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FULL LENGTH ARTICLE

The metabolic role of LncZBTB39-1:2 in the trophoblast mobility of preeclampsia

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Abstract Preeclampsia is characterized by new onset of hypertension and proteinuria after 20 weeks' gestation and is a leading cause of maternal and neonatal morbidity and mortality. The pathogenesis of preeclampsia is often associated with aberrant trophoblast function that leads to shallow placental implantation. However, the exact underlying mechanisms remain unclear. Placental LncZBTB39-1:2 expression level was investigated in 20 healthy placentae and 20 placentae with preeclampsia using qRT-PCR, and the metabolic profile of trophoblasts overexpressing LncZBTB39-1:2 *in vitro* was analysed using gas chromatography-mass spectrometry (GC–MS). In this study, we found that the expression of LncZBTB39-1:2 was significantly higher in preeclamptic placentae than in healthy placentae. Our metabolomics results have shown that tricarboxylic acid cycle intermediates and metabolites related to carbohydrate metabolism were decreased with the overexpression of LncZBTB39-1:2 in HTR8/SVneo cells. These findings were validated by detecting a lower level of intracellular ATP in HTR8/Vneo cells. Furthermore, the migration of HTR8/SVneo cells was compromised when cells were transfected with a plasmid encompassing LncZBTB39-1:2 overexpression. From these results, we conclude that abnormal levels of LncZBTB39-1:2 expression might lead to aberrant

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conditions in HTR-8/SVneo trophoblast cells. Aberrant conditions might be associated with dysregulated trophoblast migration and subsequent failure of uterine spiral artery remodelling, a pathogenesis recognised as a contributing factor in the aetiology of preeclampsia.

Introduction

Preeclampsia (PE) is defined as new onset hypertension and proteinuria after 20 weeks' gestation and is a leading cause of perinatal morbidity and pregnancy-associated mortality, especially in underdeveloped countries. Termination of pregnancy is the only treatment for PE.[1] Many theories have been established to explain the pathogenesis of PE such as inflammatory cytokines,[2] endothelial dysfunction,[3] and imbalance between proangiogenic and antiangiogenic factors.[4] At present, the "Two-stage Disorder"[5] theory of PE is accepted. Trophoblasts extensively invade the spiral arteries of the uterus during the first stage of gestation, thus remodeling the uterine spiral arteries. The vessel diameter enlarges and reduces the flow resistance, increasing uteroplacental perfusion.[6] Trophoblasts play an important role in the spiral artery remodeling. Abnormal trophoblast invasion of uterine vessels, as hypothesised to occur in PE, causes recasting obstacles for the spiral arteries and shallow placental implantation.[7] Furthermore, there is an increasing number of studies demonstrating the role of disordered cellular energy metabolism in the pathophysiology of PE. Many researchers reported that ATP level was significantly reduced in PE placentae compared to normal placentae. This phenomenon may have resulted by the accumulation of mitochondrial oxidative stress, dysregulated mitochondrial fusion, autophagy, biogenesis and abnormal lipid metabolism.[8–10] However, the specific pathological mechanisms leading to PE development remain undefined.

Yu et al. (2009) reported that Long noncoding RNA (LncRNA)H19 was related to the pathogenesis of PE[11]. Long noncoding RNA (LncRNA) is a nucleotide (longer than 200 base pairs) which is unable to code protein and does not have an open reading frame (ORF). Recently, LncRNA has been found to participate in many disease processes including cancer invasion, cancer migration, and apoptosis.[12–14] Some biological functions of trophoblasts resemble cancer cells, and some LncRNAs have been shown to play important roles in tumorigenesis[15] as well as the regulation of PE, such as LncRNAH19, LncRNASPRY4-IT1[16,17], LncRNAMEG3[18], and LncRNAMALAT.[19] These LncRNAs are involved in the cellular modulation of trophoblasts, including important processes such as proliferation, migration, apoptosis, invasion, and angiogenesis.

In this study we found that the expression of a particular type of LncRNA called LncZBTB39-1:2 (http://www.lncipedia.org) was increased in severe PE placental tissue compared with normal placental tissue, analysed using qRT-PCR. Furthermore, The LncZBTB39-1:2 transcript appears to be a splice variant of the tachykinin 3 (TAC3) gene. The encoded peptide is neurokinin B (NKB). This gene product has been studied relatively well including in the placenta of preeclampsia patients. These neuropeptides have been found to contribute to the elevation of blood pressure and in raising pressor sensitivity as observed in PE. They have also been shown to inhibit several proteins that participate in the metabolic response to oxidative and hypoxia stress.[20] Based on this reason, we applied a metabolomics approach to investigate the metabolic effects of LncZBTB39-1:2 overexpression in an HTR8/SVneo cell line with plasmids *in vitro*.

Methods

Selection criteria for participants

All experiments were approved by the Ethics Board of the First Affiliated Hospital of Chongqing Medical University and informed consent was obtained from all participants. All clinical investigations were performed according to the principles expressed in the Declaration of Helsinki.

Villi at 6—8 gestational weeks were collected from healthy pregnant women who underwent induced abortion for nonmedical causes. All chorionic villus samples with acute and chronic diseases were excluded. PE was diagnosed following the America College of Obstetricians and Gynaecologists (AOGO) criteria. Both blood pressure and proteinuria were required to meet the following criteria to define PE diagnosis: systolic blood pressure $\geq$140 mmHg and/or diastolic blood pressure $\geq$90 mmHg and/or 24-h urinary protein excretion exceeding 300 mg. Patients were not eligible for the study if they were experiencing any other pregnancy complications such as severe intrauterine growth retardation (IUGR), diabetes, chemical dependency, or fetal congenital abnormalities.

All placental tissues were collected between 2015 and 2016 from the Department of Obstetrics and Gynaecology at the First Affiliated Hospital of Chongqing Medical University, China. PE placentae were delivered from the womb by cesarean without extrusion of uterine contraction. Specimens were washed with cold normal saline and stored in liquid nitrogen prior to RNA purification.

RNA extraction

Total RNA was extracted from the placental, cells and villi using RNAiso plus reagent (Takara, Japan). The cDNA was synthesised from 1 μg of total RNA by using a reverse transcription Kit (Takara, Japan).

Real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

qRT-PCR was performed using an SYBR Green PCR Kit (Roche, Germany), according to the manufacturer's