Novel Interactome Network Construction from EV-derived circRNA in Pregnant Women Living with HIV

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Dara Brena[1], Viviane Schuch[1], Ming-Bo Huang[1], Anandi Sheth[2], Tina Tisdale[2], Christina Mehta[2], Martina L. Badell[3], Riaun Floyd[3], Amber Dawning[1], Alaijah Bashi[1], Austin Chan[4], Rana Chakraborty[5], Vincent Bond[1], **Erica Johnson[1,6*]**

[1]Department of Microbiology, Biochemistry, and Immunology, Morehouse School of Medicine, Atlanta, Georgia 30310, USA, [2]Department of Medicine, Emory University School of Medicine, Atlanta, GA, 30322, USA, [3]Department of Gynecology & Obstetrics, Emory University School of Medicine, Atlanta, GA, 30322, USA, [4]Department of Medicine, Morehouse School of Medicine, Atlanta, GA 30310, USA, [5]Department of Pediatrics, Division of Pediatric Infectious Disease, University of Miami, Miller School of Medicine, Miami, Florida, USA, [6]Department of Obstetrics & Gynecology, Morehouse School of Medicine, Atlanta, GA 30310, USA

BACKGROUND

Virologically suppressed people living with HIV (PLWH) experience a disproportionate risk for chronic non-AIDS-related morbidities (NAMs) that exhibit sex-based disparities. Women are postulated to not only have an increased risk for NAM development, but also an earlier onset and greater severity as compared to men. Concerningly, there is a 2-3 times increased risk for deleterious pregnancy and fetal/newborn outcomes. Extracellular vesicles (EVs) are central to maternal-fetal networking and HIV pathogenesis. Notably, EVs are selectively enriched for large sets of circRNAs that have considerable gene regulatory function within biological and pathological processes integral to reproduction, gynecology, and antiviral immunity.

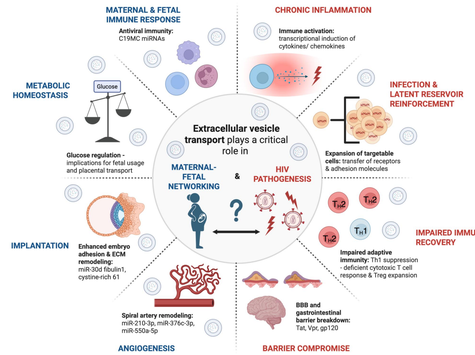


Figure 1 | Knowledge gap on the role of EVs in a pregnancy exposed to HIV. Although EVs are fundamental to both pregnancy and HIV pathogenesis, their role at this intersection remains unclear and insufficiently studied. Created with BioRender.

This study seeks to address the knowledge gap for NAMs specific to pregnancy. *We hypothesize that pregnant PLWH will have differential plasma-derived EV smRNA profiles as compared to pregnant PLWoH with implications for HIV-associated chronic inflammation and NAMs*.

METHODS

Plasma-derived EV small RNA (smRNA) cargo from pregnant PLWH (n=8) was compared to pregnant people living without HIV (PLWoH, n=10) using low-input RNA sequencing (Illumina PE150). Differential EV circRNA profiles were used to construct EV circRNA-miRNA-gene regulatory maps and EV circRNA-protein interactions (circInteractome and miRTarbase). Gene set enrichment analysis (GSEA) using Reactome on the predicted genes from the interactome networks, provided insights into the regulatory roles of the differentially expressed EV circRNAs.

Distinct EV circRNA profiles in pregnant women living with HIV were enriched in physiological pathways influential to HIV persistence, immune activation, and maternal-placental-fetal communication.

RESULTS

Table 1 | Pregnant participant demographic & clinical characteristics

Measures	PLWH (*N*=8)	PLWoH (*N*=10)
Age (years)	27.56 ± 5.73*	31.61 ± 3.88*
Race/ethnicity *n* (%)		
Black/AA, non-Hispanic	7 (87.5)	6 (60)
Black/AA, Hispanic	0 (0)	1 (10)
White/Caucasian, Hispanic	1 (12.5)	0 (0)
Multiracial, non-Hispanic	0 (0)	1 (10)
Other/unknown	0 (0)	2 (20)
Trimester *n* (%)		
First (less than 12 weeks)	3 (37.5)	1 (10)
Second (13-27 weeks)	3 (37.5)	2 (20)
Third (>28 weeks)	2 (25)	6 (60)
Other/unknown	0 (0)	1 (10)
Parity *n* (%)		
Nulliparous	1 (12.5)	2 (20)
Multiparous	5 (62.5)	5 (50)
Other/unknown	2 (25)	3 (30)
Cumulative # of pregnancies	2 (1.25, 3.75)*	3 (1.5, 8)*
Cumulative # of live births	2 (1, 3)*	4 (1, 6)*
Hypertension *n* (%)	1 (12.5)	0 (0)
Gestational diabetes *n* (%)	1 (12.5)	0 (0)
HIV parameters		
Viral suppression *n* (%)	7 (87.5)	X
CD4+ T cell count (cells/µL)	702.5 (550.5, 1105)*	NA
CD8+ T cell count (cells/µL)	554.5 (125.3, 784.8)*	NA
Nadir CD4+ T cell count (cells/µL)	615 (26, 651)*	X
Duration of infection (years)	4.15 (0.64, 10.28)*	X
Mode of transmission n (%)	5 (62.5)	X
Heterosexual	1 (12.5)	
Perinatal	2 (25)	
Other/unknown		
ART *n* (%)		
≥95% adherence	3 (37.5)	X
Regimen		X
PI-NRTI	1 (12.5)	
INSTI-NRTI	2 (25)	
INSTI	5 (62.5)	
Tobacco *n* (%)	1 (12.5)	2 (20)
Marijuana *n* (%)	4 (50)	4 (40)
Cocaine *n* (%)	1 (12.5)	0 (0)

X = not applicable, NA = not available, *mean ± SD, *median (Q1, Q3); PLWH = People living with HIV, PLWoH = People living without HIV, AA = African American, ART = Antiretroviral therapy, PI = Protease inhibitor, NRTI = Nucleoside reverse transcriptase inhibitor, INSTI = Integrase strand transfer inhibitor

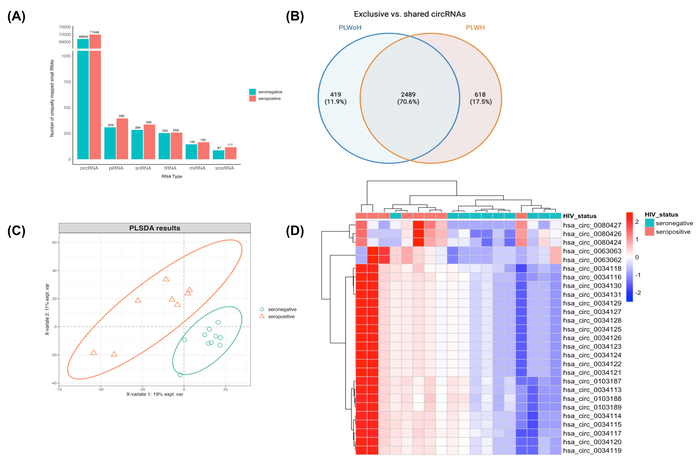


Figure 2 | Species diversity mapping and explorative profiling comparison for plasma-derived EV smRNA-seq based on HIV status in ART suppressed pregnant women. Note: PLWH (orange) and PLWoH (green). (A) Bar graph illustrates the total number and distribution of mapped unique smRNA references. (B) Exclusive vs shared total circRNA detection. circRNA presence was defined at a threshold of ≥ 3 reads in ≥ 30 % of samples. (C) Partial least squares discriminant analysis (PLS-DA), a supervised learning technique for dimensionality reduction, for EV smRNA. This analysis approach accounts for variable interaction and prediction classification relevance. The distinct grouping separation indicates that the HIV status likely alters smRNA EV cargo. (D) Heat map and hierarchical clustering analysis for all the samples and their top 20 smRNA (circRNAs).

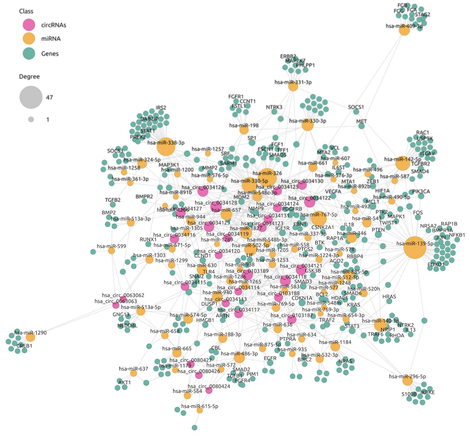


Figure 3 | Integrated circRNA-miRNA-gene network developed from differentially expressed circRNA of plasma-derived EVs in ART suppressed pregnant PLWH. The central nodes (pink) are circRNAs. The middle nodes are miRNAs (orange) that are bound by the circRNAs as determined by circInteractome with a context percentile score ≥ 90. The peripheral nodes (green) are the genes targeted by the miRNAs as determined by the miRTarbase database with support from at least 1 strong validation method such as western blot, qPCR, or reporter assay confirmation. Each connection is a specific interaction between the circRNAs, miRNAs, and target genes. Node size reflects the interaction quantity within the network (e.g. highly connected nodes appear larger vs less connected nodes appear smaller).

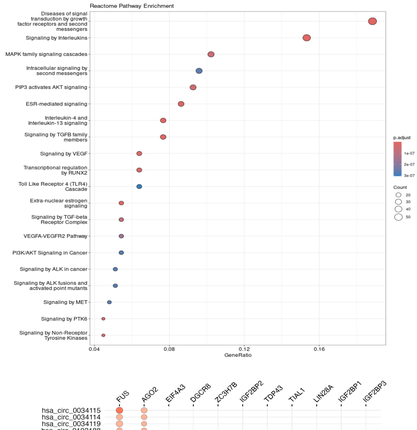


Figure 4 | Gene set enrichment analysis (GSEA) of predicted genes from the circRNA-miRNA-gene network. Gene targets of miRNAs bound by the top differentially expressed EV circRNAs of ART suppressed pregnant PLWH was assessed for physiological function through the Reactome biological pathways database. The 20 most enriched biological pathways are displayed. GSEA identifies whether a gene list is significantly overrepresented within a particular biological pathway. The gene ratio expresses the gene list representation within the complete gene set of the pathway. P-values were calculated from the enrichment scores and portray the likelihood for biological relevance of the gene list to the pathway.

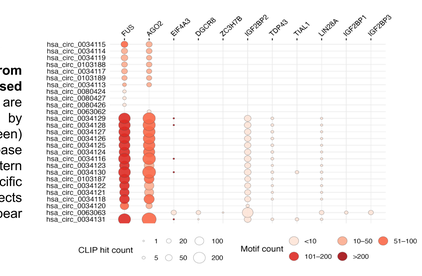


Figure 5 | RNA binding protein (RBP) motif-scanning and Crosslinking and Immunoprecipitation (CLIP) integration of differentially expressed circRNA from plasma-derived EVs in pregnant PLWH. A motif matrix of circRNA x RBP counts was generated, with the subset of the 27 identified differentially expressed circRNA, to determine candidate high-density interactions (≥20 sites). To corroborate these in silico predictions with experimental binding data, aggregated human eCLIP peak sets (all cell lines) was overlapped. This combined motif + CLIP approach highlighted FUS, AGO2 and EIF4A3 as the top RBPs likely to be engaged with the circRNAs of interest in our study of plasma-derived EVs from pregnant PLWH.

CONCLUSIONS

EV circRNA-miRNA-gene mapping and GSEA revealed a regulatory axis with roles relevant to HIV reservoir establishment, latency, replication, immune activation, immunosenescence, and maternal-placental-fetal immune cross talk.

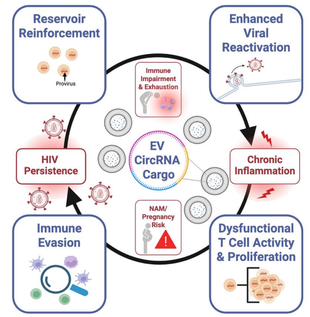


Figure 6 | Differentially expressed EV circRNAs in pregnant PLWH regulate pathways linked to immune function, HIV persistence, and chronic inflammation. Examples include: (1) Immune Evasion - IL-4/IL-13 signaling with reduced cytotoxic T-cell responses; (2) Reservoir Reinforcement – TGF-β and estrogen signaling that promotes viral latency and suppresses reactivation; (3) Enhanced Viral Reactivation and Dysfunctional T Cell Activity with subsequent Immune Evasion – TLR cascade, MAPK, and PI3K/Akt contribute to persistent inflammation and maintenance of the latent HIV reservoir. Created with BioRender.

EV circRNA size, high density of miRNA binding, and AGO2 overlap reinforces roles in scaffolding and sponging as well as directed selective EV cargo packaging.

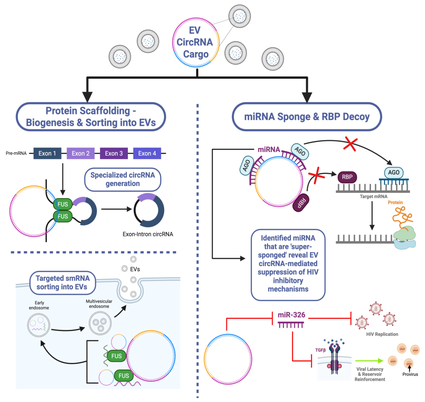


Figure 7 | Proposed molecular mechanisms of the differentially expressed EV circRNA in pregnant PLWH, including RNA-binding protein interactions (FUS, AGO2) and miRNA sponging (e.g., miR-326). in targeting RNA binding proteins (FUS and AGO) and miRNA sponging (e.g. miR-326). The EV circRNA could be involved in facilitating FUS elicited back splicing with subsequent circRNA biogenesis and in targeting smRNA species to selectively be sorted into EVs. For miRNA sponging, miR-326 was a prime example (targeted by 15 of the differentially expressed EV circRNAs) of an EV circRNA-mediated suppression of HIV inhibitory processes.

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AUTHOR CONTACT – erijohnson@msm.edu

Women make up over half of people living with HIV worldwide and face major health risks, especially during pregnancy, yet they remain underrepresented in HIV research. Our study found that biological “envelopes” in the blood, called extracellular vesicles, carry molecular ”messages” with unique patterns in pregnant women with HIV, which may drive inflammation and point to new ways to improve maternal and infant health.