MEDICAL GENETICS

Publication-based analysis of miR-210 dependent biomarkers of pre-eclampsia

Alexander Tkachenko[1,2], Roman Illarionov[1,3], Elena Vashukova[1], and Andrei Glotov[1,4]

[1]Department of Genomic Medicine, D. O. Ott Research Institute of Obstetrics, Gynecology and Reproductology, Mendeleyevskaya liniya, 3, Saint Petersburg, 199034, Russian Federation
[2]Computer Technologies Laboratory, ITMO University, Kronverkskiy pr., 49, Saint Petersburg, 197101, Russian Federation
[3]Department of Chemical and Biotechnology, Saint Petersburg State Technological Institute (Technical University), Moskovskiy pr., 26, Saint Petersburg, 190013, Russian Federation
[4]Laboratory of Biobanking and Genomic Medicine, Institute of Translational Biomedicine, Saint Petersburg State University, Universitetskaya nab., 7–9, Saint Petersburg, 199034, Russian Federation

Address correspondence and requests for materials to Andrei Glotov, anglotov@mail.ru

Abstract

MicroRNAs (miRNAs) are potential biomarkers of most pregnancy complications. In recent years, miR-210 has been shown as one of the main biomarkers, detected at different stages of pregnancy and associated with various diseases, including pre-eclampsia (PE). However, miR-210 is not reported as a marker of PE in about half of the studies. We filtered available published RNA-seq data and miRNAs associated with PE, including or excluding miR-210, obtained from the PregMiR database. For further analysis we only considered miRNAs appearing in at least four different studies. We observed that miR-152, miR-1 and miR-193b were only detected in studies with a changed miR-210 level, whereas miR-27a, miR-29a, miR-130a and miR-519b were detected in studies without miRNA-210 differential expression. Common biomarkers of PE are miR-182, miR-126, miR-155, miR-181a, miR-18a, miR-195, miR-21, miR-223, miR-335, miR-517c, miR-518b, miR-518e and let-7f. Based on the obtained data and taking into account the direction of differential miRNA expression, it can be assumed that the most likely mechanisms of PE development in the early pregnancy stage are either upregulation of miR-210, miR-152, miR-518b and downregulation of miR-126; or upregulation of miR-126 and downregulation of miR-182 and miR-518b. Late stages of PE are determined by miR-210, miR-152, miR-518b, miR-21, miR-155, miR-181a, miR-182, miR-193b-3p, miR-517c, miR-518e (upregulation) and miR-126, miR-18a, miR-195, miR-223, let-7f (downregulation); or miR-27a, miR-29a, miR-130a and miR-519d, miR-517c, miR-518e miR-155, miR-126, miR-181a, miR-195 (upregulation) and miR-223, miR-18a, miR-182 (downregulation). The presented results allow speculation about the influence of certain miRNAs on PE development in the context of the presence or absence of miR-210 differential expression, but additional experimental studies are required to evaluate the findings.

Keywords: microRNA, pregnancy complications, miR-210, pre-eclampsia, bioinformatics analysis.

Introduction

Pre-eclampsia (PE) is a complication of pregnancy characterized by the onset of hypertension and proteinuria after 20 weeks of gestation. It is one of the leading causes of maternal and neonatal mortality and morbidity worldwide. Despite numerous studies, the origin and pathogenesis of PE remain unclear and accurate prognostic biomarkers of PE are lacking to date. There is evidence that PE is not a single disorder but a syndrome with many etiologies (Sibai, Dekker and Kupferminc, 2005).

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Authors' information: Alexander Tkachenko, Junior Researcher, orcid.org/0000-0001-7985-0216; Roman Illarionov, PhD student, orcid.org/0000-0003-2711-748X; Elena Vashukova, PhD, Researcher, orcid.org/0000-0002-6996-8891; Andrei Glotov, Dr. of Sci. in Biology, Head of Department, orcid.org/0000-0002-7465-4504

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Short (21–25 nucleotides) noncoding RNAs, miRNAs negatively regulate gene expression by binding to the 3′-untranslated region of their target mRNAs (Mott and Mohr, 2015). Important roles are played by miRNAs in diverse biological processes, including pregnancy. Many studies have identified aberrant expression of different miRNAs in the placentas and blood from women with PE. Also, certain miRNAs were shown to be potential prognostic biomarkers for PE (Choi et al., 2013).

The most identified differentially expressed miRNA in PE is miR-210 — a hypoxia-responsive miRNA which is upregulated in many different types of cells under hypoxic conditions (Takizawa et al., 2012). The gene encoding miR-210 (*MIR210*) is located on the short arm of chromosome 11 (11p15.5) within the intron of long noncoding RNA AK123483. The *MIR210* promoter contains binding sites of Hypoxia-Inducible Factor 1-alpha (HIF1α), which induces expression of miR-210 placenta, endothelium and trophoblast cells. Expression of *MIR210* can be induced by Tumor Necrosis Factor alpha (TNFα), Nuclear Factor kappa B (NF-κB) and after activation of Toll-like Receptor 3 (TLR3). Targets of miR-210 are involved in mitochondrial metabolism, angiogenesis, DNA damage response, cell proliferation, and apoptosis (Bavelloni et al., 2017). Although miRNA-210 is most often mentioned in studies related to oncology, different pregnancy complications are associated with miRNA-210: gestational hypertension (Hromadnikova et al., 2017), gestational diabetes mellitus (Poirier, Desgagne, Guerin and Bouchard, 2017), and preterm birth (Östling, Kruse, Helenius and Lodefalk, 2019). In most studies (Pineles et al., 2007; Zhu et al., 2009; Enquobahrie et al., 2011; Gunel et al., 2011; Ishibashi et al., 2012; Betoni et al., 2013; Ura et al., 2014; Weedon-Fekjaer et al., 2014; Jiang et al., 2015; Li et al., 2015; Zhang et al., 2015; Munaut et al., 2016; Vashukova et al., 2016; Jairajpuri et al., 2017; Awamleh et al., 2019), miR-210 was found to be upregulated in the placenta, but miR-210 was also identified among differentially expressed miRNA in the plasma or serum of PE patients. Moreover, increased levels of miR-210 were detected in plasma samples before clinical manifestations of PE. However, miR-210 is not reported as a marker of PE in about half of the studies using both blood and placenta samples (Guo et al., 2009; Mayor-Lynn et al., 2011; Noack et al., 2011; Yang et al., 2011; Wang et al., 2012; Wu et al., 2012; Choi et al., 2013; Li et al., 2013; Akehurst et al., 2015; Yang et al., 2015; Sandrim et al., 2016; Gunel et al., 2017; Pei-Yin et al., 2017; Xu et al., 2017; Lykoudi et al., 2018; Martinez-Fierro et al., 2018; Timofeeva et al., 2018; Yoffe et al., 2018), so there is no clear understanding of regulatory pathways associated with miRNA-210 in PE.

The aim of our study is to compare and determine the influence of certain miRNAs on PE development in the context of the presence or absence of miR-210 differential expression. These findings may contribute to our understanding of molecular mechanisms involved in PE and identifying specific diagnostic markers for PE.

Materials and methods

Data analysis of miRNAs linked with preeclampsia. We obtained miRNAs linked with preeclampsia from the manually curated pregnancy pathology database PregMiR (https://pregmir.ott.ru/). We included miRNA genes in the analysis only if their differential expression between samples with preeclampsia and normal samples was significant (adjusted p-value < 0.05). In order to compare gene regulation by miRNA in studies that list miR-210 among differentially expressed miRNA genes and studies that do not contain information about miR-210, we filtered the PregMiR database and further grouped miRNAs according to gestational age. We also performed a search for the identification of preeclampsia miRNA expression profiling studies. We undertook a web-based search in Gene Expression Omnibus (GEO, www.ncbi.nlm.nih.gov/geo/) using search term ("preeclampsia" [MeSH Terms] OR preeclampsia [All Fields]) AND "Homo sapiens" [porgn] AND ("Non-coding RNA profiling by array"[Filter] OR "Non-coding RNA profiling by high throughput sequencing" [Filter]). All the relevant studies were further reviewed manually to include studies of miRNA and no other types of noncoding RNAs. For further analysis we only considered miRNAs appearing in at least four different studies that used both blood and placenta samples.

Target genes prediction. Predicted targets for miRNAs were obtained from miRDB database v.6.0. Genes with target prediction score equal to 80 and above were included in further analysis.

GO enrichment analysis. R packages ClusterProfiler v.3.14.0 (Yu, Wang, Han and He, 2012) and DOSE 3.12.0 (Yu, Wang, Han and He, 2014) were applied to perform GO enrichment analysis (biological process terms), KEGG enrichment and DisGeNET enrichment analysis. In order to adjust p-values, false discovery rate (FDR) was calculated using the Benjamini and Hochberg method. FDR <0.05 was selected as the cutoff value for functional and pathway enrichment analysis of differentially expressed genes.

Results and discussion

Base data analysis

We conducted analysis of different studies with and without differential expression of miR-210 in PE. After filtering the data in the PregMiR database, we obtained 16 and 20 studies with and without differential expres-

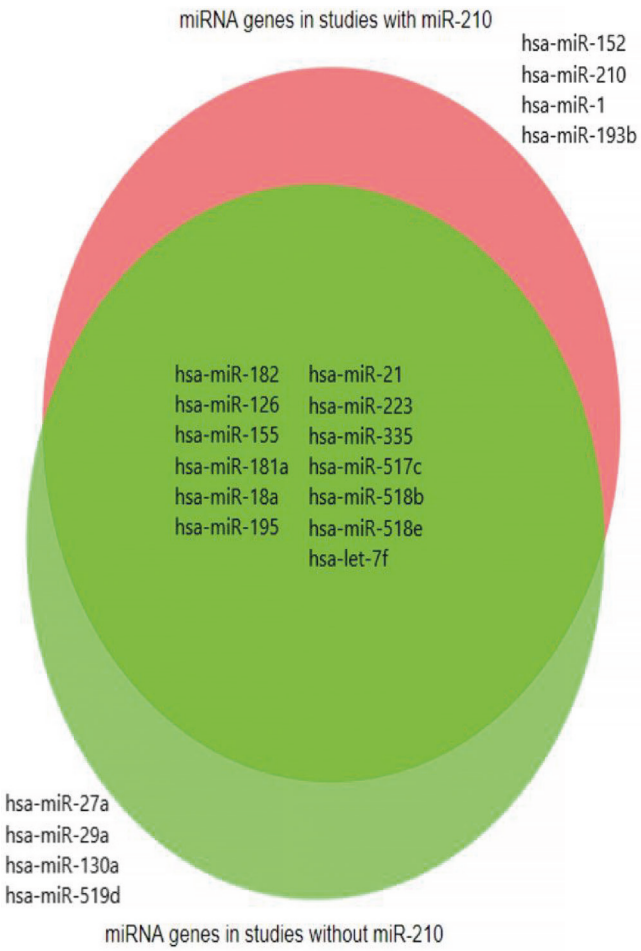


Fig. 1. Venn diagram of miRNAs appearing in at least four different studies of miRNA in preeclampsia.

sion of miRNA-210 in PE, respectively, at different stages of pregnancy.

The search for publicly available datasets for comparison of miRNA between preeclampsia and normotension resulted in four studies conducted with microarrays and three studies — with high-performance sequencing. Among the search results, only data from GSE114349 (RNA sequencing of miRNA from Azamleh et al., 2019) was included in Supplement 1, as other datasets did not have significantly differentially expressed genes (GSE103542, GSE96983), were already listed in PregMiR (GSE15789, GSE119799) or did not have an accompanying publication (GSE84260, GSE96983).

Most studies were performed with placental miRNAs; circulating miRNAs appeared in only 18 studies, with only 5 of them in the early stages of pregnancy. Placental miRNAs are not reliable biomarkers for pregnancy complications, as Menon et al. showed that miRNA expression changes during pregnancy (2018).

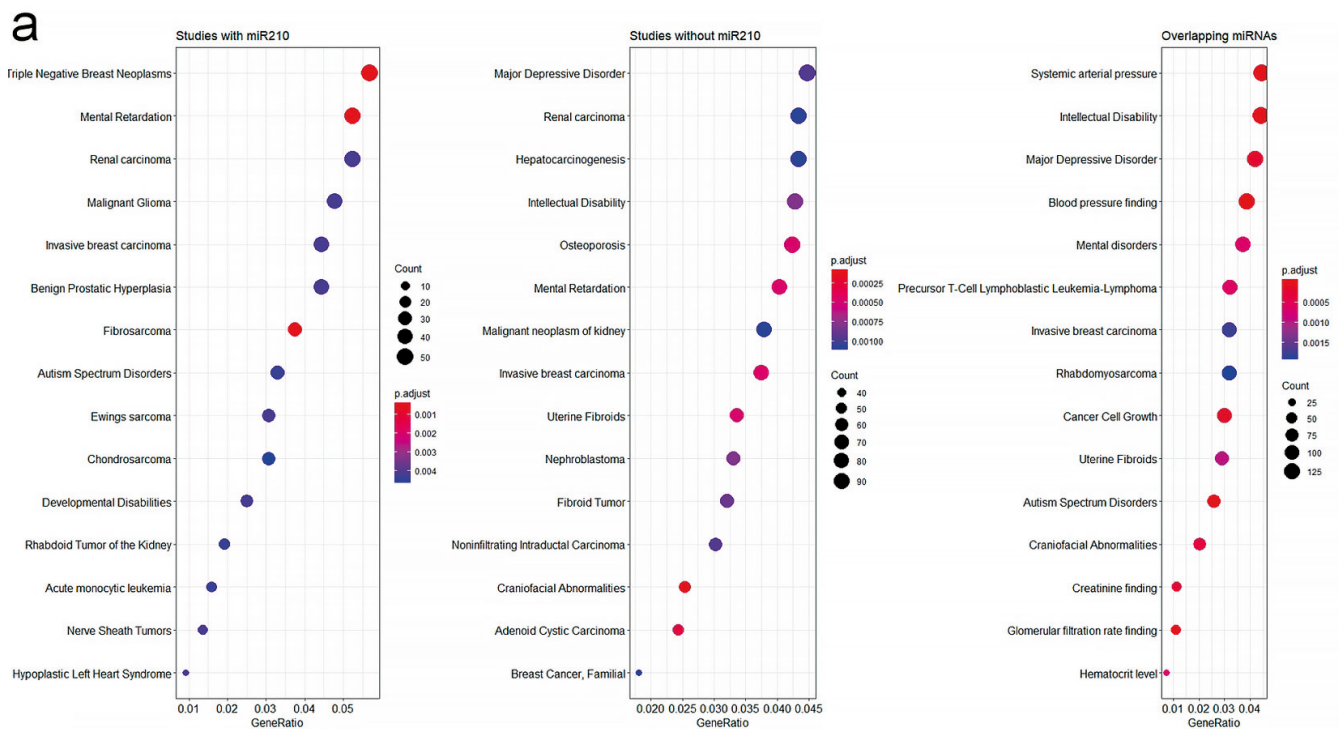
Combining data from PregMir and analysis of GEO data, we obtained 239 differentially expressed miRNAs in studies with miRNA-210 (Suppl. 1) and 126 — in studies without miR-210 differential expression reported (Suppl. 2), amounting to 320 unique miRNAs. Four different miRNA genes appeared in at least four different studies mentioning miR-210 and four miRNA genes — in studies without miR-210 in listed differential expression results, and 15 miRNAs appeared in both cases (Figure 1). Among them three miRNAs (miR-1, miR-193b, miR-195) were only differentially expressed in placenta samples and not in serum or plasma samples. Targets of these miRNAs were enriched in 32 and 79 KEGG pathways (89 for overlapping miRNAs appearing both in studies with and without miR-210 differential expression), 118 and 179 disease profiles from DisGeNET (147 for overlapping), 458 and 771 Gene Ontology biological process (815 for overlapping miRNAs) profiles for studies with and without miR-210, respectively. Dotplots for 15 most enriched gene lists are provided in Figure 2a–c.

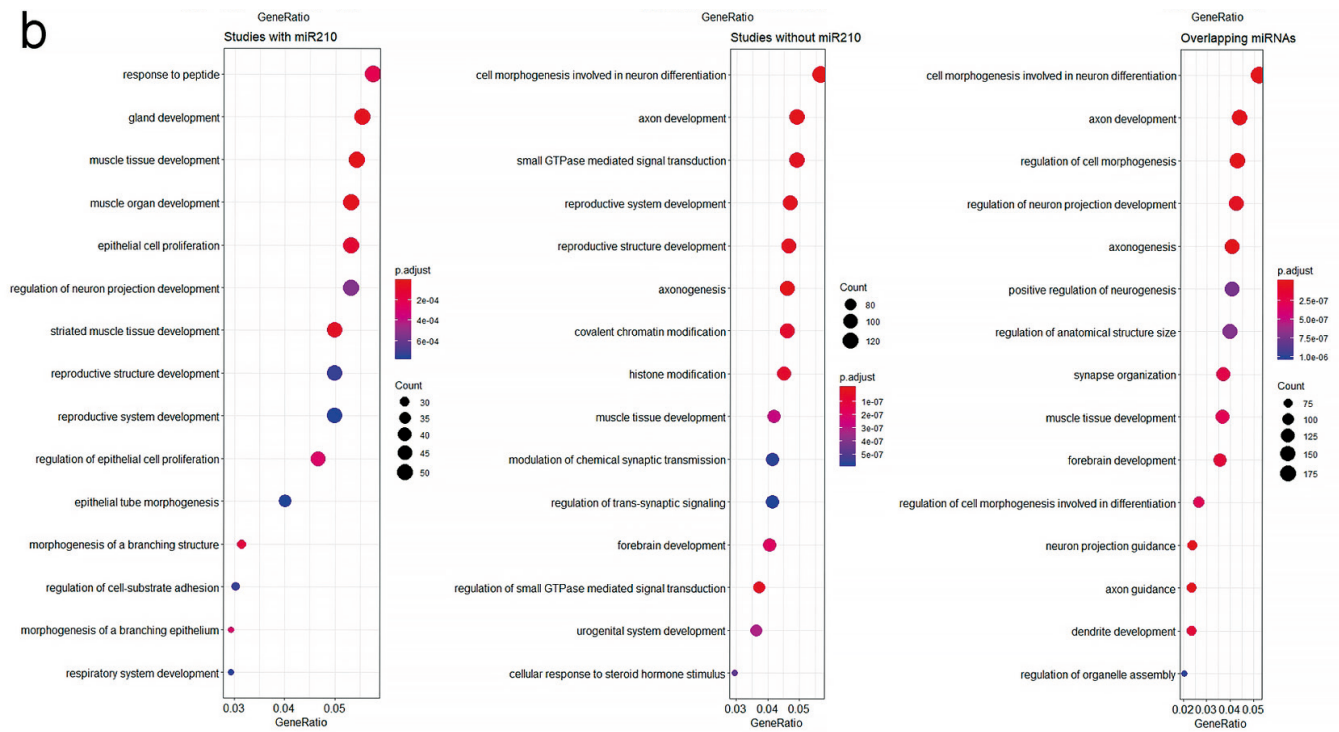
Interestingly, only targets of miRNAs that are co-occurring with miR-210 in studies of pre-eclampsia are enriched in anoxia-associated genes (adjusted p-value 0.005), which is consistent with the hypoxia-related role of miR-210. Both RNA groups that appear with and without miR-210 context are associated with systemic arterial pressure (adjusted p-value $2.92^{*}10^{-9}$), as would be expected from PE markers.

C19MC cluster

Of these miRNAs, one miRNA (519d) in studies without miR-210 Yang et al., 2011; Li et al., 2013; Yang et al., 2015) and three (517c, 518b, 518e) in all studies (Zhu et al., 2009; Guo et al., 2011; Mayor-Lynn et al., 2011; Yang et al., 2011; Ishibashi et al., 2012; Li et al., 2013; Ura et al., 2014; Xu et al., 2014; Yang et al., 2015; Vashukova et al., 2016) were mapped to the C19MC cluster, the largest human miRNA gene cluster, whose expression is almost exclusively confined to the placenta. The cluster spans ~100 kb on chromosome 19q13.41 and contains 54 predicted miRNA genes, 43 of which have been cloned and sequenced (Poirier et al., 2017). The C19MC cluster is regulated by genomic imprinting with only the paternally inherited allele being expressed in placenta, while the maternal one displays a methylation imprint (Poirier et al., 2017). The precise biological function of the C19MC cluster is unknown. It was shown that miRNAs of the C19MC have characteristics of oncogenes and may play a role in trophoblast cell proliferation, invasion, migration, intercellular communication and viral resistance (Poirier et al., 2017). It was reported that altered expression of 517c in the decidua is associated with recurrent pregnancy loss (Dong et al., 2014), and altered expression of miR-518b in the placenta—with low fetal birth weight (Ostling et al., 2019).

It is of note that the functioning of this cluster differs between two compared groups of miRNA studies


a
Studies with miR210
Triple Negative Breast Neoplasms
Mental Retardation
Renal carcinoma
Malignant Glioma
Invasive breast carcinoma
Benign Prostatic Hyperplasia
Fibrosarcoma
Autism Spectrum Disorders
Ewings sarcoma
Chondrosarcoma
Developmental Disabilities
Rhabdoid Tumor of the Kidney
Acute monocytic leukemia
Nerve Sheath Tumors
Hypoplastic Left Heart Syndrome
0.01 0.02 0.03 0.04 0.05
GeneRatio
Count
10 20 30 40 50
p.adjust
0.001 0.002 0.003 0.004
Studies without miR210
Major Depressive Disorder
Renal carcinoma
Hepatocarcinogenesis
Intellectual Disability
Osteoporosis
Mental Retardation
Malignant neoplasm of kidney
Invasive breast carcinoma
Uterine Fibroids
Nephroblastoma
Fibroid Tumor
Noninfiltrating Intraductal Carcinoma
Craniofacial Abnormalities
Adenoid Cystic Carcinoma
Breast Cancer, Familial
0.020 0.025 0.030 0.035 0.040 0.045
GeneRatio
p.adjust
0.00025 0.00050 0.00075 0.00100
Count
40 50 60 70 80 90
Overlapping miRNAs
Systemic arterial pressure
Intellectual Disability
Major Depressive Disorder
Blood pressure finding
Mental disorders
Precursor T-Cell Lymphoblastic Leukemia-Lymphoma
Invasive breast carcinoma
Rhabdomyosarcoma
Cancer Cell Growth
Uterine Fibroids
Autism Spectrum Disorders
Craniofacial Abnormalities
Creatinine finding
Glomerular filtration rate finding
Hematocrit level
0.01 0.02 0.03 0.04
GeneRatio
p.adjust
0.0005 0.0010 0.0015
Count
25 50 75 100 125


b
GeneRatio
Studies with miR210
response to peptide
gland development
muscle tissue development
muscle organ development
epithelial cell proliferation
regulation of neuron projection development
striated muscle tissue development
reproductive structure development
reproductive system development
regulation of epithelial cell proliferation
epithelial tube morphogenesis
morphogenesis of a branching structure
regulation of cell-substrate adhesion
morphogenesis of a branching epithelium
respiratory system development
0.03 0.04 0.05
GeneRatio
p.adjust
2e-04 4e-04 6e-04
Count
30 35 40 45 50
GeneRatio
Studies without miR210
cell morphogenesis involved in neuron differentiation
axon development
small GTPase mediated signal transduction
reproductive system development
reproductive structure development
axonogenesis
covalent chromatin modification
histone modification
muscle tissue development
modulation of chemical synaptic transmission
regulation of trans-synaptic signaling
forebrain development
regulation of small GTPase mediated signal transduction
urogenital system development
cellular response to steroid hormone stimulus
0.03 0.04 0.05
GeneRatio
Count
80 100 120
p.adjust
1e-07 2e-07 3e-07 4e-07 5e-07
GeneRatio
Overlapping miRNAs
cell morphogenesis involved in neuron differentiation
axon development
regulation of cell morphogenesis
regulation of neuron projection development
axonogenesis
positive regulation of neurogenesis
regulation of anatomical structure size
synapse organization
muscle tissue development
forebrain development
regulation of cell morphogenesis involved in differentiation
neuron projection guidance
axon guidance
dendrite development
regulation of organelle assembly
0.02 0.03 0.04 0.05
GeneRatio
p.adjust
2.5e-07 5.0e-07 7.5e-07 1.0e-06
Count
75 100 125 150 175

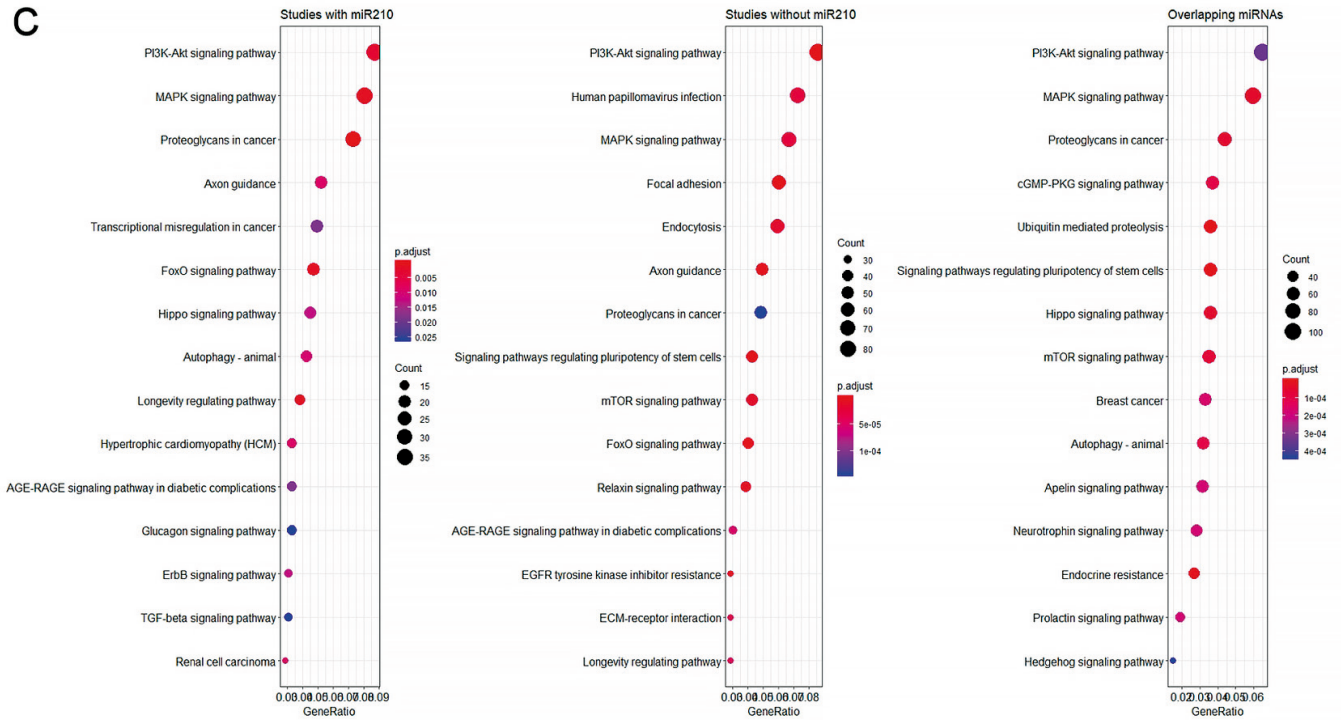


Fig. 2. a) Dotplot that depicts top 15 enriched DisGeNET pathways in miRNA targets of miRNA that do and do not co-occur with miR-210 in studies of preeclampsia. Dot sizes reflect sizes of gene sets, color corresponds to Bonferroni-Hochberg adjusted p-values. GeneRatio shows the proportion of miRNA target genes that are present in reference gene sets used to enrichment analysis. b–c) Same as a but for GO biological process and KEGG pathways, respectively.

in the direction of miR-518b expression change. In the context of miR-210 differential expression, miR-518b is upregulated in PE (Zhu et al., 2009; Ura et al., 2014; Xu et al., 2014) while it has decreased expression in studies where miR-210 levels are unchanged (Guo et al., 2011; Mayor-Lynn et al., 2011). This fact is important for understanding possible mechanisms of PE development, especially in early pregnancy terms when miR-518b is already detected in the plasma, as it can be used for routine screening of pregnant women according to changed miRNA expression profiles. It can be speculated that the risk group for PE is comprised of patients with upregulation of both miR-210 and miR-518b or downregulation of miR-518b on the background of unchanged miR-210 expression level.

miRNAs in studies with miR-210

Among analyzed miRNAs, miR-152, miR-1, and miR-193b are detected only in studies with miRNA-210. Upregulation of miR-152 is found in PE placentas (Zhu et al., 2009; Jiang et al., 2015) and blood serum from PE patients (Ura et al., 2014; Li et al., 2015). One of the targets of miR-152 is the *HLA-G* gene (Zhu et al., 2010). HLA-G protects trophoblast cells from lysis by Natural Killer (NK) cells and contributes to the tolerance of the mother's immune system to the fetus (Zhu et al., 2010). A reduced level of *HLA-G* mRNA was detected in PE placentas (Zhu et al., 2010). Overexpression of miR-152 in trophoblast cells is associated with a reduction of HLA-G levels and increasing trophoblast lysis by NK cells (Zhu et al., 2010) that results in defective remodeling of uterine spiral arteries by the trophoblast. Also, miR-152 has been found to target placental growth factor (PLGF), which is synthesized in trophoblast cells and plays an important role in placental vessels development (Cai et al., 2016). In pregnancies complicated by PE, the level of PLGF is reduced (Cai et al., 2016).

Results about the expression of miR-1 are conflicted and inconsistent. In one study the level of miR-1 is increased (Zhang et al., 2015), while in others it is reduced in PE placentas (Zhu et al., 2009; Enquobahrie et al., 2011; Vashukova et al., 2016). It is possible that miR-1 affects the risk of PE development through its effect on calcium signaling (Enquobahrie et al., 2011) and through its influence on the expression of metallopeptidase inhibitor 3, which is involved in the regulation of trophoblast invasion (Lim et al., 2005; Xiang et al., 2013). Given the heterogeneous results and the lack of expression of this microRNA in early pregnancy stages

and in blood samples, it is difficult to consider it as an important biomarker of PE.

In all reported studies miR-193b-3p is upregulated in PE placentas (Ishibashi et al., 2014; Xu et al., 2014; Vashukova et al., 2016; Awamleh et al., 2019). There were no studies where miR-193b differential expression was detected in blood samples. Overexpression of miR-193b-3p represses proliferation, invasion, migration and growth of cancer cells (Zhou et al., 2016). In experiments in vitro, upregulation of miR-193b-3p has been found to inhibit the migration and invasion of trophoblast cells by targeting TGF-β2 (transforming growth factor-β), which demonstrated significantly lower levels of mRNA and protein in PE placentas (Zhou et al., 2016).

miRNAs in studies without miR-210

The miRNAs miR-27a, miR-29a, miR-130a and miR-519d were detected in more than four studies without miRNA-210 differential expression and are upregulated in all the studies reported here.

Upregulation of miR-27a was found in plasma and placental tissue in studies associated with PE (Hu et al., 2009; Li et al., 2013; Yang et al., 2015). Its gene is located on the chromosome 19p13.13. miR-27a is a member of the miR-23 ~ 27 ~ 24 cluster, participating in the process of angiogenesis by targeting angiogenesis inhibitor SEMA6A, which controls the repulsion of neighboring endothelial cells. miR-27a plays an anti-adipogenic role disrupting the function of mitochondria, and it is associated with angiogenesis in cardiovascular diseases and endothelial apoptosis in cardiac ischemia. Its role in inflammation is demonstrated by increased expression of pro-inflammatory cytokines, such as IL-10, when activated in TlR2 or TlR4-activated macrophages. It was also shown that the knockdown of miR-27a reduces the regulation of pro-inflammatory cytokines IL-6 and TNF-α, which are associated with PE (Maharaj et al., 2016).

The miR-29 family is associated with adult cardiovascular diseases, is expressed in human endothelium, and is involved in the process of angiogenesis. miR-29a is associated with pathways that are dysregulated in PE: the TGF-β signaling pathway, estrogen signaling pathway, focal adhesion, and PI3K-Akt signaling pathway. Changes in these signaling pathways can lead to dysregulation of the endothelial function of the fetus, which is noted in PE (Zhou et al., 2017). In all studies reported here miR-29a was upregulated (Yang et al., 2011; Li et al., 2013; Yang et al., 2015), thus disrupting angiogenesis, causing dysregulation in the placentation.

A pro-angiogenic miRNA, miR-130a regulates homeobox proteins homeobox A5 (HOXA5) and growth arrest homeobox (GAX) (Chen and Gorski, 2007), which are involved in the development of the vasculature of the placenta. Research has shown that miR-130a attenuates endothelial cell damage by suppressing PTEN and activating the PI3K-Akt-NOS3 signaling pathway (Song et al., 2016). Increased regulation of miRNA-130a was found in all studies (Guo et al., 2011; Li et al., 2013; Yang et al., 2015), which suggests that it plays an important role in the pathogenesis of PE.

All of those miRNAs (miR-27a, miR-29a, miR-130a and miR-519d) were detected in plasma and placenta in later stages of pregnancy, which is probably due to their defined roles in the development of late PE forms.

Overlapping miRNAs

In most studies miR-223 is significantly downregulated in PE placentas (Zhu et al., 2009; Betoni et al., 2013; Choi et al., 2013; Weedon-Fekjaer et al., 2014; Xu et al., 2014; Vashukova et al., 2016;) and blood (Yang et al., 2011; Li et al., 2013). Only in two studies without miR-210, miR-223 was upregulated in the placenta (Guo et al., 2011; Mayor-Lynn et al., 2011). The expression of miR-223 is regulated by several transcriptional factors, such as CCAAT-enhancer-binding proteins (C/EBP)-α and -ß and nuclear factor I-A (NFI-A) (Kapinas and Delany, 2011). Originally, miR-223 was characterized as a hematopoietic regulator that affects the development of hematopoietic stem cells, myeloid, erythroid and lymphoid cells (Johnnidis et al., 2008). Further studies have shown that miRNA-223 is involved in osteoclastogenesis (NF-IA), granulopoiesis (IKKα, NF-IA, E2F), erythropoiesis (LMO2), cell invasiveness (MEF2C), tumor suppression (EPB4IL3), tumorigenesis (STMN1, FBW7, KRAS, EGF, EGFR2, MMP9, SEPTIN6), inflammation (NLRP3, Pknox1), glucose uptake (GLUT-4), VSMC proliferation (IGF-1R), and VSMC contractile phenotype (Rho B, MEF2C), which are associated with oncology, type 2 diabetes, cardiovascular diseases and diseases of the musculoskeletal system (Taïbi et al., 2014). There have also been studies where miR-223 is differentially expressed in various pregnancy complications, with the exception of PE, such as preterm birth (Mayor-Lynn et al., 2011; Tang et al., 2015; Enquobahrie et al., 2016; Gray et al., 2017; Winger et al., 2017; Menon et al., 2019).

Involvement and the mechanism of action of let-7f in PE are still unclear. However, the role of let-7 family members in proliferation, apoptosis and inflammation suggests their contribution to the onset of PE via deregulation of these processes (Vashukova et al., 2016). In studies without miR-210, miR-let-7f was mostly downregulated (three cases out of five) (Yang et al., 2011; Li et al., 2013; Gunel et al., 2017) and was downregulated in the studies with miR-210 (Vashukova et al., 2016).

The miRNAs miR-335 and miR-21 are hypoxia-upregulated (Doridot et al., 2013). Jiang et al. (2015) has shown that miR-335 suppresses the migration and

invasion of trophoblast cells by regulation of endothelial nitric oxide synthase (NOS3), which catalyzes the formation of nitric oxide (NO) from L-arginine in endothelial cells. NO is involved in trophoblast invasion, development and function of the placenta (Jiang et al., 2015). *NOS3* has been identified as one of the susceptibility genes for PE. G894T (rs1799983) and T-786C (rs2070744) polymorphism in *NOS3* predisposes to PE by decreasing the enzyme level and reducing the production of NO (Zeng et al., 2016). PE is associated with a decrease in the activity of NOS3, which leads to increased blood pressure (Zeng et al., 2016). However, the results of studies are conflicted, since in all studies, miRNA-335 increased and decreased in two cases (Hu et al., 2009; Ura et al., 2014; Jiang et al., 2015; Zhang et al., 2015). Therefore, the effect of miR-335 remains unclear.

miR-21 enhances trophoblast proliferation and invasion by modulating the nodal signaling pathway. Targets of miR-21 are VEGF and HIF-1α, which are involved in the regulation of angiogenesis. Research has shown that miR-21 also targets zinc finger transcription factor SP1, which is important in placental functions. SP1 controls the expression of cystathionine-γ-lyase (CSE), responsible for the synthesis of hydrogen sulfide (H2S), which has a vasodilator effect important for correct placentation (Doridot et al., 2013). During gestational diabetes mellitus (Wander et al., 2017) and pregnancy-induced hypertension complicated with heart failure (Kan et al., 2019), miR-21 is differentially expressed. Sanders et al. (2015) showed that miR-21, isolated from cervical cells, was significantly overexpressed in women who had a shorter gestation. The expression of miRNA-21 was increased in all studies with miRNA-210 (Jiang et al., 2015; Jairajpuri et al., 2017), and studies have produced contradictory results (one upregulated (Li et al., 2013) and two downregulated (Choi et al., 2013; Gunel et al., 2017)).

An important role in angiogenesis is played by miR-126 and miR-155 by enhancing the function of vascular endothelial growth factor VEGF. Targets of miR-126 are phosphoinositide-3-kinase regulatory subunit 2 (PIK3R2) and sprouty-related EVH1 domain, containing protein 1 (SPRED1), which downregulate VEGF via MAP kinase and PI3 pathways (Hong, Li and Xu, 2014). miR-155 binds to the 3' UTR region of CYR61 (cysteine-rich protein 61), inducing VEGF (Zhung et al., 2014). It was shown that VEGF is also involved in the regulation of trophoblast invasion, proliferation and differentiation. (Shore et al., 1997). Interestingly, in studies with miR-210, miR-126 was downregulated in three cases out of four (Zhu et al., 2009; Ishibashi et al., 2012; Ura et al., 2014), and in studies without miR-210, it was upregulated in four cases out of five (Hu et al., 2009; Guo et al., 2011; Yang et al., 2015; Lykoudi et al., 2018). It can be assumed that in these cases, the pathogenesis of PE occurs via different pathways. In all studies, miR-155 was upregulated (Pineles et al., 2007; Lykoudi et al., 2015; Xu et al., 2015; Jairajpuri et al., 2017).

A member of the miRNA 17-92 cluster, miR-18a is involved in the regulation of trophoblast cell activity. The target for miRNA-18a is Smad2, associated with TGF-β signaling, which modulates trophoblast cell invasion (Xu et al., 2014). Also, miRNA-18a regulates the expression of estrogen receptor α-3' (ESRα-3'), which is involved in trophoblast apoptosis (Zhu et al., 2015). As in the case of miR-126, miR-18a has different regulation between studies with (downregulation) and without (upregulation) miR-210 (Zhu et al., 2009; Li et al., 2013; Xu et al., 2014; Yang et al., 2015; Vashukova et al., 2016; Jairajpuri et al., 2017).

Also, miR-182 affects trophoblast invasion via binding to RND3 3' UTR region (Fang et al., 2018). It is possible that miR-182 plays a role as an immune response enhancer by stimulation of T helper cells via upregulation of IL-2 (Serebelli, Satoh and Chan, 2012). In one of eight studies (the list without miR-210), miR-182 was downregulated in plasma in early terms (Yoffe et al., 2018); in the rest of the studies (Pineles et al., 2007; Noack et al., 2011; Jiang et al., 2015; Li et al., 2015; Zhang et al., 2015) it was increased, which allows us to make an assumption about the importance of miR-182 in PE.

SPP1 and ITGB3, which are targets for miR-181a, are components of the focal adhesion signal pathway. Focal adhesions are dynamic macromolecular complexes comprised of integrins which bind the extracellular matrix (ECM) to the actin cytoskeleton and have been demonstrated to play an important role in embryo implantation and placentation. Many of the components of the focal adhesion signal pathway link integrin-mediated signals with other signaling pathways, such as mTOR, PI3K, and MAPK signaling pathways (Su et al., 2014). Additionally, miR-181a is involved in the invasion and migration of trophoblasts via deregulation of insulin-like growth factor 2 mRNA-binding protein 2 (IGF2BP2) (Wu et al., 2018). In all studies, miR-181a was upregulated (Hu et al., 2009; Zhu et al., 2009; Wu et al., 2012; Jiang et al., 2015; Zhang et al., 2015; Awamleh et al., 2019). Given its relationship with pathways involved in PE, miR-181a can be considered a potential biomarker of PE.

Involvement of miR-195 occurs in inhibition of cell invasion and migration (FASN, CCNE1), inhibition of cell proliferation (HSPA4L, CCNE1, AKT3), angiogenesis and inhibition of trophoblast invasion (VEGF), vascular homeostasis (SMURF1), trophoblast apoptosis and cell cycle control (WEE1), which lead to impaired placentation and, therefore, to PE (Gunel et al., 2018). The difference in the expression of miR-195 in studies associated and unassociated with miR-210 suggests different mechanisms of PE in two groups of studies.

Differential expression of miR-195 was found only in placenta samples where it was downregulated in studies with miR-210 (Zhu e al., 2009; Xu et al., 2014; Vashukova et al., 2016), and upregulated in studies without miR-210 (Hu et al., 2009).

Conclusion

The search for new biomarkers of severe pregnancy complications remains a relevant task. Different miRNAs are among promising candidates for biomarkers of pregnancy complications. One of the most popular biomarkers, miR-210, is a hypoxia-responsive miRNA which is associated with various pregnancy complications including PE, gestational diabetes, preterm birth and others (Hromadnikova et al., 2017; Poirier, Desgagne, Guerin and Bouchard, 2017; Östling, Kruse, Helenius and Lodefalk, 2019). However, miR-210 is not reported as a marker of PE in about half of the studies, which may indicate the presence of different pathogenetic mechanisms of PE development.

Here we have shown that targets of miRNAs co-occurring with miR-210 in studies of PE are enriched in anoxia-associated genes, which is consistent with the hypoxia-related role of miR-210. All reviewed studies report miR-152 co-expression with miR-210. Research has found miR-152 to target placental growth factor (PLGF), the main biomarker of PE, which is synthesized in trophoblast cells and plays an important role in placental angiogenesis (Cai et al., 2016). Apparently, these mechanisms of pathogenesis of PE have a common basis. This suggests that both miR-210-associated biomarkers and PLGF can serve as predictors of early PE. On the other hand, at least half of the cases of PE require additional biomarkers. Therefore, our study aims to determine the effect of certain miRNAs on PE development in the context of the presence or absence of miR-210 differential expression.

Based on data from studies of miRNA expression, we can assume several different mechanisms of PE development. The first is related to early detection of upregulated miR-210 and co-expressed miR-152, miR-518b and downregulation of miR-126 in plasma of pregnant women followed by upregulation of miR-21, miR-155, miR-181a, miR-182, miR-193b-3p, miR-517c and miR-518e and downregulation of miR-18a, miR-195, miR-223 and let-7f in placentas in later stages. The second mechanism of PE development may be associated with detection of such biomarkers as miR-126 (upregulated) and miR-182, miR-518b (downregulated) in blood plasma in early pregnancy. And the third mechanism is apparently determined by changes of specific miRNAs not associated with miR-210 in late stages of pregnancy: miR-27a, miR-29a, miR-130a and miR-519d (upregulated); and common biomarkers: miR-517c, miR-518e, miR-155, miR-126, miR-181a, miR-195 (upregulated) and miR-223, miR-18a, miR-182 (downregulated).

Currently there are few studies of miRNA-seq on early stages of pregnancy. We understand that this study is theoretical in nature. All of this is a certain limitation of the work and our findings need to be tested in experimental studies. However, despite this we believe that our results elucidate the mechanisms of PE pathogenesis and can be useful in screening programs in the future.

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SUPPLEMENTS

Supplement 1. Differentially expressed miRNAs in studies with miR-210 associated with pre-eclampsia

Trimester	Type of biomaterial	Upregulated	Downregulated	Reference
1	serum	miR-25, miR-32, miR-152, miR-193a-3p, miR-204, miR-210, miR-215, miR-296-5p, miR-518b, miR-650, miR-520a, miR-1233	miR-15b, miR-126, miR-144, miR-335, miR-376a, miR-668	Ura B, Feriotto G, Monasta L et al (2014) Taiwan J Obstet Gynecol.
2	serum	miR-152, miR-183, miR-210		Li Q, Long A, Jiang L et al (2015) Biomedical Reports
2–3	serum	miR-210, miR-210-5p, miR-574-5p, miR-1233		Munaut C, Tebache L, Blacher S et al (2016) Biomedical Reports.
3	serum	miR-152, miR-182, miR-183, miR-210		Li Q, Long A, Jiang L et al (2015) Biomedical Reports.
	serum	miR-210		Gunel T, Zeybek YG, Akçakaya P et al (2011) Genet Mol Res.
	plasma	miR-21, miR-155, miR-210, miR-215, miR-650	miR-18a, miR-19b1	Jairajpuri DS, Malalla ZH, Mahmood N et al (2017) Gene.
	placenta	miR-210	miR-1, miR-34c-5p, miR-139-5p, miR-328, miR-500, miR-584, miR-1247	Enquobahrie DA, Abetew DF, Sorensen TK et al. (2011) Am J Obstet Gynecol.
	placenta	miR-182, miR-210	miR-7, miR-101, miR-128, miR-140-5p, miR-196b, miR-199b-5p, miR-223, miR-363, miR-493, miR-520c-3p, miR-520f, miR-551b, miR-585	Betoni JS, Derr K, Pahl MC et al. (2013) Hypertens Pregnancy.
	placenta	miR-10b, miR-18a, miR-19a, miR-20a, miR-22, miR-126, miR-142-3p, miR-144, miR-146b-5p, miR-185, miR-193b, miR-210, miR-451, miR-517c, miR-518c, miR-518f, miR-519e, miR-520a-3p, miR-525-5p, miR-526b, miR-590-5p	miR-224	Ishibashi O, Ohkuchi A, Ali MM et al. (2012) Hypertension.
	placenta	miR-17, miR-21, miR-96, miR-135a, miR-152, miR-181a, miR-182, miR-210, miR-335, miR-451a, miR-516, miR-584	miR-32, miR-126, miR-196, miR-362-3p, miR-377	Jiang F, Li J, Wu G et al. (2015) Mol Med Rep.
	placenta	miR-210	miR-1301, miR-223-3p, miR-224-5p	Weedon-Fekjaer MS, Sheng Y, Sugulle M et al (2014) Placenta.
	placenta	miR-154, miR-155, miR-181b, miR-182, miR-183, miR-200b, miR-210		Pineles BL, Romero R, Montenegro D et al. (2007) Am J Obstet Gynecol.
	placenta	miR-30a-3p, miR-152, miR-181a, miR-210, miR-296, miR-362, miR-517, miR-518b, miR-519e, miR-584, miR-638	miR-1, miR-18a, miR-19a, miR-18b, miR-10b, miR-32, miR-101, miR-126, miR-144, miR-154, miR-150, miR-195, miR-204, miR-218, miR-223, miR-363, miR-374, miR-377, miR-411, miR-450, miR-542-3p, miR-590, miR-625	Zhu XM, Han T, Sargent IL et al (2009) Am J Obstet Gynecol.

Trimester	Type of biomaterial	Upregulated	Downregulated	Reference
	placenta	miR-515-3p, miR-31, miR-210, miR-518a, miR-524, miR-518c, miR-520a, miR-515-5p, miR-516a-5p, miR-519e, miR-193b, miR-4532, miR-518f, miR-527, miR-518e	miR-195, miR-223, miR-1, miR-34c, miR-let-7f, miR-98, miR-135b	Vashukova ES, Glotov AS, Fedotov PV et al (2016) Mol Med Rep.
	placenta	miR-30a-3p, miR-524, miR-17-3p, miR-151, miR-193b, miR-210, miR-518b	miR-195, miR-223, miR-218, miR-17, miR-18a, miR-19b1, miR-92a1, miR-379, miR-411	Xu P, Zhao Y, Liu M et al (2014) Hypertension.
	placenta	miR-20a, miR-19b, miR-424, miR-125b-1-3p, miR-355, miR-1469, miR-181a, miR-210, miR-1, miR-16, miR-182	miR-29a-3p, miR-200c, miR-744, miR-1826, miR-584, miR-363, miR-335	Zhang C, Li Q, Ren N et al (2015) Placenta.
	placenta	miR-193b-5p, miR-193b-3p, miR-210-5p, miR-210-3p, miR-365a-3p, miR-365b-3p, miR-181a-2-3p, miR-365a-5p, miR-27a-5p, miR-33b-3p, miR-520a-3p		Awamleh Z, Gloor GB, Han VKM (2019) BMC Med Genomics

Supplement 2. Differentially expressed miRNAs without miR-210 associated with pre-eclampsia

Trimester	Type of biomaterial	Upregulated	Downregulated	Reference
1	plasma	miR-221, miR-4433b, miR-let-7g	miR-10b, miR-25, miR-99b, miR-143, miR-151a, miR-182, miR-191, miR-146b, miR-486	Yoffe L, Gilam A, Yaron O et al (2018) Scientific Reports.
2	serum	miR-512-3p, miR-518f-3p, miR-520d-3p, miR-520c-3p		Martinez-Fierro ML, Garza-Veloz I, Gutierrez-Arteaga C et al (2018) Arch Gynecol Obstet.
3	plasma	miR-1183		Gunel T, Hosseini MK, Gumusoglu E et al (2017) Placenta.
	serum	miR-29a, miR-125a-5p, miR-125b, miR-136, miR-517b, miR-517c, miR-518e, miR-519d, miR-519a, miR-520h, miR-520g, miR-521, miR-542-3p, miR-let-7a-star, miR-let-7f-1-star	miR-185, miR-223, miR-320c, miR-1260, miR-1272, miR-let-7d, miR-let-7f	Yang Q, Lu J, Wang S et al (2011) Clin Chim Acta.
	plasma	miR-10a, miR-15b*, miR-18a, miR-19a, miR-21, miR-23a, miR-23b, miR-24, miR-26b, miR-27a, miR-29a, miR-29b, miR-29c, miR-30a, miR-30b, miR-34a, miR-99a, miR-100, miR-101, miR-114, miR-125a-5p, miR-125b, miR-130a, miR-144, miR-145, miR-182, miR-199a-5p, miR-221, miR-299a-5p, miR-378, miR-424, miR-512-5p, miR-515-3p, miR-517b, miR-517c, miR-518b, miR-518c, miR-519a, miR-519d, miR-519e, miR-520h, miR-521, miR-525-3p	miR-15b, miR-19b, miR-25, miR-107, miR-185, miR-223, miR-320c, miR-451, miR-let-7f	Li H, Ge Q, Guo L et al (2013) Biomed Res Int.
	serum	miR-155		Xu Yang, Yiling Ding et al. (2017) Medicine (Baltimore)
	plasma	miR-206-5p		Akehurst C, Small HY, Sharafetdinova L et al (2015) J Hypertens.
	plasma	miR-24, miR-26a, miR-103, miR-130b, miR-181a, miR-342-3p, miR-574-5p		Wu L, Zhou H, Lin H et al (2012) Reproduction.
	plasma	miR-346, miR-582-3p		Pei-Yin Tsai, Mei-Tsz Su et al. (2017) Int J Mol Sci
	plasma	miR-423-5p, miR-519a-3p, miR-629-5p, miR-let-7c-5p		Timofeeva AV, Gusar VA, Kan NE et al (2018) Placenta.
	plasma	miR-18a, miR-18b, miR-27a, miR-29a, miR-93, miR-126, miR-130a, miR-135b, miR-142-3p, miR-149, miR-188-5p, miR-203, miR-205, miR-224, miR-301a, miR-517c, miR-518a-3p, miR-518e, miR-519d		Yang S, Li H, Ge Q et al (2015) Mol Med Rep.
	plasma	miR-885-5p		Sandrim V, Luizon M, Palei A et al (2016) BJOG.
	plasma		miR-23c, miR-425, miR-let-7b, miR-let-7f-1	Gunel T, Hosseini MK, Gumusoglu E et al (2017) Placenta.
	placenta	miR-125b, miR-126, miR-130a, miR-141, miR-223, miR-517a, miR-517c, miR-518e	miR-22, miR-29c, miR-30d, miR-143, miR-518b, miR-525	Guo L, Yang Q, Lu J et al. (2011) PLoS ONE.

Trimester	Type of biomaterial	Upregulated	Downregulated	Reference
	placenta	miR-16, miR-20b, miR-26b, miR-27a, miR-29b, miR-30e, miR-126, miR-141, miR-181a, miR-195, miR-222, miR-335, miR-450a, miR-451, miR-486-3p, miR-519b-3p, miR-520g, miR-522, miR-565, miR-7f	miR-214, miR-423-5p, miR-491-5p, miR-508-5p, miR-523-3p, miR-612, miR-658	Hu Y, Li P, Hao S et al. (2009) Clin Chem.
	placenta	miR-18a, miR-18b,miR-27a, miR-29a, miR-93, miR-126, miR-130a, miR-135b, miR-142-3p, miR-149, miR-188-5p, miR-203, miR-205, miR-224, miR-301a, miR-517c, miR-518a-3p, miR-518e, miR-519d		Yang S, Li H, Ge Q et al (2015) Mol Med Rep.
	placenta	miR-20b, miR-512-3p, miR-516a-5p, miR-524-3p, miR-2277	miR-34c-5p, miR-146a, miR-151-3p, miR-192	Wang W, Feng L, Zhang H et al (2012) J Clin Endocrinol Metab.
	placenta		miR-10b, miR-18b, miR-21, miR-23, miR-23c, miR-30c-1, miR-33b, miR-125a-3b, miR-191, miR-345, miR-370, miR-422a, miR-425, miR-509-3-5p, miR-513b, miR-550a, miR-614, miR-650, miR-662, miR-718, miR-933, miR-1225-3p, miR-1273c, miR-1275, miR-1539, miR-2116, miR-3162, miR-3180-5p, miR-let-7b, miR-let-7f-1	Gunel T, Hosseini MK, Gumusoglu E et al (2017) Placenta
	placenta	miR-25, miR-26a, miR-26b, miR-92b, miR-95, miR-191, miR-197, miR-198, miR-202, miR-204, miR-296-5p, miR-296-3p, miR-342-3p	miR-21, miR-223	Choi SY, Yun J, Lee OJ et al. (2013) Placenta.
	placenta		miR-346, miR-582-3p	Pei-Yin Tsai, Mei-Tsz Su et al. (2017) Int J Mol Sci
	placenta	miR-155		Xu Yang, Yiling Ding et al. (2017) Medicine (Baltimore)
	placenta	miR-223, miR-378, miR-431, miR-493, miR-496, miR-720	miR-24, miR-30d, miR-146b-5p, miR-145, miR-146a, miR-181d, miR-495, miR-512-3p, miR-517a, miR-518b, miR-539, miR-526b, miR-654-3p, miR-let-7e,	Mayor-Lynn K, Toloubeydokhti T, Cruz AC et al (2011) Reprod Sci.
	placenta	miR-124, miR-130b, miR-155*, miR-302c, miR-367, miR-383, miR-431, miR-455-5p, miR-500a, miR-518a-5p, miR-875-3p, miR-1183, miR-1197, miR-1204, miR-1305, miR-1914, miR-1915, miR-3143, miR-3157, miR-3186-5p, miR-3200-5p, miR-3616-5p, miR-3670, miR-3928, miR-3941	miR-30a, miR-103-2, miR-126, miR-412, miR-516a-3p, miR-548o, miR-631, miR-19a, miR-542-3p, miR-544b, miR-548w, miR-663b, miR-885-3p, miR-1248, miR-3652, miR-3942, miR-3943	Lykoudi A, Kolialexi A, Lambrou GI et al (2018) Placenta.
	placenta		miR-127-3p, miR-423-5p, miR-519a-3p, miR-532-5p, miR-539-5p, miR-629-5p, miR-let-7c-5p	Timofeeva AV, Gusar VA, Kan NE et al (2018) Placenta.
	placenta	miR-104, miR-128a, miR-133b, miR-182, miR-302, miR-let-7b		Noack F, Ribbat-Idel J, Thorns C et al (2011) Journal of Perinatal Medicine.
	cord plasma		miR-346	Pei-Yin Tsai, Mei-Tsz Su et al. (2017) Int J Mol Sci