Neuroendocrinology Letters Volume 36 No. 7 2015
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

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

Zora Lasabová [1,2], Martin Vážan [1,2], Jana Zibolenová [3], Iveta Švecová [4]

1 Biomedical Center Martin, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Slovakia
2 Department of Molecular Biology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Slovakia
3 Department of Public Health, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Slovakia
4 Clinic of Gynecology and Obstetrics, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Slovakia

Correspondence to: Assoc. Prof. Zora Lasabová, MSc., PhD.
Biomedical Center Martin, Jessenius Faculty of Medicine in Martin
Comenius University in Bratislava
Malá hora 4C, 03601 Martin, Slovak Republic.
TEL: +421 43 2633 803; E-MAIL: lasabova@jfmed.uniba.sk

Submitted: 2015-10-26 Accepted: 2015-11-12 Published online: 2015-12-28

Key words: **preeclampsia; miRNA; relative gene expression; apoptosis; placenta**

Neuroendocrinol Lett 2015; **36**(7):695–699 **PMID:** 26859593 NEL360715A04

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

ORIGINAL ARTICLE

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 miRNA 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 $2^{-\Delta\Delta Ct}$ 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).

Tab. 1. Basic characteristics of the patient and control cohorts.

Characteristic	PEE (n=11)	Control (n=7)	*p*-value
Age (years)	27.6±4.9	26.6±2.8	$p>0.05$
gestational age (weeks)	33.2±3.0	40±1.4	$p<0.05$
pregnancy BMI	30.1±7.1	27.4±4.8	$p>0.05$
prepregnacy BMI	24.3±6.0	22.1±3.8	$p>0.05$
neonatal birth weight (g)	2109±720	3327±481	$p<0.01$
Cesarean section	8	2	

Overexpression of miR-155 in placenta from PEE patients

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 RQ_{mean} values (PEE RQ_{mean} 17.2 vs. RQ_{mean} 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,

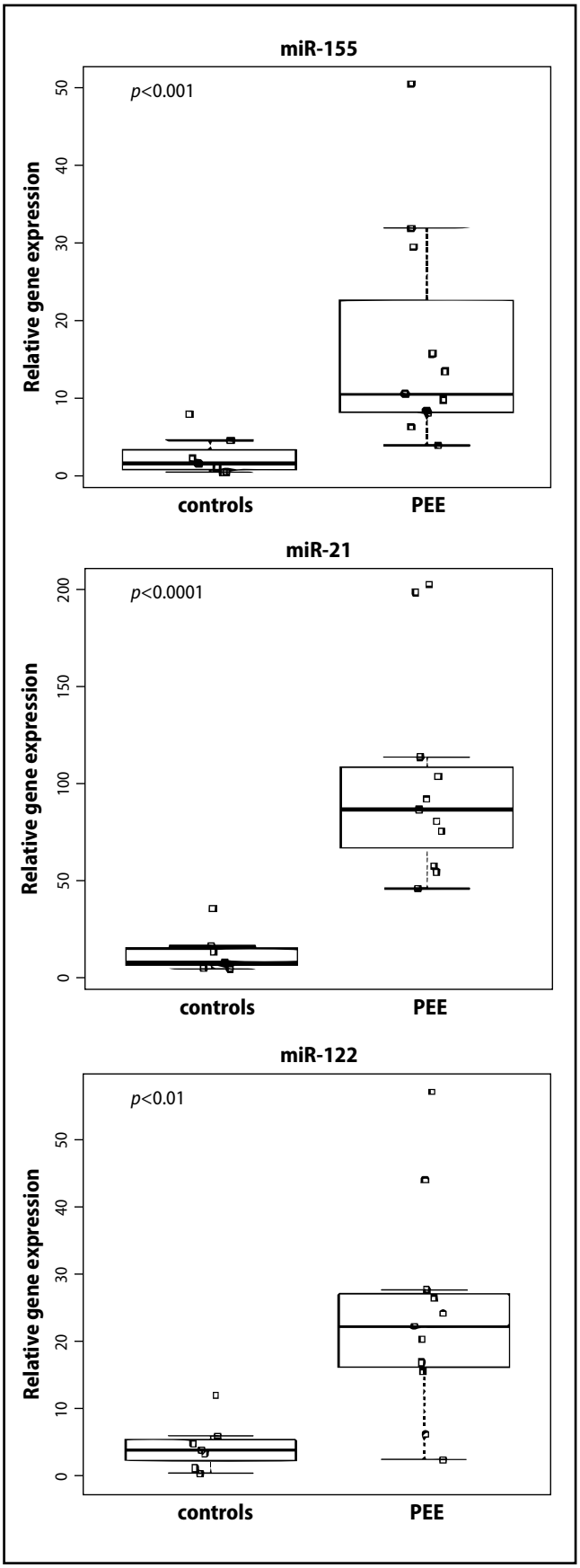

Fig. 1. Relative gene expression of apoptosis-related miRNAs miR-155, miR-21 and miR-122 in term placenta samples from patients with preeclampsia (PEE) and controls. All values are normalized against the level of RNU.

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). Downregulation 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.

ACKNOWLEDGMENT

This study was supported by Centre of Excellence in Perinatology Research (IMTS 26220120016) cofinanced from EU sources and Grant VEGA 1/0102/15.

REFERENCES

1 Betoni JS, Den K, Pahl MC, Rogers L, Muller CL, Packard RE, *et al.* (2013). MicroRNA analysis in placentas from patients with preeclampsia: comparison of new and published results. Hypertens Pregnancy. **32**: 321–339.
2 Buscaglia LE, Li Y (2011). Apoptosis and the target genes of microRNA-21. Chin J Cancer. **30**: 371–380.
3 Chen X, Liu Y, Xu X, Chen H (2011). Decreased Cyr61 under hypoxia induces extravillous trophoblasts apoptosis and preeclampsia. J Huazhong Univ Sci Technolog Med Sci. **31**: 235–240.
4 Choi SY, Yun J, Lee OJ, Han HS, Yeo MK, Lee MA, *et al.* (2013). MicroRNA expression profiles in placenta with severe preeclampsia using a PNA-based microarray. Placenta. **34**: 799–804.
5 Cindrova-Davies T, Herrera EA, Niu Y, Kingdom J, Giussani DA, Burton GJ (2013). Reduced cystathionine γ-lyase and increased miR-21 expression are associated with increased vascular resistance in growth-restricted pregnancies: hydrogen sulfide as a placental vasodilator. Am J Pathol. **182**: 1448–1458.
6 Day Y, Qiu Z, Diao Z, Shen L, Xue P, Sun H, *et al.* (2012). MicroRNA-155 inhibits proliferation and migration of human extravillou trophoblast derived HTR-8/SV neo cells via downregulating cyclin D1. Placenta. **33**: 824–829.
7 Enquobahrie DA, Meller M, Rice K, Psaty BM, Siscovick DS, Williams MA (2008). Differential placental gene expression in preeclampsia. Am J Obstet Gynecol. **199**: 566.e1–566e.11.
8 Friedman RC, Farh KK, Burge CB, Bartel DP (2009). Most mammalian mRNAs are conserved targets of microRNAs. Genome Res. **19**: 92–105.
9 Fu G, Brkic J, Hayder H, Peng C (2013). MicroRNAs in human placental development and pregnancy complications. Int J Mol Sci. **2013**: 5519–5544.
10 Hrtánková M, Siváková J, Sumichrastová P, Lukáč P, Višňovský J (2014). Principles and limits of clinical methods in the diagnosis of fetal hypoxia. Ceska Gynekol. **79**: 326–31.

11 Ishihara N, Matsuo H, Murakoshi H, Laoag-Fernandez JB, Samoto T, Maruo T (2002). Increased apoptosis in the syncytiotrophoblast in human term placentas complicated by either preeclampsia or intrauterine growth retardation. Am J Obstet Gynecol. **185**: 158–166.
12 Lasabova Z, Zigo I, Svecova I, Szabo G, Stanclova A, Skerenova M, *et al.* (2014). Association of specific diplotypes defined by common rs1800682 and rare rs34995925 single nucleotide polymoprhisms within the STAT1 transcription binding site of the FAS gene promoter with preeclampsia. Gen Physiol Biophys**. 33**: 199–204.
13 Li X, Li C, Dong X, Gou W (2014). MicroRNA-155 inhibits migration of trophoblast cells and contributes to the pathogenesis of severe preeclampsia by regulating endothelial nitric oxide synthase. Mol Med Rep. **10**: 550–554.
14 Liang Y, Ridzon D, Wong L, Chen C (2007). Characterization of microRNA expression profiles in normal human tissues. BMC Genomics. **8**:166–172.
15 Livak KJ, Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C (T)) method. Methods. **25**: 402–408.
16 Ma L, Liu J, Shen J, Liu L, Wu J, Li W, *et al.* (2010). Expression of miR-122 mediated by adenoviral vector induces apoptosis and cell cycle arrest of cancer cell. Cancer Biol Ther. **9**: 554–561.
17 Mayor-Lynn K, Toloubeydokhti T, Cruz AC, Chegini N (2011). Expression profile of microRNAs and mRNAs in human placentas from pregnancies complicated by preeclampsia and preterm labor. Reprod Sci. **18**: 46–56.
18 Morales-Prieto DM, Chaiwangyien, M, Ospina-Prieto S, Schneider U, Herrmann J, Gruhn B, *et al.* (2012). MicroRNA expression profiles of trophoblasti cells. Placenta **33**: 725–734.
19 Mouillet JF, Chu T, Sadovsky Y (2011). Expression Patterns of placental microRNAs. Birth Defect Res. **91**: 737–743.
20 Nassirpour R, Mehta PP, Yin MJ (2013). miR-122 regulates tumorigenesis in hepatocellular carcinoma by targeting AKT3. Plos One. **8**: e79655.
21 Pineles BL, Romera R, Montenegro D, Tarca AL, Han YM, Kim YM, *et al.* (2007). Distinct subsets of microRNAs are expressed differentially in human placentas of patients with preeclampsia. Am J Obstet Gynecol. **196**: 261.e1–6.
22 Stubert J, Koczan D, Richter DU, Dietrich M, Ziems B, Thiesen HJ, *et al.* (2014). miRNA expression profiles determined in maternal sera of patients with HELLP syndrome. Hypertens Pregnancy. **33**: 215–235.
23 Visnovsky J, Kudela E, Nachajova M, Danko J (2015). The examination of superior mesenteric artery circulation in fetus during pregnancy. J Matern Fetal Neonatal Med. 10: 1–5.
24 Whitehead CL, Teh WT, Walker SP, Leung C, Larmour L, Tong S (2013). Circulating microRNAs in maternal blood as potential biomarkers for fetal hypoxia in-utero. PLoS One. **8**: e78487.
25 Xu LF, Wu ZP, Chen Y, Zhu QS, Hamidi S, Navab R (2014). MicroRNA-21 (miR-21) regulates cellular proliferation, invasion, migration, and apoptosis by targeting PTEN, RECK and Bcl-2 in lung squamous carcinoma. Gejiu City, China. PLoS One **9**: e103698.
26 Zhang BG, Li JF, Yu BQ, Zhu ZG, Liu BY, Yan M (2012). MicroRNA-21 promotes tumor proliferation and invasion in gastric cancer by targeting PTEN. Oncol Reports. **27**: 1019–1026.
27 Zhang Y, DiaoZ, Su L, Sun H, Li R, Cui H, Hu Y (2010). MicroRNA-155 contributes to preeclampsia by down-regulating CYR61. Am J Obstet Gynecol. **202**: e1–7.