Overexpression of miR-21 and miR-122 in preeclamptic placentas

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Abstract

OBJECTIVE: Preeclampsia is a pregnancy-associated disease with the impact of genetic, epigenetic and environmental factors. Increased apoptosis was observed in cells from preeclamptic placentas. MicroRNAs are involved in the regulation of apoptosis and are abundant in placenta. In this study, we focused on the analysis of differential gene expression of apoptosis-associated miRNAs in preeclamptic placenta samples compared to the samples obtained from healthy pregnant women.

METHODS: MicroRNA was extracted from placental samples of patients with preeclampsia and physiological course of the pregnancy. The gene expression of miR-155, miR-122 and miR-21 in placenta and control samples was estimated by relative quantitation (RQ) using TaqMan probes, normalized against RNU44. The RQ mean values were statistically evaluated by Man-Whitney test.

RESULTS: Using the relative gene expression analysis, we could observe a significant increase in gene expression of miR-155 (p<0.001), miR-21 (p<0.0001) and miR-122 (p<0.01) in preeclamptic placentas.

CONCLUSION: The apoptosis-associated miRNAs miR-21 and miR-122 are dysregulated in the term preeclamptic placentas. The increased miRNA expression suggest the downregulation of potential targets mRNAs, which can contribute to the pathogenesis of preeclampsia. The identification of their targets in placenta will improve our understanding of their role in preeclampsia.

INTRODUCTION

Preeclampsia (PEE) is a pregnancy-associated disease characterized by maternal symptoms such as de novo hypertension and proteinuria in which both fetal and maternal factors are contributing. The invasion of trophoblasts of the placenta into maternal decidua is essential for physiological pregnancy. Proper apoptosis is critical for normal placental development and increased apoptosis and hypoxia are associated with PEE (Ishihara et al. 2002; Hrtankova et al. 2014). Global gene
expression analysis on different tissues revealed that small non-coding microRNAs are abundant in placenta where they exhibit distinctive expression profiles (Liang et al. 2007). MicroRNAs (miRNAs) are short non-coding RNA molecules involved in the post-transcriptional gene regulation. Recent studies, mostly microarray-based miRNA profiles have suggested that dysregulation of miRNAs in placental tissues is involved in the pathogenesis of PEE (Mouillet et al. 2011; Betoni et al. 2013). Among the miRNA identified to be aberrantly expressed in PEE were miRNAs involved in the regulation of apoptosis such as miR-155. The regulating pathways of miR-155 are well known. An increased expression of miR-155 has been found in the placentas of women with preeclampsia at delivery (Pineles et al. 2007). There are more experimentally confirmed targets of miR-155 involved in the extravillous trophoblast apoptosis, regulation of placental cell proliferation and inhibition of cell invasion of trophoblast cells (Zhang et al. 2010; Chen et al. 2011; Dai et al. 2012; Li et al. 2014). However, the role of the other miRNAs involved into regulation of apoptosis such as miR-21 and miR-122 is not so well characterized in placenta. There are controversial reports about the role of miR-21 in preeclampsia and placental development. The PNA-based microarray profiling showed that miR-21 is underexpressed in severe PEE, although these results were not validated by realtime PCR (Choi et al. 2013). In pregnancies complicated by severe preterm fetal growth restriction there was observed upregulation of hypoxia and apoptosis regulated miR-21 in blood from women of affected babies (Whitehead et al. 2013). In addition, the overexpression of miR-21 was described in placentas with abnormal Doppler waveforms (Cindrova-Davies et al. 2013). The miR-122 was reported as overexpressed in sera of patients with HELLP syndrome (Stubert et al. 2014), although it was not detected as differentially expressed by microarray profiling. In our experimental approach, we decided to use the analysis of very well documented overexpression of miR-155 in placenta as model miRNA upregulated in placenta and to characterize the gene expression of apoptosis-associated miR-21 and miR-122 in preeclamptic placentas by relative quantitation analysis.

PATIENTS AND METHODS

Patients

PEE is defined as the onset of gestational hypertension and proteinuria after 20 weeks of gestation. Hypertension was defined as two or more recordings of a diastolic blood pressure of ≥90 mmHg taken ≥4 hours apart. Proteinuria was defined as the excretion of ≥300 mg of protein over 24 hours (Lasabova et al. 2014). Human placentas were collected from the maternal site of placenta (Enquobahrie et al. 2009). The project was approved by the institutional ethical committee and informed written patient consent was obtained before sample collection. Gestational age was calculated from the last menstrual period and was confirmed by routine ultrasonography at 11 to 12 weeks of gestation. The control placentas were delivered from normotensive healthy singleton pregnancies with no history of cigarette smoking, diabetes autoimmune disease, or thrombophilia.

MicroRNA extraction and real-time PCR

MicroRNA was extracted from placental samples of patients with preeclampsia and with physiological course of the pregnancy using mirVana miRNA Isolation Kit (Ambion, USA) according the manufacturer instructions. The RNA concentration was determined by spectrophotometry and the quality by PAGE. For reverse transcription and real time PCR were used TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, USA) and TaqMan MicroRNA Assay (Applied Biosystems, USA). Briefly, 200 ng of mRNA were reverse-transcribed into cDNA using TaqMan MicroRNA Reverse Transcription Kit (ABI, Applied Biosystems, USA) according to the manufacturer’s protocol. TaqMan MicroRNA Assays with stem loop technology were applied for the real-time PCR of miR-155, miR-122 and miR-21, all normalized against RNU44. The real-time PCR was performed in triplicates with multiple negative controls on 7500 Fast Real Time PCR System (Applied Biosystems, USA). The value for relative gene expression RQ was obtained by the 2–ΔΔCt method (Livak & Schmittgen 2001) using the 7500 Software v2.0.1.

Statistical analysis

All data were statistically analyzed using R version 3.1.1 (http://www.r-project.org). Mean RQ values were calculated using descriptive statistics from the obtained RQ values calculated by 7500 Software v2.0.1. Differences in maternal age, gestational week and relative gene expression were compared by Mann-Whitney test. Data in figures are presented as median ± interquartile range (IQR). Statistical significance was defined as p<0.05.

RESULTS

Clinical characteristics

In this study, 11 placental samples from women with preeclampsia (8 of them delivered by cesarean section) and from 7 healthy controls (5 from noninduced vaginal delivery and two from cesarean section) were analyzed. All samples were obtained at term. The maternal age was comparable within groups (p=0.31). The gestational age of PEE group was significantly shorter than in control group gestational age (p<0.05). The pregnancy and prepregnancy BMI were not significantly different between groups, however as expected, the neonatal birthweight is considered to be significantly different (Table 1).
Overexpression of miRNAs in preeclampsia

We first examined the previously as overexpressed in preeclamptic term placentas reported miR-155 normalized against RNU44. We used 7 control samples from healthy. Two were from healthy pregnancies terminated with caesarean section (CS) and five from noninduced vaginal deliveries. The relative expressions (RQs) of miR-155 in CS and vaginal delivery were not significantly different ($p=0.07$), all 7 samples were included as reference healthy samples compared to 11 preeclamptic samples. The values of relative gene expression obtained from preeclamptic placentas were significantly different when compared to the RQ values of controls ($p<0.001$; Figure 1) The expression of miR-155 in placentas from PEE patients was 6.5 fold increased after comparison of the RQmean values (PEE RQmean 17.2 vs. RQmean of controls 2.6).

Overexpression of miR-21 and miR-122 in placentas from PEE patients

The RQs for miR-21 and miR-122 of control samples from two CS and five noninduced vaginal pregnancies were comparable, normalized against RNU44 and not statistically significant different ($p>0.05$). These seven placental samples were analyzed as control samples compared with 11 PEE samples. The relative gene expression of miR-21 ($p<0.0001$) and miR-122 ($p<0.01$) was significantly higher in samples from patients with PEE than in controls (Figure 1). The expression of miR-21 and miR-122 in preeclamptic placentas was 10.8 fold (96.8 vs. 8.93) and 5.5 fold (23.9 vs. 4.4) increased, respectively.

DISCUSSION

The mechanism of PEE seems to be a combination of genetic, epigenetic and environmental factors. Although, the fetal sonography is an important clinical tool for the monitoring of the fetus and mother (Visnovsky et al. 2015), the understanding of molecular mechanisms can help to identify markers which play important role in development of PEE. In our pilot study, we have been concentrated on the gene expression analysis of selected apoptosis-related miRNAs and reported about the significant increase in miR-155, miR-21 and miR-122 in term placenta samples from patients with preeclampsia (PEE) and controls. All values are normalized against the level of RNU.
miR-122 and miR-21 expression in preeclamptic term placental samples compared to the healthy controls. Recent studies show that miRNAs may partly regulate implantation and placentation as well as different processes such as angiogenesis, apoptosis or cell cycle of placental cells (Fu et al. 2013). Nowadays, the mostly used approach of array-based miRNA profiling has produced a lack of consistent results. The reasons can be different such as differences in gestational age, mode of delivery, variations in sampling site and microarray platform as well as the strategy of data analysis. The structure of placenta is multicellular with cells responsible for immune response, angiogenesis and vasculogenesis, the cells are in different stage of differentiation and apoptosis. So the results from such type of studies can be apparently diversified and even occasionally controversy concerning quantitation of gene expression. As one single miRNA is very often involved in several molecular pathways in placenta and different targets have been reported, we used this miR-155 as model for the study of miRNA overexpression in preeclamptic placentas. For miR-155, there are more experimentally validated targets such as CYR61, CCND1 or NOS3 (Chen et al. 2011; Day et al. 2012; Li et al. 2014). Down-regulation of CYR61 by miR-155 induces apoptosis in extravillous trophoblast (Chen et al. 2011), decrease of CCND1 protein amounts leads to G1 arrest (Day et al. 2012) and downregulation of NOS3 results in inhibition of cell invasion in trophoblast cells (Li et al. 2014). The miR-155 is an important regulatory miRNA in placental development and preeclampsia. In our samples, we were able to confirm the upregulation of miR-155 in preeclamptic placentas by comparative quantitation and specific stem loop primer; therefore we conclude the correct sampling of our probes for miRNA analysis in preeclamptic term placentas. Because of controversy in the profiling results of miR-21 and miR-122 in preeclamptic samples, the goal of our study was to analyze gene expression of these miRNAs in samples with a validated miR-155 expression as a model miRNA. The miR-122 and miR-21 have been reported as involved into apoptosis, preferentially in cancer (Ma et al. 2010, Buscagila & Li 2011). Both have targets which are as well proapoptotic as antiapoptotic. They are expressed in placental tissue (Morales-Prieto et al. 2013). In preeclampsia, the downregulation of miR-21 has been reported (Choi et al. 2013); however, the miR-122 was not reported as differentially expressed in PEE (Zhao et al. 2013). In our samples with upregulated miR-155, both the miR-21 as well miR-122 were significantly overexpressed. The increased miRNA expression suggest the downregulation of potential targets mRNAs, which can contribute to the pathogenesis of preeclampsia. There are many targets reported for miR-122 and miR-21 (Buscaglia & Li 2011), however, it remains to determine which their targets in placenta are. While collecting the placental samples, it should be considered that these samples are not free of blood cells. During the pregnancy, the maternal immune system has to tolerate the semiallogenic fetus expressing paternal antigens without immune rejection and the placenta is highly perfused. It should be taken in account that the “contamination” with blood cells may influence the results of miRNA gene expression analysis. As potential biomarker of hypoxia, the overexpression of miR-21 was reported in blood samples obtained at delivery (Whitehead et al. 2013). The studies dealing with the placental gene expression should probably analyze also the immune cells as controls.

The identification of potential target genes is essential for further elucidation of the biological role of particular miRNAs in the pathogenesis of PEE. Induction of apoptosis and perturbation of angiogenesis has been proposed as one of the key features of PEE, and miRNA is strongly implicated in apoptosis and angiogenesis. Further studies are needed to understand the deregulation of miRNAs in PEE and to develop noninvasive tools for early detection of PEE.

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