Expression and clinical significance of LXRα and SREBP-1c in placentas of preeclampsia

1 Introduction

The incidences of preeclampsia (PE) among primipara and multipara are 3%-7% and 1%-3%, respectively [1]. Its pathophysiologic alterations involve lesions of multiple organs and systems, and PE represents as one of the leading causes of maternal mortality.

Liver X receptor alpha (LXRα) is a ligand-activated transcription factor that belongs to the nuclear receptor superfamily. Studies have shown that LXRα is closely related with lipid metabolism, injury of vascular endothelial, proliferation and invasion of trophoblasts. As the key receptor for maintaining homeostasis of total cholesterol (TC) and triglyceride (TG), LXRα acts through transcription regulation of key target genes involved in lipid absorption, transport, synthesis, metabolism and excretion [3]. It is already known that LXRα is expressed in placentas, and compared with normal pregnancies, it is significantly up-regulated in placentas of PE patients, which is positively correlated with the extent of hypoxia [4,5]. Its abnormal expression may be important for PE progression.

Sterol regulatory element-binding protein-1c (SREBP-1c) belongs to the nuclear transcription factor family that was initially identified from the nuclear extracts of cervical cancer HeLa cells. The mammal genes encoding SREBP include SREBP-1a, -1c and SREBP-2. SREBP-1c and SREBP-2 are the major isoforms expressed in animal tissues, in which SREBP-1c constitutes up to 90% of SREBP-1 in the body. SREBP-1c could activate transcription of multiple genes encoding for enzymes involved in synthesis of TC, TG, fatty acid (FA) and phospholipid, such as 3-hydroxy-3-methylglutaryl-CoA synthase 1 (HMGCSE1), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) and squalene epoxidase (SE) that are related with TC synthesis, acetyl-CoA carboxylase alpha (ACC), fatty acid synthase (FAS) and stearoyl-CoA desaturase that are related with FA synthesis. HMGCR is the rate-limiting enzyme for lipid synthesis while ACC is the rate-limiting enzyme for fatty acid synthesis. Thus SREBP-1c plays an irreplaceable role in the process of fat synthesis. Vasarhelyi B et al. had proved that SREBP-1c is a key adjustment factor to
keep dynamic balance of fatty acid in placenta through investigating differential gene expression in placentas of PE pregnancies by means of gene chip technology [6]. Besides, SREBP-1c, acyl-CoA synthetase long-chain family member 3 (ACSL3), FAS are all targets of LXRα and are all closely related with lipid metabolism. In particular, SREBP-1c is the chief transcription factor that regulates the transcription of genes involved in fat synthesis, which could be up-regulated by LXRα.

In our study, we applied RT-PCR and IHC methods to investigate the expression of LXRα and its target gene SREBP-1c and their correlations, to explore the clinical value of these two molecules in the pathology of PE. Our results would help to understand more about PE placental pathologic changes and its pathogenesis mechanisms.

2 Materials and Methods

2.1 Patients

Samples were obtained from 116 cases of pregnancies undergoing cesarean section at our hospital from 2011 Oct to 2012 Aug, including 60 cases of PE pregnancy group (29 mild cases and 31 severe cases) and 56 cases of normal pregnancy group. No women with chronic hypertension, diabetes, liver or kidney diseases or other diseases that influence lipid metabolism were included. The body mass indexes (BMI) of these candidates are all in the normal range. No other complications occurred during their pregnancies. All subjects had cesarean deliveries. The diagnostic standard of PE was according to the 7th edition of the “Obstetrics and Gynecology” [2].

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the authors’ institutional review board or equivalent committee.

Informed consent: Informed consent has been obtained from all individuals included in this study.

2.2 Methods

2.2.1 Tissue samples

All tissue samples were obtained during cesarean section, and two pieces of placental tissues, of approximately 1cm³, were immediately taken off the maternal side. Omit the infarcted, organized and calcificated regions. One copy of placenta tissues were fixed in 10% neutral paraform aldehyde for 24hrs after repeatedly rinsed by physiological saline, followed by conventional paraffin embedding and serial section at a thickness of 2.5μm. The other copy of placenta tissues were put into the RNA enzyme-inactivated cryopreserved tubes and snap-frozen in liquid nitrogen within 5 minutes ready for RNA extraction.

2.2.2 RT-PCR method for detecting the mRNA expression level of LXRα and SREBP-1c in placentas

(1) Total RNA of placentas was isolated as per the instructions of the Trizol kit (ordered from the American Invitrogen company). (2) cDNA was synthesized according to the reverse transcription kit (ordered from the Fermentas company). (3) PCR amplification procedures: All set up to 20 μl reaction system; PCR reaction conditions for LXRα, SREBP-1c and the internal reference gene β-actin were all as follows: 94ºC 5min; 36 cycles of 94ºC 30sec, 58ºC 30sec, 72ºC 4min; 72ºC 7min. Primers were all designed and synthesized by Shanghai Sangon biological engineering technology service co., LTD. The sequences of forward (F) and reverse (R) primers for LXRα, SREBP-1c and β-actin were as follows respectively: L LXRα-F: 5’-GCGAGGGCTGCAAGGGATTCT-3’, LXRα-R: 5’-ATGGGCGGCCGTCGACGCG-3’, with the product length of 376 bp; SREBP-1c-F: 5’-GCCTATTTAACCCACCCTATG-3’, SREBP-1c-R: 5’-TGGCACTGACTCTTCCTTGAT-3’, with the product length of 251bp; β-actin-F: 5’-ATGGGGAGCCAGGCTGAGCGG-3’, β-actin-R: 5’-TGGCAGCTGACTCTTCTTGAT-3’, with the product length of 251bp; β-actin-F: 5’-CTGGGAGCAAGGAGCGAGGA-3’, β-actin-R: 5’-AAGGAAGGCTTGGAAGAGTGC-3’, with the product length of 564bp. (4) The above RT-PCR products were further run for gel electrophoresis, imaging and quantification by the Quantity One software to determine the ratios (RIs) of absorbance value of purpose stripes with that of the internal reference gene β-actin, which were considered as the relative
gene expression levels in each sample (experiments were repeated 3 times for each gene).

2.2.3 IHC technology for detecting the protein expression of LXRα and SREBP-1c in placentas

Experiments were performed by using the 2nd general 2-step detection system (non-biotin, PV-9000 purchased from Beijing Zhongshan Golden Bridge Company) according to the instructions of the manufacturer. The concentrations of working liquid of rabbit anti-human polyclonal antibody LXRα and SREBP-1c were 1:100, 1:200 diluted in PBS, respectively. PBS was used instead of primary antibody in the negative control group. Yellow and/or brownish-yellow granules appeared within cells or matrix was considered as positive cells. Ten 400× power fields were randomly shot for each section. Integral optical density (IOD) was calculated by Image-Pro plus 6.0 software for each images, and the mean value of IOD was used as the relative expression value of the indicated proteins in placentas (experiments were repeated 3 times for each protein).

2.3 Statistical analysis

Statistical analysis was performed with SPSS version 17.0. All data were expressed as ± s. The comparison between measurement data sets was done by t-test. Bivariate correlation analysis was performed by using Pearman linear correlation analysis.

3 Results

3.1 Clinical characters

Among the objects of our study, there were no significant differences in age and BMI between the PE groups and the normal group (P>0.05). However, there were significant statistic differences in gestational age, systolic pressure, diastolic pressure, and birth weight between the indicated two groups (P<0.01 or P<0.05) (Table 1).

### Table 1: Clinical characteristics of the control and PE patient groups

| Group       | n   | Age/year | gestational age/week | BMI/(Kg/m²) | systolic pressure/mmHg | diastolic pressure/mmHg | birth weight/Kg |
|-------------|-----|----------|----------------------|-------------|------------------------|-------------------------|-----------------|
| Normal      | 56  | 28.83±4.65 | 39.61±1.10          | 29.66±3.61  | 113.23±9.87            | 72.77±7.21              | 3.31±0.28       |
| PE          | 60  | 28.72±4.86 | 37.62±1.38***       | 30.84±4.65  | 155.24±9.88***         | 98.24±7.81***           | 2.75±0.39**     |
| Mild PE     | 29  | 29.13±4.12 | 38.12±0.91**        | 30.61±3.91  | 145.21±7.98***         | 94.68±6.69***          | 2.98±0.30       |
| Severe PE   | 31  | 28.22±4.11 | 37.14±1.20***       | 30.96±4.23  | 165.23±8.91***         | 102.33±6.12**          | 2.61±0.29***    |

Note: a – The P-values are given for severe PE compared to mild PE group. b – The P-values are given for PE and/or mild PE groups compared to normal group. P values * <0.05 and ** <0.01.

### Table 2: The expression levels of LXRα and SREBP-1c in placentas of each group

| Group       | n   | LXRα mRNA | SREBP-1c mRNA | LXRα protein | SREBP-1c protein |
|-------------|-----|-----------|---------------|--------------|------------------|
| Normal      | 56  | 0.33±0.12 | 0.36±0.17     | 65887.56±43863.11 | 51667.78±15874.12 |
| PE          | 60  | 0.55±0.21*** | 0.56±0.19*** | 96874.18±30124.16** | 71845.64±14667.12*** |
| Mild PE     | 29  | 0.48±0.14**  | 0.49±0.15**  | 87142.11±12846.11*** | 65892.15±10651.41**   |
| Severe PE   | 31  | 0.62±0.16*** | 0.60±0.18*   | 106871.12±20121.11** | 76741.22±12684.11**   |

Note: a – The P-values are given for severe PE compared to mild PE group. b – The P-values are given for PE and/or mild PE groups compared to normal group. P values * <0.05 and ** <0.01
3.2 Expression of LXRα and SREBP-1c in placenta tissues of pregnancies

3.2.1

As shown by RT-PCR, the mRNA levels of LXRα and SREBP-1c were gradually upregulated along the normal pregnancy group, mild PE and severe PE groups (Figure 1-2). The differences were of statistical significance (P<0.01 or P<0.05) (Table 2).

3.2.2

The results from the IHC staining showed that LXRα and SREBP-1c were mainly located in cell membrane and cytoplasm of cytotrophoblasts, syncytiotrophoblasts and vascular endothelial cells. They were hardly observed in cell membrane or cytoplasm of villus stroma core and the decidual cells. Yellow or brown staining indicated positive expression. The protein levels of LXRα and SREBP-1c emerged gradually increased expression along the severity of PE (Figure 3). Also, LXRα and SREBP-1c protein levels were gradually upregulated along the normal pregnancy group, mild PE and severe PE groups. The differences were of statistical significance (P<0.01 or P<0.05) (Table 2).

3.2.3

After that, the correlation of LXRα and SREBP-1c expression was calculated: LXRα mRNA expression positively correlated with SREBP-1c mRNA and LXRα protein (r=0.521, P<0.01; r=0.422, P<0.01). The expression of SREBP-1c mRNA positively correlated with its protein level (r=0.598, P<0.01). There were positive correlations between the expression of LXRα protein and SREBP-1c protein (r=0.612, P<0.01) (Table 3).

Table 3: The correlation analysis of LXRα and SREBP-1c expression

| Item            | SREBP-1c mRNA | LXRα protein |
|-----------------|---------------|--------------|
| LXRα mRNA       | 0.521**       | 0.422**      |
| SREBP-1c protein| 0.598**       | 0.612**      |

Note: P values ** <0.01.
It is well known that SREBP-1c is a target of LXRα. The promoter of SREBP-1c gene contains the LXR reaction element (LXRE). LXRα forms obligate heterodimers with the nuclear receptors retinoid X receptors (RXRs) and thereafter binds to the LXRE in the upstream promoter of SREBP-1c gene, thus controlling the transcription of SREBP-1c, and further regulate expression of the downstream genes involved in de novo fatty acid metabolism, TG synthesis and cholesterol homeostasis. It was shown that ligands or agonists of LXR could elevate the protein levels of LXR and SREBP-1c, meanwhile upregulating the mRNA expression of ACC and FAS. As an agonist of LXRα, T0901317 could enhance the promoter activity of SREBP-1c by three times and upregulate its expression, thus to induce significantly increased serum levels of TC, TG, low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) [79]. Schultz JR et al. reported that treating mice with oragonists of LXRα led to increased levels of serum TC, TG and VLDL. The mRNA expression of hepatic SREBP-1c and SCD-1 showed dose-dependent upregulation, twofold for ACC, fourfold for FAS and nonuple for SCD-1 in detail. However, in LXRα knockout mice, the transcription or expression of these above genes was decreased. Agonists of LXR such as P2P2 and 23f could partially antagonize raised TG by LXRα [11].

In the present study, we found that mRNA and protein expression of both LXRα and SREBP-1c significantly increased gradually with the extent of PE among normal pregnancy, mild PE and severe PE groups, indicating that up-regulated LXRα could contribute for promoting the transcription and translation of the target gene, SREBP-1c, which further influences the synthesis of enzymes related with each other, at least reflected by their similar expression pattern in PE. Future mechanical studies are needed to investigate the regulatory mechanisms. We believe that better understanding the LXRα/SREBP-1c axis in PE would benefit the overcoming of abnormal lipid deposition within vascular endothelial cells and cytotrophoblast. This impairs recasting of the uterine spiral artery, causing shallow implantation of the trophocyte in placenta, eventually induces ischemic anoxia of uterus and placenta, finally leads to the higher incidence of the maternal complications and perinatal mortality.

Taken together, LXRα and SREBP-1c in placentas might mediate the lipid metabolism disorder of the pregnancies, and play a regulatory role during PE. Future study on inhibitors or antagonists of LXRα/SREBP-1c and effective controlling their expression in placentas might provide novel clues for preventing and treating PE.

Conflict of interest statement: Authors state no conflict of interest

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