Novel Exosomal HIV miRNAs May Contribute to Chronic Immune Activation

Mark A. Bernard, Simon C. Yue, Asha Anandaiah, Henry Koziel, and Souvenir D. Tachado

Pulmonary, Critical Care and Sleep Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, United States

Background

HIV-associated chronic immune activation promotes AIDS pathogenesis and contributes to development of complications such as atherosclerosis, malignancy and opportunistic infections. Despite effective highly active antiretroviral therapy (HAART), chronic HIV infection is associated with chronic immune activation, yet the stimuli inducing inflammation and the mechanisms accounting for its persistence remain incompletely understood.

MicroRNAs (miRNAs) are increasingly recognized as important regulators of a wide range of cellular functions, such as proliferation, differentiation, metabolism, apoptosis and tumor progression. miRNAs are 18-22 nucleotides (nts) in length that regulate gene expression by binding to mRNAs leading to translational blockade. However, recent data suggest that miRNAs may have a more direct effect on cellular function, independent of gene regulation. To date, only four HIV-encoded miRNAs are identified and mapped to HIV TAR, Env, Nef and U3.

The goal of this study is to identify novel HIV miRNAs and examine regulation of human macrophages through a novel non-canonical function of HIV miRNAs.

Methods

- **Candidate short hairpins were identified in HIV LTR using UNAfold software.**
- **Small RNAs derived from the GU-rich tract (ssRNA40, vmiR88, vmiR99) were synthesized.**
- **Real Time qRT-PCR assays for miRs were developed using miRCURY LNA Universal RT miRNA PCR technology (Exiqon, Woburn, MA).**
- **Cell extracts of human alveolar macrophage (AM cells; 15-days post infection or clinically HIV-positive) and cell lines (U937 and HIV-infected subclone U1) assayed for microRNAs by qRT-PCR.**
- **Synthetic HIV miRNAs were formulated and applied to cells. Medium was analyzed for cytokine release by ELISA. TNF mRNA (normalized to GAPDH) was analyzed from cell extracts by Real Time RT-PCR.**
- **TLR receptor was identified by siRNA knockdown (TLR8 siRNA SMARTpool or ON-TARGETplus Non-targeting siRNA #1; Dharmacon Inc., Boulder, CO) in the cytokine release assay with stimulation by synthetic vmiR99 ligand (1.0 μg/mL).**
- **Serum exosomal RNA from HIV+ persons was purified using ExoQuick reagent and SeraMir Exosome RNA Purification Columns (System Biosciences) and analyzed by qRT-PCR.**
- **VmiRs were confirmed by cloning and sequencing.**

Results

Fig. 1. Short hairpins (shRNA), predicted by UNAfold RNA folding software, are expected intermediates in biogenesis of mature microRNAs (miR).

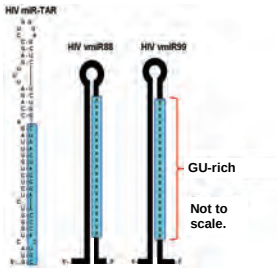


Fig. 2. Novel HIV miRNA detected in cell extracts (A, B) and in exosomal preparations (C, D) of PMA-stimulated chronically-infected U1 cells (A, C) and acutely-infected AM cells (B, D).

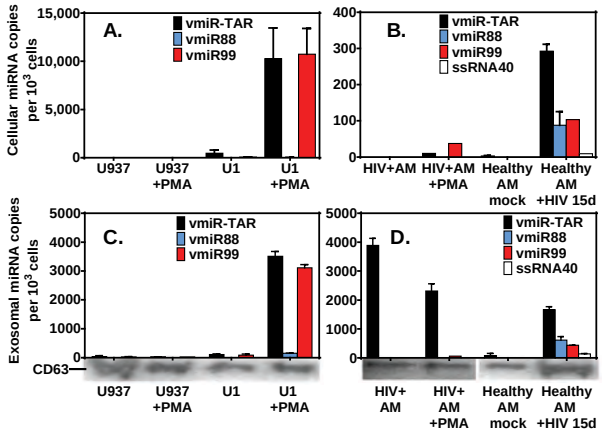


Fig. 3. Signaling by novel HIV vmiRs stimulate downstream TNFα release by primary alveolar macrophages (A) that is inhibited by pre-treating cells with specific antagomir or by pre-duplexing vmiR99 with antagomir (B) and is dependent upon Toll-like Receptor TLR8 (C).

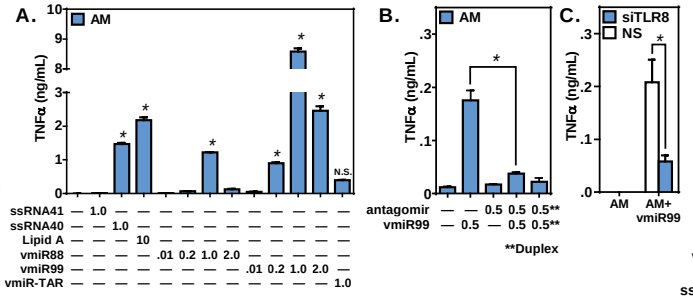


Fig. 4. HIV vmiR99 elicits rapid release of pre-formed TNFα protein by THP-1 macrophages that precedes stimulation of *TNF* gene expression.

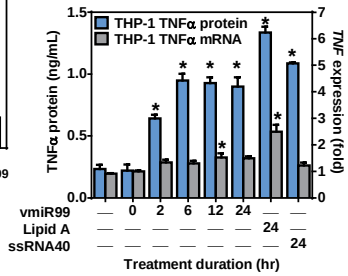
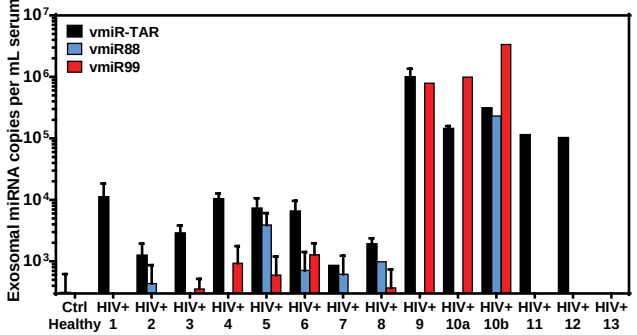


Fig. 5. Novel HIV-produced miRNAs are associated with exosome preparations from sera of asymptomatic HIV+ persons with undetectable viral load. Peripheral blood CD4+ T-lymphocyte count <200 cells/mm³.



Summary

- **Novel HIV-encoded microRNAs (vmiR88 and vmiR99) were identified.**
- **Novel HIV-encoded microRNAs were detected in HIV-infected primary alveolar macrophages and cell lines.**
- **HIV vmiRs stimulate rapid TNFα release by human macrophages through endosomal TLR8.**
- **Induction of TNFα depends on GU base composition**
  - **GU-rich (vmiR88 and vmiR99) are stimulatory.**
  - **GU-poor vmiR-TAR is not stimulatory.**
  - **blocked by novel specific antagomir.**
- **HIV vmiRs are more potent than Lipid A (TLR4 agonist) in primary AM cells.**
- **Clinical samples (HIV+ serum) contain exosomes with vmiR88, vmiR99 and vmiR-TAR, suggesting potential route for intercellular pro-inflammatory stimulation of uninfected bystander cells.**

Conclusion

- **HIV microRNA ("inflam-miRs") may contribute to chronic immune activation that underlies AIDS pathogenesis leading to comorbidities such as atherosclerosis, pulmonary hypertension and malignancy.**

Supported by National Institutes of Health Grant R01-HL092811.