Vaginal Bacteria Regulate Micro-RNAs Targeting the HIV-Host Interactome

Raina Fichorova1, Hidemi Yamamoto1, Yashini Govender1, Andrea Thurman2, Sharon Anderson3, Jennifer Deese1, Charles Morrison3, Pai-Lien Chen3, Jonathan Dreyfuss4, Robert Barbieri1, Robert Salata5, Gustavo Doncel2

1Brigham and Women’s Hospital and Harvard Medical School, MA, USA, 2Eastern Virginia Medical School, VA, USA, 3FHI 360, NC, USA, 4Joslin Diabetes Center, MA, USA 5Case Western Reserve University, OH, USA

BACKGROUND
Understanding the molecular mechanisms underlying the role of the vaginal microbiome in HIV acquisition risk is an essential step toward safer and more effective HIV prevention. We hypothesized that micro(mi)-RNAs regulated by the resident microbiota can interfere with host pathways exploited by the virus. MiRNAs are endogenous short non-coding RNA molecules that are stably carried in circulation by extracellular vesicles and exert post-transcriptional epigenetic regulation with emerging significance in HIV infection. Their role in the anti-viral mucosal barrier function is unknown.

METHODS
The study utilized 288 cervicovaginal specimens from 141 healthy reproductive-age women collected during the luteal phase of the menstrual cycle, for which data were available on age, race, ethnicity, sexual activity, contraception and vaginal hygiene practices. All subjects were confirmed negative for sexually transmitted infections at the time of sampling. Vaginal microbiota was classified by Nugent scores categorized (0-3 scores – normal, 4-6 – intermediate, and 7-10 – bacterial vaginos, BV) and by metagenome classification (Fig. 1 and 2). Differential expression (DE) was determined using Bioconductor DESeq2. miRNA target prediction was performed using miранAtap Bioconductor package. For miRNA target gene sets, we used both the TargetScan conserved miRNA targets and nonconserved miRNA targets, which are obtained from Harmonizome (n=1829).

RESULTS
• Cervicovaginal miRNA profiles varied by both Nugent score categories (0-3 scores – normal, 4-6 – intermediate, and 7-10 – bacterial vaginos, BV) and by metagenome classification (Fig. 3 and 4).
• Higher microbiome diversity was associated with higher number of significantly dysregulated miRNAs (308 in BV versus 69 in Nugent 4-6 compared to Nugent 0-3, FDR<0.1, p<0.01). The gene ontology predictions based miRNAs dysregulated by any of the dysbiotic conditions tested by either Nugent or metagenome identified enrichment for 191 genes previously validated as part of the HIV-host interactome facilitating infection.
• Gene clusters identified with highest stringency included antigen processing and presentation, proteasome and chaperonin pathways, T cell activation and T-cell receptor signaling pathways. Top enrichment scores were achieved for oxireductase activities and the TCP-1 ring complex which interacts with the HIV Vif.
• The miRNAs dysregulated by BV overlapped with 61% of the miRNAs which were up or down regulated in G. vaginalis-dominated compared to L. crispatus-dominated metagenomes. Genes targeted by these overlapping dysregulated miRNAs showed enrichment for 93 genes representing the HIV interactome. Fig. 5 shows the top 10 overrepresented pathways identified by Fisher exact test in the HIV-related gene sets from the MSigDB Collections.

CONCLUSIONS
• The vaginal microbiota plays a role in defining the miRNA cargo of mucosa-derived extracellular vesicles that can exert epigenetic control over protein expression
• miRNAs dysregulated by dysbiotic conditions diagnosed by abnormal Nugent score or metagenomic dominance of bacterial species characteristic of bacterial vaginosis may underly the risk of HIV
• These findings open the door to new strategies for HIV prevention.

Author Contact Information: rfichorova@bwh.harvard.edu
Acknowledgements: This research was supported by the National Institute of Child Health and Development 1R01HD099091-01