Circulating EV-miRNA profile in pregnant women with opioid use disorder treated with buprenorphine

Maricedes Acosta-Martinez[1], Christelle Jean-Baptiste[1], Radha Panchal[1], Zijian Shao[2], Omar Abuzeid[3], Cassandra Heiselman[3], David Garry[3]
Renaissance School of Medicine at Stony Brook University
Department of Physiology & Biophysics[1] Department of Molecular Pharmacology[2], Department of Obstetrics & Gynecology, Division of Maternal Fetal Medicine[3]

Introduction

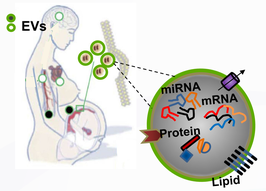

Fig. 1 Extracellular vesicles (EVs) are released from every tissue including the placenta and their cargo, such as microRNAs reflects the pathophysiological state of the cell of origin.

- If untreated, maternal opioid use disorder (OUD) can impair placental function and increase the risk of negative health outcomes such as preterm labor, fetal death, and neonatal opioid withdrawal syndrome (NOWS), the later associated with poor postnatal growth and impaired neurodevelopment.
- Medications for opioid use disorder (MOUD), particularly buprenorphine, are one of the mainstays of treatment during pregnancy, however the effects of buprenorphine treatment on maternal and fetal physiology is poorly understood.
- Because circulating extracellular vesicle-derived miRNAs have both a maternal and fetal (placental) origin, they may serve as biomarkers of maternal and fetal health.

Objective & Hypothesis

- To examine the effects of buprenorphine treatment on maternally-derived plasma EV-miRNA composition.
- We hypothesized that plasma derived EV-miRNAs from pregnant women receiving buprenorphine treatment will reveal insight into the pathways related to the adverse health outcomes associated with prenatal opioid exposure.

Study Design

Recruitment and Sample Collection:

- A total of 24 subjects were included: women stable with buprenorphine treatment (n=12) and healthy controls (n=12).
  - Inclusion criteria: 1) viable pregnancy, 2) 18 years of age or older, 3) planning delivery at SBU Hospital.
  - Exclusion criteria: 1) multiple gestation, 2) fetal aneuploidy or anomaly, 3) pre-existent diseases (i.e. HTN, DM), 4) later develop of obstetric complications (i.e. PE, GDM).
- 3rd trimester plasma samples were collected, aliquoted and stored at -80°C until use.
- **EV and total RNA isolation**. Exosomal RNA was isolated from plasma samples using the exoRNeasy Mini Kit (QIAGEN). This column-based kit incorporated two stages; the exosome purification stage and the RNA isolation stage. (Figure 2)
- **Small RNA sequencing** EV-associated miRNA signatures were determined using low-bias small RNA sequencing (RealSeq Biosciences®). DNA libraries were prepared from 10 ul RNA and 20 cycles of PCR. The libraries were pooled to equal nanomolarity concentrations, purified and size selected. Sequencing was done on the NextSeq500 with single 75 bp reads. (Figure 3)
- **miRNAs differential presence analysis:** Read counts were log-10 normalized and 2-way ANOVA comparisons between groups performed with Benjamini and Hochberg method used to control the false discovery rate (FDR). MIENTURNET webtool was used for microRNA-target enrichment analysis and miRNA-associated diseases were identified using RNADisease v4.0

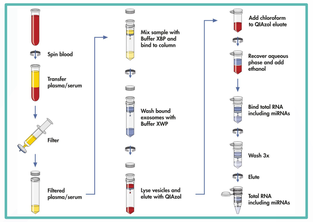

Fig. 2. **EV and total RNA isolation**.

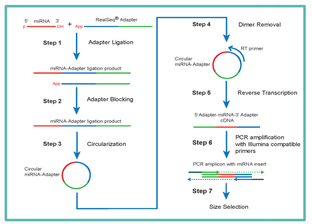

Fig. 3. **Small RNA sequencing**

Results

Table 1. Maternal & Infant Demographics.

Maternal demographics	Control (n = 12)	Buprenorphine (n =12)	P value
Maternal age (years)	30.3 ± 1.4	31.9 ± 1.0	0.37
First trimester BMI (kg/m^2)	27.0 ± 0.8	25.0 ± 1.1	0.17
GA blood collected (weeks)	30.7 ± 2.7	31.4 ± 2.9	0.57
GA at delivery (weeks)	39 ± 1.4	39 ± 0.9	0.92
Cesarean birth	3 (25%)	4 (33%)	>0.9999
Gestational Age at delivery	39 ± 1.4	39 ± 0.9	0.92
Infant Demographics			
Female newborn	7 (58%)	6 (50%)	>0.9999
Newborn weight (grams)	3524 ± 121.5	3232 ± 111.5	0.09
Length of infant hospitalization	1.8 ± 0.2	7.5 ± 0.6*	<0.0001
APGAR score at 1 min	8.9 ± 0.1	8.7 ± 0.1	
APGAR score at 5 min	9.0 ± 0	9.0 ± 0	
Maximum newborn NOWS score	NA	8.2 ± 0.8	
Newborns requiring pharmacological treatment	NA	2 (17 %)	

Data are presented as number (%) or mean ± standard error of mean (SEM). *P*-values are based on t-test for continuous variables and Fisher's exact test for categorical variables. Statistical significance is denoted by *.

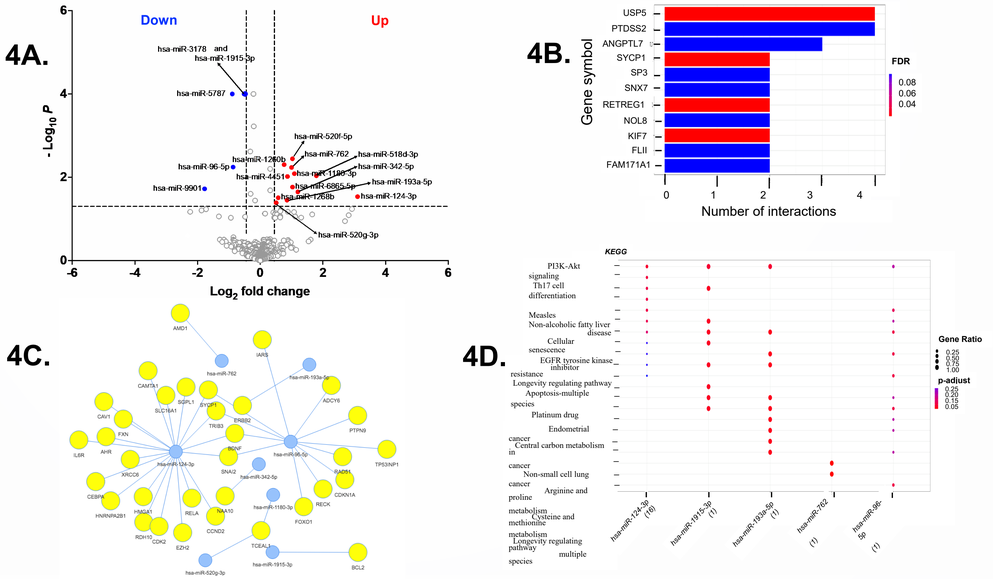

Fig. 4. Identification and pathway analysis of circulating EV-miRNAs associated with buprenorphine exposure in pregnancy. **A.** Volcano plot showing down-regulated (blue) and up-regulated (red) miRNAs in EVs from buprenorphine-exposed subjects compared to controls. **B.** miRNA-target enrichment analysis showing top target genes by number of interactions for buprenorphine EV-miRNAs. The color of the bars represent adjusted p values. **C**. EV-miRNAs differentially expressed in buprenorphine exposed subjects. Blue circles indicate miRNAs, yellow circles indicate their target genes. **D.** Dot plot of functional enrichment analysis for target genes of miR-124-3p, -1915-3p, -193a-5p, -762, and -96-5p. Color dots represent adjusted p values.

- A total of 508 EV-miRNAs were detected, of which 17 were differentially abundant between buprenorphine and control groups, (*p*-value <0.05, and absolute log2fold change of ≥ 0.5 and ≤ -0.5; **Fig. 4A**).
- Among the EV-miRNAs enriched in the buprenorphine group, hsa-miR-520f-5p, hsa-miR-520g-3p, and hsa-miR-518d-3p, belong to the placenta-specific, chromosome 19 miRNA cluster (C19MC).
- The most significant miRNA targets identified by MIENTURNET miRTarbase, which uses data from experimentally validated miRNA-target interactions, included USP5, SYCP1, RETREG1 and KIF7 (p<0.01 and FDR <0.05; **Fig. 4B**)
- Among the EV-miRNAs associated with buprenorphine exposure, miR-124-3p and miR-96-5p have several interactions with other elements in the network (**Fig. 4C**) hence, the are likely to have more influence on the network.
- Top targeted biological pathways linked to the EV-miRNAs from buprenorphine exposed pregnant subjects included the PI3K-akt signaling pathway, pathways in cancer, cellular senescence, and longevity regulating pathways (**Fig. 4D**).
- RNA-disease prediction analysis revealed hsa-miR-520g-3p, hsa-miR-124-3p, has-miR-1260b to be associated with preeclampsia.

Conclusion

- There are unique alterations in circulating EV-miRNAs in buprenorphine treated pregnant patients with OUD
- Based on its significant enrichment in EVs from buprenorphine-treated subjects (fold change > 3) and its link to pathways such as cell senescence, hypothesized to play a role in placental pathologies, miR-124-3p features as a good candidate for future studies aimed at investigating its potential as a biomarker that predicts neonatal complications such as NOWS severity.

Acknowledgements

This work was supported with funding from Stony Brook Medicine's Targeted Research Opportunity Program (FUSION Award: authors MAM & DG) and the INDUCER program (CJ-P).