (miRNAs) play multiple roles in carcinogenesis, immune responses and organ development by posttranscriptional regulation of their target genes, and in many cases, they have been shown to modulate intracellular signaling pathways in cells [28-31]. Recent evidence demonstrated that miRNAs exert a strong influence over organ morphogenesis by targeting hormones and growth factors such as VEGF. miRNA profiling studies demonstrate the dynamic expression of miR-126-3p, -150, and -504 during lung organogenesis, and they were all validated targeting on the VEGF 3'-UTR [32-34]. miR-16 and miR-424 regulate cell-intrinsic angiogenic activity of endothelial cells by modulating VEGF and Flk-1 expression [35]. All these data suggested that miRNAs potentially involve in the lung development by influencing VEGF signaling pathway. Recent experimental studies have suggested that various adverse event *in utero* influenced miRNAs levels in multiple organs, subsequently led to alteration of the expression of their target genes [36, 37]. No studies yet reported have explored the effect of protein malnutrition during pregnancy on fetal lung miRNAs levels.

Thus, evidence exists to support the hypothesis that fetal pulmonary alveolarization and angiogenesis would be impaired in experimental IUGR and disturbed signaling through VEGF-VEGFR pathway is associated with defective development of lung. To test our hypothesis, we developed an IUGR model in rats by maternal protein restriction, whereby the animals share many of the fundamental fetal complications of human IUGR [38]. We quantified indexes of alveolar formation at E20 (saccular stage of lung development) using morphometric methods. We used molecular methods to measure mRNA transcript levels and protein abundances of the key molecules of VEGF signaling pathway, and selected microRNAs as upstream regulators. We also investigated the effect of protein restriction on VEGF signaling pathway in placenta. Our results shown that IUGR led to hypoplastic lung and decreased VEGF-signaling molecules accompanied with increased miR-126-3p levels in the lung. In contrast, IUGR fetus only presented decreased expression of placenta VEGF, with no significant changes in microRNAs. Thus we speculated that protein restriction *in utero* disrupted VRGF signaling through different mechanisms in the lung and placenta, and over-expression of miR-126-3p may down-regulate VEGF-signaling molecules, then contribute to the hypoplastic lung in IUGR.

Materials and methods

Animals

The animal study protocols were approved by the Animal Research Committee of China Medical University. All animals were individually housed under specific pathogen-free conditions in an environmentally-controlled clean room. Food and water were provided *ad libitum* throughout the study. Timed-pregnant Wistar rats were obtained from the Experimental Animal Center (Shengjing Hospital, China Medical University), with the morning of the vaginal plug defined as gestational day 0 (E0). Then pregnant rats were randomly divided into 2 groups: animals in the undernourished group received an isocaloric low-protein diet (8% protein) from day 0 of pregnancy until full term as described before [38]; control animals were

Table 1. Primer sequence and length of the amplicons of the genes studied with real-time RT-PCR

Target Genes	Accession No.	Left primer sequence	Right primer sequence	Amplicon length (nt)
Flt-1	NM_019306	TGTCCTCAACTGCACCGTCAC	CCGCTGCCTGATAGATGCTCTC	100
Flk-1	NM_013062	TAGCACGACAGAGACTGTGAGGT	TGAGGTGAGAGAGATGGGTAGG	113
β-actin	NM_031144	ACTATTGGCAACGAGCGGTT	TGTCAGCAATGCCTGGGTACA	190
RT-primer	GCTGTCAACGATACGCTACGTAACGGCATGACAGTGTTTTTTTTTTTTTTTTTTTTTTV			
rno-miR-126a-3p	MIMAT0000832	TCGTACCGTGAGTAATAATGC	GCTGTCAACGATACGCTACG	81
rno-miR-16	MIMAT0000785	GTAGCAGCACGTAAATATTGG	GCTGTCAACGATACGCTACG	81
rno-miR-150	MIMAT0000853	CTCCCAACCCTTGTACCAGT	GCTGTCAACGATACGCTACG	80
U6	K00784	CTCGCTTCGGCAGCACA	AAGGCAGCAGGTCGTATAGT	94

β-actin and U6 were used as the housekeeping genes, and mRNA PCR data was normalized to β-actin expression, while miRNA PCR data was normalized to U6 expression.

maintained on standard diet (20% protein) during gestation. This produced IUGR in 56.12% of fetuses [38]. On the 20th day of gestation (E20), a set of pups (n = 6) were delivered by caesarian section, and the placental samples and fetal lungs were removed *en bloc*, weighed, snap-frozen in liquid nitrogen, and stored at -80°C. DNA was extracted from snap-frozen lung samples with the Universal Genomic DNA Extraction Kit (Takara, Dalian, China), according to the manufacturer's protocol and quantified in duplicate using UV absorbance at 260 nm. Total protein was determined using Protein Quantitative Detection Kit (Bio-Rad, Hercules, CA).

Histology and morphometric analysis

Another set of E20 pups (n = 6) were delivered by caesarian section and the chest was opened. After tracheal cannulation, 4% paraformaldehyde was injected at < 10 cm/H_2O for alveolar distention, and the fetal lungs and placentae were harvested, fixed and embedded in paraffin. 4-μm lung sections were stained with hematoxylin and eosin, Periodic acide Schiff (PAS), and Masson staining. At least three lung sections from each animal were assessed for morphometric analysis. Ten peripheral arterioles with diameters ranging from 50 to 100 μm in three lung sections from each animal were selected and the vascular parameters were measured with the assistance of image analysis software (Image Pro-Plus; Media Cybernetics, Washington, DC). Immunohistochemical staining on lung and placenta sections were performed using standard techniques with anti-VEGF (19003-1-AP, Proteintech), Flt-1 (ab32152, Abcam), or Flk-1 (21079, Signalway) antibody.

Quantitative RT-PCR

RNA was extracted using miRVana miRNA Isolation Kit (Ambion, Austin, TX, USA) according to the manufacturer's instructions. The concentration and purity of RNA were measured in duplicate using a spectrophotometer (Nano Vue; GE Healthcare, Buckinghamshire, England). Equal amounts (500 ng) of extracted RNA containing miRNAs was polyadenylated by Poly (A) Polymerase (NEB, Ipswich, MA, USA) and reverse transcribed to cDNA at 37°C for 15 min and 85°C for 5 s with the RT primer and the PrimeScript RT reagent kit (Takara, Dalian, China) in a final volume of 20 μl following the manufacturer's protocol. To minimize variation in the reverse transcription reaction, all RNA samples from a single experimental setup were reverse transcribed simultaneously. cDNA was then subjected to real time PCR using a SYBR® Premix Ex Taq™ (Takara) in 20 μl of reaction solution with the primers described in **Table 1**. Real time PCR was performed on Light Cycle 480 (Roche, Basel, Switzerland). The relative mRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method after normalization with GAPDH as a housekeeping gene, whereas the relative miRNA level was calculated after normalization with U6 RNA as an endogenous control [39].

Immunoblotting assay

Frozen tissues were washed with ice-cold PBS and homogenized in RIPA lysis buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 5 mM EGTA, 1 mM PMSF, 1% NaDC, 0.1% SDS, 1% Triton X-100, 50 mM NaF, 0.5 mM Na_3VO_4). Lysates were centrifuged at 14,000 g for 15 min at 4°C and supernatants collected. Protein concentration was measured using BCA protein

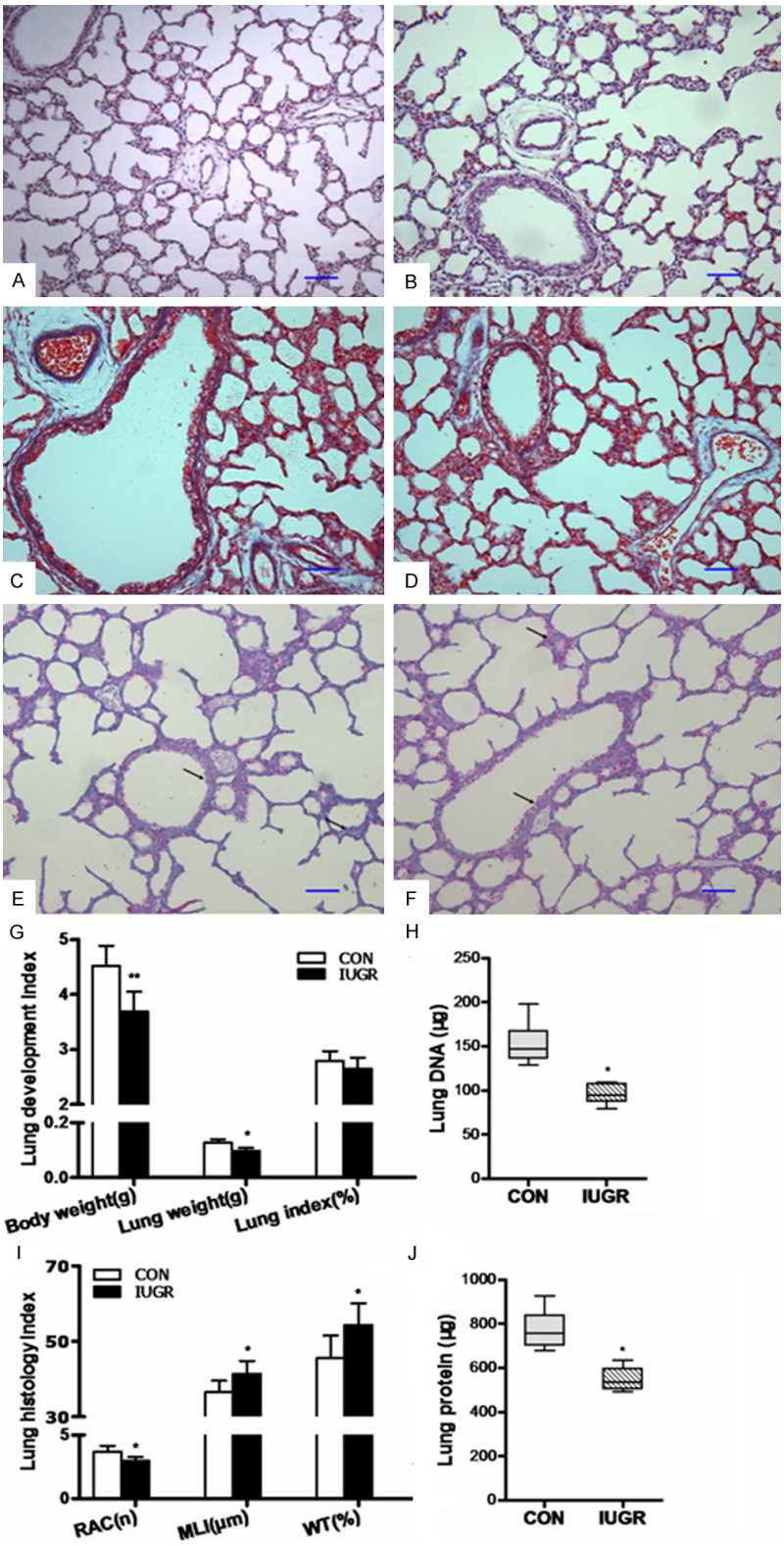

Figure 1. The influence of maternal protein restriction on offspring Lung histology. (A, B) Representative photomicrographs of hematoxylin and eosin–stained lung sections from CON (A) and IUGR groups (B). (C, D) Masson stain revealed there were no differences in collagen deposits and lung fibrosis between CON (C) and IUGR groups (D). (E, F) PAS stain for glycogen granules in the fetal lungs. Compared with CON group (E), glycogen granules were more abundant in the fetal lung with IUGR (F). Black arrows: glycogen granules. All panels original magnification ×200 (scale bar, bar = 50 μm). (G-J) Compared with controls, lungs of IUGR fetus were hypoplastic as evidenced by decreased lung weight (G), reduced DNA (H) and protein content (J), as well as decreased RAC and increased MLI (I) due to deficient airway branching. Data are shown as means ± SD. Significant differences compared with controls are indicated by *($P < 0.05$) and **($P < 0.01$).

assay kit (Beyotime, Haimen, China). Equal amounts of proteins were separated on 6-12% SDS-polyacrylamide gel electrophoresis and transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore, MA, USA). The filter was then blocked with 5% BSA and probed with antibodies against VEGF and Flt-1 respectively. Blot was then exposed to horseradish peroxidase-conjugated secondary antibody and visualized using ECL plus reagent (GE Healthcare, USA). Quantity One software (Bio-Rad) was applied for analysis of the optical density of the protein bands. The relative expression quantity of target protein was illustrated as the percentage of the optical density (OD) of target protein, adjusted with the corresponding GAPDH OD.

Statistical analysis

All experiments were repeated in triplicate and data were expressed as mean ± standard error of the mean (SEM). Statistical significance was assessed by the Student's t-test. All

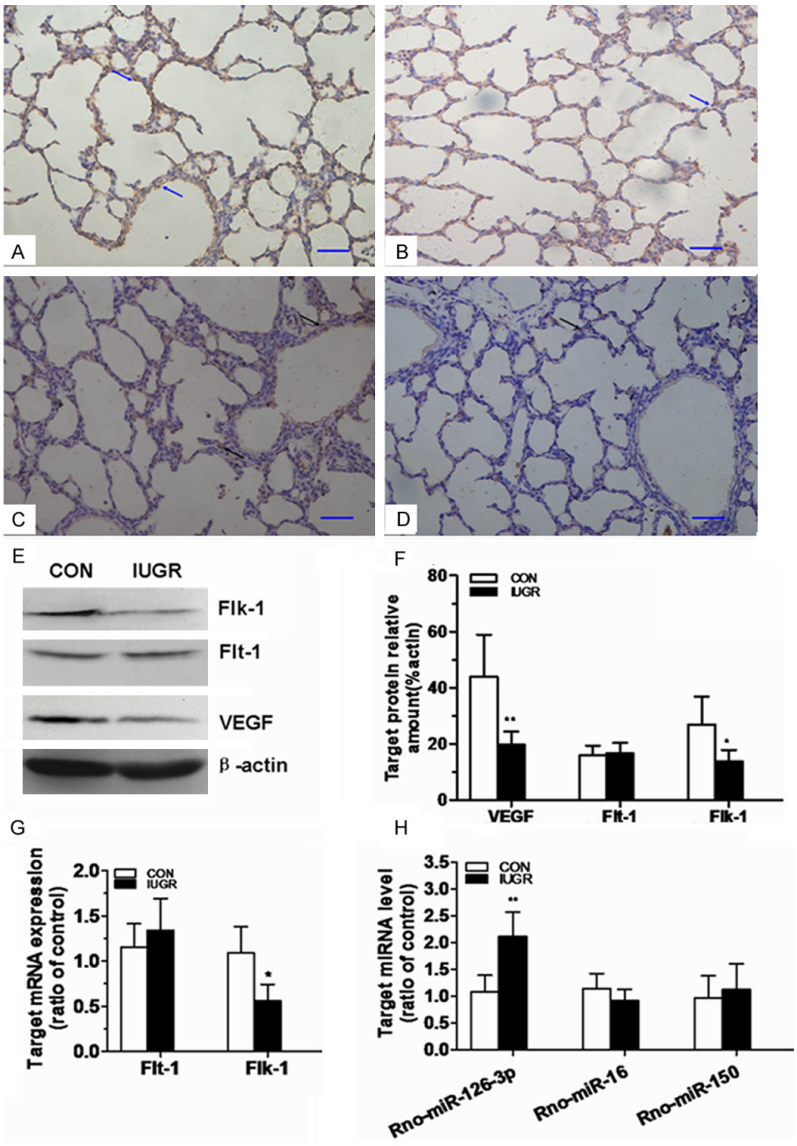

Figure 2. Effect of IUGR on VEGF, receptors and regulating miRNAs in the fetal lungs. (A, B) Representative photomicrographs of IHC-stained lung sections from CON (A) and IUGR (B) groups. VEGF was strongly expressed in the airwayepithelium, but VEGF is also detectable in some mesenchymal structures (blue arrowhead). (C, D) Representative micrographs from CON (C) and IUGR (D) fetal lungs after immunohistochemical staining for Flk-1. The Flk-1 signalis localized to the mesenchymal cells and the luminal airway epithelium (black arrowhead). (Original magnification ×200, scale bar = 50 μm). (E, F) Representative immunoblotting and densitometric analysis of VEGF and receptors protein expression in the fetal lungs of both groups. Protein expression level was normalized relative to the expression of β-actin. (G) The expression levels of VEGF receptors mRNA in the fetal lungs of both groups were determinated by quantitative RT-PCR, and results were expressed relative to the control, after normalized with β-actin as housekeeping gene. (H) Regulating miRNAs levels in fetal lungs of both groups were determinated by quantitative RT-PCR, and results were expressed relative to the control, after normalized with U6 snRNA as internal control. Data are presented as means ± SD for 6 observations per group. Significant differences compared with controls are indicated by *($P < 0.05$) and **($P < 0.01$).

statistical calculations were performed with SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Two-sided $P < 0.05$ was considered significant.

Results

Lung histology

Malnutrition fetus weighed significantly less compared to control animals, so did the lung weight. However, lung weight, expressed as a percentage of body weight between the two groups was not different (**Figure 1G**). The IUGR group had significantly decreased alveolar number manifested by lower RAC, and significantly increased mean linear intercept (MLI) of alveoli, when compared with the lungs from control rats (**Figure 1I**). Total DNA and protein were diminished at embryonic day 21 (E21) in IUGR fetal lungs in comparison with controls, revealing decreased cell mass (**Figure 1H-J**). The arterial vessels were mildly thicker in IUGR groups, evidenced by increased MT% (**Figure 1I**). Masson staining showed there are no differences in collagen deposits and lung fibrosis between two groups (**Figure 1C**, **1D**), while PAS stain revealed more glycogen granules in IUGR fetal lung (**Figure 1E**, **1F**).

Effect of malnutrition on VEGF signalling pathway in the developing lung

VEGF immunoreactivity was mainly present in bronchial epithelial cells and type 2 pneumocytes in lungs by IHC. Positive VEGF staining was also noted in mesenchymal (**Figure 2A**, **2B**). Positive immunoreactivity for Flk-1 was found in mesenchy-

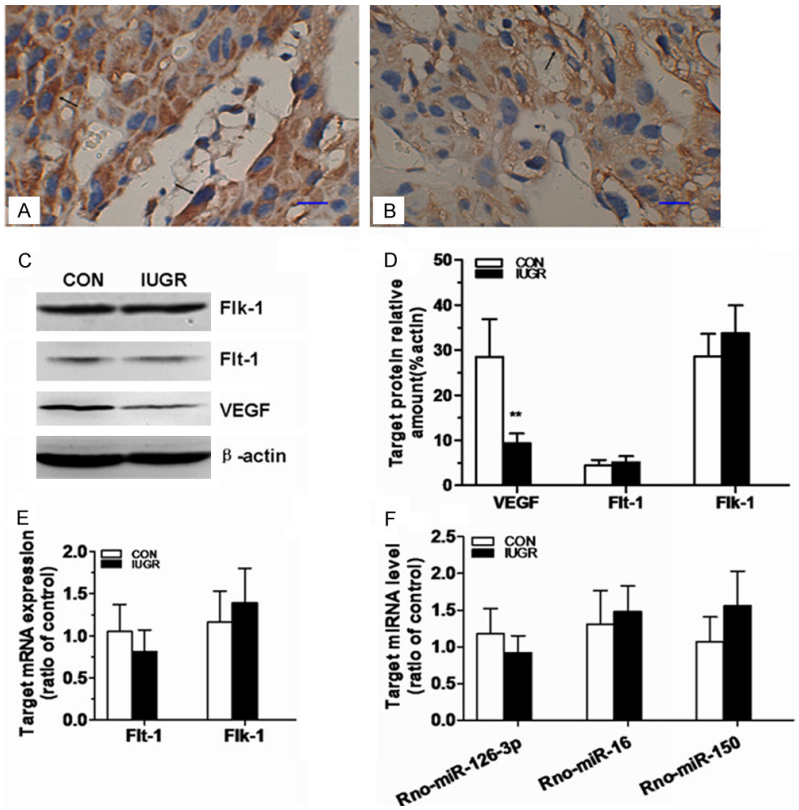

Figure 3. Effect of intrauterine protein restriction on VEGF, receptors and regulating miRNAs in the placenta. (A, B) Representative micrographs from CON (A) and IUGR (B) placentas after immunohistochemical staining for VEGF. VEGF was strongly expressed in the decidual cells, vascular endothelial cells and trophoblastic cells (black arrowhead). (Original magnification ×400, scale bar = 100 μm). (C, D) Representative Western blotting and densitometric analysis of VEGF and receptors protein expression in the placentas of both groups. Protein abundance was normalized relative to the expression of β-actin. (E) Flk-1 and Flt-1 mRNA levels in both groups were determinated by quantitative RT-PCR, and results were expressed relative to the control, after normalized with β-actin as housekeeping gene. (F) Regulating miRNAs levels in the placentas of both groups were also determinated by quantitative RT-PCR, and results were expressed relative to the control, after normalized with U6 snRNA as internal control. Data are presented as means ± SD for 6 observations per group. Significant differences compared with controls are indicated by *($P < 0.05$) and **($P < 0.01$).

mal cells immediately adjacent to the epithelium as well as in vascular structures of the mesenchyme. Flk-1 was also expressed in the luminal airway epithelium (**Figure 2C**, **2D**). No unwanted background staining was observed in the negative control without the primary antibody (data not shown). The expression of VEGF and Flk-1 in IUGR groups seemed decreased significantly compared with control group. We didn't calculate the gray value because evaluation of staining intensity by IHC is subject to observer variability, we proceed to assess VEGF and receptors expression in lungs by RT-PCR and/or western blot, as the latter two methods are more quantifiable and reproducible methods. According to RT-PCR analysis, malnutrition induced marked reduction in Flk-1 mRNA levels. In contrast, IUGR did not induce an alteration in Flt-1 mRNA levels (**Figure 2G**). Generally, comparable changes in VEGF and Flk-1 protein concentrations were also observed by immunoblotting analysis (**Figure 2E**, **2F**).

Effect of malnutrition on the miRNA in the developing lung

According to real time PCR analysis, maternal undernutrition significantly upregulated rno−miR-126-3p levels in the lung, whereas no significant differences were observed in the levels of miR-150 and miR-16 between IUGR and control rats (**Figure 2H**).

Effect of malnutrition on VEGF signalling pathway in the placenta

IUGR are often associated with abnormalities in placental structure and function. Maternal protein restriction has been shown to have deleterious effects on placental development. VEGF signalling pathway is essential for the placental vascular development. We further investigate the effect of maternal protein-restriction on VEGF and receptors in placenta. The positive expression of VEGF was mainly in the decidual cells, vascular endothelial cells and trophoblastic cells of placentas by IHC (**Figure 3A**, **3B**). The expression of VEGF in IUGR groups seemed decreased significantly compared with control group. To obtain accurate information of the expression alteration, we measured the mRNA and protein levels of VEGF

and receptors using RT-PCR and/or immunoblotting assay. In general, mRNA and protein levels were comparable. VEGF expressions were also significantly downregulated in the IUGR rats as compared with normal placenta, though Flt-1 and Flk-1 expressions did not differ between two groups (**Figure 3C-E**).

Effect of malnutrition on the regulating miRNAs in the placenta

On the basis of PCR analysis, we compared the expression of the three miRNAs in the placentas from IUGR fetuses and the control group. The expression of miR-126-3p and miR16 were apparently identical in both groups. The expression of miR-150 had a tendency to be higher but the variation did not achieve statistical significance (**Figure 3F**).

Discussion

IUGR indicates that the fetus has failed to achieve its full growth potential. Decreased fetal growth rates reflect an adaptation to the adverse intrauterine environment temporarily, but may lead to permanent alterations of structure or function with unfavorable long term consequences. The impact of impaired fetal development *in utero* on lung function in humans and animals has long been recognized. A recent study reported that school-aged children born with IUGR demonstrated poorer lung function compared with age-matched control children [12]. The aim of the present study was therefore to elucidate the defective development of lung and related underlying patterns of gene expression in IUGR fetus. Our results revealed that protein malnutrition *in utero* (one of the most widely used animal model of IUGR) induced alterations in lung maturation, including reduced total mRNA and protein content, decreased alveolarization, thicker arterial vessels, consistent with previous reports. Furthermore, we also found decreased VEGF and receptors in lung and over-expression of miR-126-3p which maybe at least part of the cause for decreased lung VEGF. These changes may persist throughout life and contribute to the increased incidence of respiratory infections in IUGR patients.

Rat lung development occurs in several distinct stages [40-42]: embryonic phase (days 0-13) in which lobar division takes place; pseudoglandular phase (days 13-18), in which epithelial tubes of air passages are formed but have little or no lumen; canalicular phase (days 18-20), in which bronchioles are produced and a lumen can be recognized in many tubules; saccular phase (days 20-full term), in which alveolar ducts and air sacs are formed; and alveolar stages (from full term to postnatal) in which true alveoli are formed. Each of the five developmental stages is coordinated by a multitude of signaling molecules and pathways [43]. VEGF, a specific endothelial cell mitogen [44], is indispensable for lung development. Experimental studies have shown that in addition to promotes vessel growth. VEGF coordinates the development of airway epithelial cells and alveolarization. VEGF signals mainly via two endothelial specific tyrosine kinase receptors, Flt-1 and Flk-1; the former mainly mediates the role of VEGF to cause the organization of endothelial cells into vascular structures [23] and the latter responsible for endothelial cell mitogenesis and migration [45, 46]. The importance of VEGF for normal lung development is highlighted by the transgenic mice studies that fetal mice deficiency of the VEGF isoforms died of respiratory distress syndrome (RDS); whereas intrauterine delivery of VEGF stimulated production of surfactant proteins and protected preterm mice against RDS [22]. Disruption of the VEGF-VEGFR signaling with the aid of the VEGFRS inhibitor not only reduced pulmonary arterial density but also led to reduced alveolarization and immature lung formation [26, 47-49]. As far as we know, no observation of the impact of malnutrition on VEGF signaling in fetal lung has been reported. Our result revealed that intrauterine protein restriction reduced VEGF and Fllk-1 in fetal lung. These findings are consistent with the interpretation that reduced VEGF signaling through Flk-1 impaired embryonic lung epithelial to endothelial crosstalk and branching morphogenesis, resulted in a lack of alveolarization and dysmorphic vasculature, which are in accordance with previous reports [50].

miRNAs are a class of small (approximately 22 nt), single-stranded, non-coding RNAs that can bind the 3'-untranslated regions (UTR) of target mRNAs and thus specifically regulate their stability or translational efficiency [51]. Various miRNAs have been implicated in regulating various physiological and pathological process-

es, including development [52], differentiation [53], proliferation and apoptosis [54], and oncogenesis [55]. In the lung epithelia, inactivation of Dicer, the enzyme responsible for producing mature miRNAs, results in the inhibition of lung epithelial branching, revealing the importance of miRNAs for lung development [56]. miRNA profiling studies demonstrate the dynamic expression of miR-126-3p and -504 during lung organogenesis, and they were all validated targeting on the VEGF 3'-UTR [32-34]. Overexpression of miR-16 and miR-424 reduced Flk-1 expression and regulated cell-intrinsic angiogenic activity of human umbilical vascular endothelial cells and CNE cells (a human nasopharyngeal carcinoma cell) [35, 57]. Therefore, we further selected miR-126-3p, -150 and -16 as the upstream regulators of VEGF and FLK-1 and measured them to confirm the correlation between miRNAs and VEGF. We found that, the expression of miR-126-3p in lung was significantly upregulated. However, the expression of miR-150 and -16 in lung was similar to that in control fetuses. These data suggest that the low expression levels of VEGF in lung may be partially regulated by miR-126-3p. Further investigation is necessary to investigate the temporal and spatial expression pattern of miR-126-3p and how it modulates VEGF expression in lung.

Our last objective of the present study was to investigate whether protein restriction *in utero* may exert similar deleterious effects on VEGF signaling in the placenta. VEGF family and receptors are essential for the placental vascular development. Previous reports showed paradoxical views of the effect imposed by IUGR. In some cases of IUGR, studies show poor placental vascular development and increased placenta growth factor (PlGF) with no apparent changes in VEGF expression, which support the hypothesis of "placental hyperoxia" in IUGR [58, 59]. In contrast, others reported increased expression of VEGF and receptors due to "placenta hypoxia" in IUGR [60, 61]. Studies of animal model also showed inconsistent conclusions. Regnault et al discovered that VEGF mRNA was elevated first, and then restored in mid-pregnancy (90 days post coitus) in an ovine model of placental insufficiency-IUGR, while VEGFR-1 and VEGFR-2 mRNA was significantly reduced. On the contrary, Timothy R reported decreased placental VEGF in late-pregnancy (135 days post coitus) using a similar IUGR model, possibly due to different investigation time points [62]. Moreover, to date, little is known about how gestational protein insufficiency affects the expressions of VEGF in the placenta. The present study showed, for the first time, that intrauterine protein restriction downregulated the placental VEGF expression, without significant changes in the expression of receptors and regulating miRNAs, which suggested that protein malnutrition disrupted VEGF signaling through different mechanisms in the fetal lung and placenta. Further studies are required to elucidate the underlying mechanistic basis for the alteration of placental VEGF.

Overall, the results of this study clearly show that intrauterine malnutrition results in abnormal fetal pulmonary development with decreased alveolarization. These defects are associated with decreased VEGF and receptors, partly due to the over-expression of miR-126-3p. If persistent into postnatal life, these defects might contribute to increased susceptibility to lung disease in IUGR patients.

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Disclosure of conflict of interest

None.

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