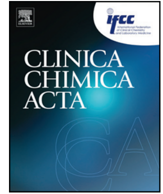
Clinica Chimica Acta 463 (2016) 193–199

Contents lists available at ScienceDirect

Clinica Chimica Acta

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



Distinct expression profiles of lncRNAs between early-onset preeclampsia and preterm controls

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ARTICLE INFO

Article history:
Received 9 May 2016
Received in revised form 31 October 2016
Accepted 31 October 2016
Available online 02 November 2016

Keywords:
Early-onset preeclampsia
LncRNA
Microarray
Real-time PCR
MMP9

ABSTRACT

Early-onset preeclampsia (EOPE), which is the most severe form of the syndrome, confers a high risk of neonatal morbidity and perinatal death. We aim to study the roles of long non-coding RNAs (lncRNAs) in the pathogenesis of early-onset preeclampsia (EOPE). Therefore, we examined the expression profiles of lncRNAs between early-onset preeclampsia and preterm controls using microarray analysis. Quantitative real-time PCR (qRT-PCR) was performed to verify the selected differentially expressed lncRNAs. In total, we identified 15,646 upregulated and 13,178 downregulated lncRNAs in the placenta of EOPE patients compared to the preterm controls. Gene ontology and pathway analysis revealed that compared to the preterm controls, many of the processes overrepresented in the EOPE patients were related to cell migration and cell motility. A selection of the differentially expressed lncRNA transcripts was confirmed using qRT-PCR, particularly RP11-465L10.10, which is associated with the MMP9 gene. These data may offer a background/reference resource for future functional studies of lncRNAs related to EOPE.

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1. Introduction

Preeclampsia (PE) is a major health issue in pregnant women and their infants [1]. Recently, the relationship between the gestational week of onset and the severity of PE has attracted more attention [2,3]. Early-onset preeclampsia that develops prior to 34 weeks of gestation causes significant effects on maternal-foetal morbidity and mortality, particularly when it occurs early on in the pregnancy [4,5]. Following the delivery of the placenta, preeclampsia begins to resolve. However, neonates face significant problems secondary to the prematurity that results from the induced early delivery.

The genetic basis for preeclampsia is complex. A number of biological molecules are differentially expressed in early- and late-onset PE. However, these molecules are protein-centric, and the main problems concerning the treatment, prediction and prevention of early-onset preeclampsia have not been elucidated [6]. Accumulating evidence suggests that poor placental development is particularly associated with early-onset preeclampsia [7–9]. Therefore, it is necessary to further study the mechanism of placental development association with early-onset preeclampsia from the placenta.

Long non-coding RNAs (lncRNAs), which are defined as non-coding RNAs >200 nucleotides in length, were believed to have no functional significance when they were first discovered [10]. However, increasingly lncRNAs have been found to play critical roles in various disorders [11–14], including cardiovascular diseases, cancers and neurodegenerative diseases. Recent studies have reported that lncRNAs may be involved in the pathophysiological mechanisms of preeclampsia [15], and some lncRNAs such as H19, SPRY4-IT1 and MEG3 might have a biological function in regulating the behaviour of trophoblast cells [16–18]. However, the lncRNA profile of early-onset preeclampsia is still unclear. Increased understanding of the lncRNAs involved in early-onset preeclampsia could have a profound impact on our understanding of placental development and therapies for pregnancy complications, which may provide opportunities for interventions in early-onset preeclampsia.

In this study, microarray experiments were performed to determine the expression of lncRNAs in the placenta of individuals with early-onset preeclampsia. We further explored the expression of candidate lncRNAs between individuals with early-onset preeclampsia and controls. The results of this study may provide new insights into the pathophysiology of early-onset preeclampsia.

2. Methods and materials

2.1. Ethics statement

This study was approved by the Medical Ethics Committee of the Obstetrics and Gynecology Hospital Affiliated to Nanjing Medical University (project No. NJFY-201253). Patients attending our hospital for a

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http://dx.doi.org/10.1016/j.cca.2016.10.036
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caesarean section read information about the purpose of the study, and written informed consent was obtained from each participant.

2.2. Patient information and sample collection

Placental biopsies used for the microarray analysis and quantitative PCR were obtained during caesarean sections from preterm control patients (n = 32) and early-onset preeclampsia patients (n = 32). Preeclampsia was diagnosed following the criteria of the American College of Obstetricians and Gynecologists (2013), which was defined as new onset hypertension (systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg on two occasions at least 4 h apart) and proteinuria (the excretion of 300 mg or more of protein in a 24-hour urine collection) after 20 weeks of gestation in women who previously had normal blood pressure. Early-onset preeclampsia was defined as preeclampsia that developed prior to 34 weeks of gestation. A total of 32 early onset preeclampsia samples were obtained from pregnant women (30–36 weeks) at the time of an elective caesarean section. The indications for the caesarean sections in the control group of pregnant women (30–36 weeks) were either the presence of breech and transverse presentation with premature rupture of the membranes or placenta previa with bleeding. We selected patients who underwent preterm delivery of the placenta as the control group to exclude the possible influence of the number of gestational weeks. The exclusion criteria were as follows: multiple gestations, maternal infections, smoking, chemical dependency, known major foetal or chromosomal anomalies, intrahepatic cholestasis during pregnancy, assisted reproductive technology (ART) treatments and any other confounding pathology (diabetes mellitus, renal disease, chronic hypertension, hyperthyroidism and hypothyroidism). The patient characteristics, such as the maternal reproductive data, deliveries and infant's outcomes, are presented in Table 1. The placentas were prepared within 10 min of delivery. The dissected tissue was immediately snap frozen in liquid nitrogen and stored at −80 °C until RNA extraction.

2.3. Total RNA extraction from the placental tissue

To the RNA extraction, the ground, frozen tissues were resuspended in TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA purification was performed with the RNeasy minikit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. After DNase digestion, the total RNA was then eluted using RNase-free water and treated with turbo DNase (Invitrogen, Carlsbad, CA, USA) to remove DNA contaminants.

Table 1
Clinical characteristics of EOPE and control groups.

Clinical features	C (n = 32)	EOPE (n = 32)	P value
Maternal age (mean ± SD)	29.2 ± 3.7	30.7 ± 5.6	0.222
Maternal BMI (mean ± SD)	26.0 ± 2.2	28.9 ± 3.8	<0.001
Week gestation (mean ± SD)	33.8 ± 1.7	33.1 ± 1.7	0.123
Mode of delivery CS (%)	100%	100%	
Birth weight (g) (mean ± SD)	2253.4 ± 395.8	1624.3 ± 290.1	<0.001
BP systolic (mm Hg)	N/A	167.7 ± 16.7	
BP diastolic (mm Hg)	N/A	108.4 ± 12.6	
Proteinuria (g/24 h)	N/A	6.0 ± 2.9	
FGR	0	21 (65.6%)	<0.001
HELLP	0	2 (6.2%)	0.151
Apgar score 1 min	9.9 ± 0.2	9.7 ± 0.8	0.211
Apgar score 5 min	10 ± 0	9.9 ± 0.3	0.321

C, preterm control; EOPE, early-onset preeclampsia; BMI, body mass index; BP, blood pressure; FGR, foetal growth restriction; HELLP, hemolysis, elevated liver enzymes, and low platelets syndrome; P value, early-onset preeclampsia compared with control group.

The quantity and quality of the RNA was evaluated using the Nanodrop and Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA).

2.4. Microarray analysis

Finally, 12 randomly and blindly selected samples (6 early-onset preeclampsia patients and 6 preterm controls) were hybridized and two biological replicates for each condition were used. The information for these patients is shown in Table 2. The expression profiles of the placental LncRNAs were detected using the Arraystar Human LncRNA Microarray v3.0, which was simultaneously used to detect the expression profiles of human genome-wide protein-coding transcripts (Glover et al. 2015). The Arraystar Human LncRNA Microarray v3.0 is designed for the global profiling of human lncRNAs (Kangcheng, Shanghai, China). Quantile normalization and subsequent data processing were performed using the GeneSpring GX v11.5.1 software package (Agilent Technologies, CA, USA). The statistically significant differentially expressed lncRNAs were identified using Volcano Plot filtering. The threshold value used to screen the differentially expressed lncRNAs was a fold change ≥2.0 or ≤−2.0 ($P < 0.05$).

2.5. Gene ontology (GO) and pathway analysis of the dysregulated lncRNAs

A gene ontology analysis was designed to determine the functional trends that were associated with the lncRNAs differentially expressed between the early-onset PE and control groups. These differentially expressed lncRNAs exhibited a significance value of $P < 0.05$. The candidate genes were mapped to GO terms in the database (http://www.geneontology.org/), and then the number of genes was calculated for each term potential molecular functions term of these target genes in the pathways that were identified using the KEGG (Kyoto Encyclopedia of Genes and Genomes) database (http://www.genome.ad.jp/kegg/) and BioCarta (http://www.biocarta.com). The recommended P-value cut-off was <0.05.

2.6. Validation of the microarray findings

A total of 1 μg of RNA from each sample was reverse transcribed to cDNA using a random hexamer primer with the Thermo Scientific™ RevertAid First Strand cDNA Synthesis kit (Thermo Scientific, MA, USA). Primers for each lncRNA were designed according to Primer 3 and confirmed using the Basic Local Alignment Search Tool (BLAST) of NCBI to ensure a unique amplification product. Real-time PCR was performed on an Applied Biosystems ViiA™ 7 Dx (Life Technologies, MA, USA) using the SYBR green method according to the manufacturer's instructions. The PCR reaction conditions were as follows: a denaturation step at 95 °C for 10 min, followed by 40 PCR cycles at 95 °C for 15 s and 60 °C for 1 min. The relative gene expression levels were quantified based on the cycle threshold (Ct) values and normalized to the internal control housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The $2^{-\Delta\Delta Ct}$ method was used to comparatively quantify the levels of mRNA.

2.7. Statistical analysis

The samples were randomized to control for the pre-analytical variables, and an individual blinded to the experimental groups performed the sample preparation and microarray experiments. The statistical significance of the lncRNA datasets was performed using a one-way ANOVA corrected for multiple comparisons followed by the Student's *t*-test with a significance of $P < 0.05$. Data from the quantitative real-time PCR experiments were analysed with Student's unpaired or paired *t*-tests where appropriate. The values are presented as the mean ± standard deviation (SD).

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