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

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BACKGROUND

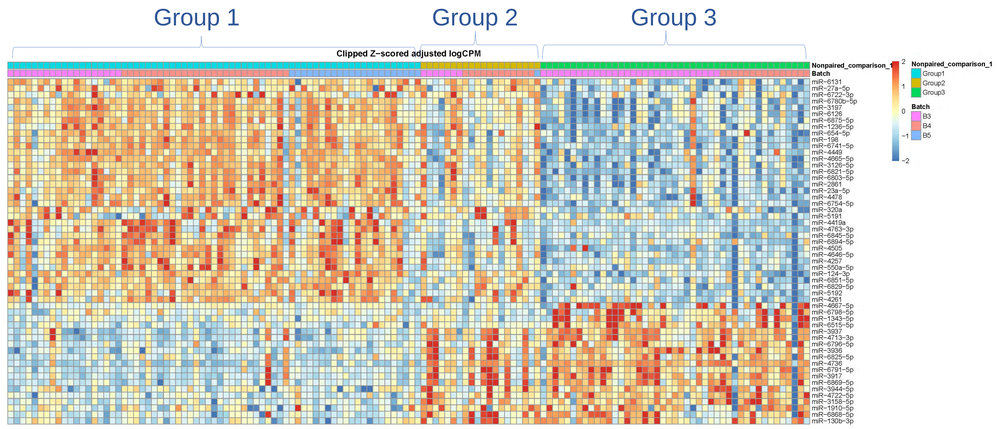
Understanding the molecular mechanisms underlying the role of the vaginal microbiome in HIV acquisition risk is an essential step toward safer and more effective HIV prevention. We hypothesized that micro(mi)-RNAs regulated by the resident microbiota can interfere with host pathways exploited by the 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 and microbiome sequencing (Fig. 1). Levels of miRNAs were quantified in extracellular vesicles isolated from the cervicovaginal secretions using the EdgeSeq global transcriptome platform (Fig. 2). Differential expression (DE) was determined using Bioconductor DESeq2. miRNA target prediction was performed using miRNAtap 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).

We identified miRNAs dysregulated by vaginal dysbiosis that may facilitate immune imbalance and cellular pathways associated with HIV risk in reproductive age women.

Fig. 3.DE miRNAs distinguishing dysbiosis by Nugent score (Gr. 1= BV, Gr. 2= intermediate, Gr. 3= norm)



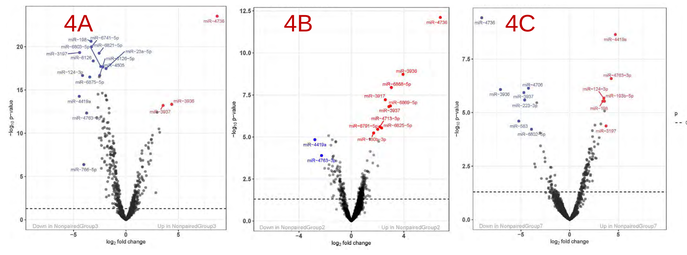


Fig. 4. DE miRNAs by (A) BV (Nugent score>7), (B) intermediate Nugent score 4-6; and (C) , *Gardnerella vaginalis*-dominated versus *Lactobacillus* crispatus-dominated metagenomes.

Fig.1. Microbiome clustering by Nugent score

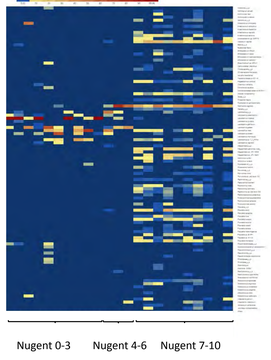


Fig. 2. **EdgeSeq NextGen Platform Process**

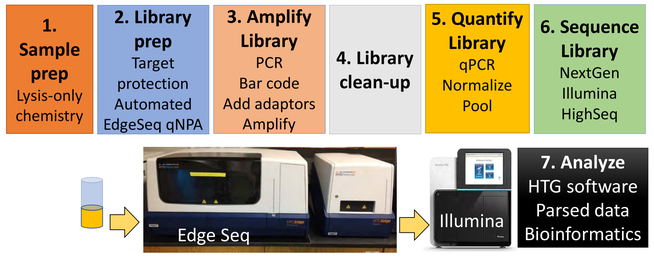
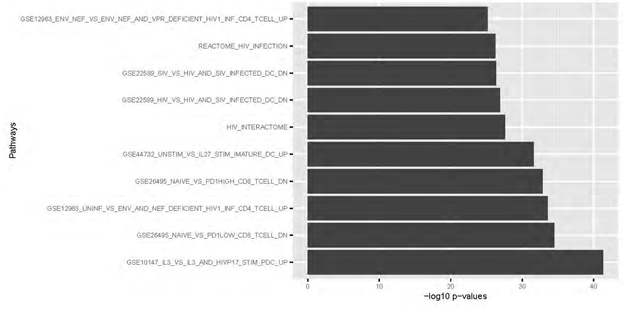


Fig. 5. HIV-related pathways targeted by dysbiosis-dysregulated miRNA



RESULTS

- Cervicovaginal miRNA profiles varied by both Nugent score categories (0-3 scores – normal, 4-6 – intermediate, and 7-10 – bacterial vaginosis, 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 preventions.

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