Unique Circulating MicroRNA Profiles and Endothelial Function in HIV Infection

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Introduction

- MicroRNAs (miRs) are non-coding RNAs that regulate gene expression.
- Studies of non-HIV infected patients have demonstrated an association between the expression of circulating miRs (particularly endothelial-specific, inflammation-specific, and cardiac muscle-enriched miRs) and occurrence of cardiovascular disease (CVD).
- Therefore, miRs could serve as potential biomarkers given their stability in blood and characteristic expression in various diseases.
- It has also been reported that cellular miRs may modulate HIV replication and pathogenesis
- The role of circulating miRs as potential biomarkers in HIV infection and HIV-associated CVD has not been defined.

Objectives

- In this study we aim to determine:
  - Which circulating, plasma-derived miRs are differentially expressed in HIV infection.
  - The association between circulating miRs and flow-mediated dilatation of the brachial artery (FMD), a physiologic measure of endothelial function and a strong predictor of future CVD events.

Methods

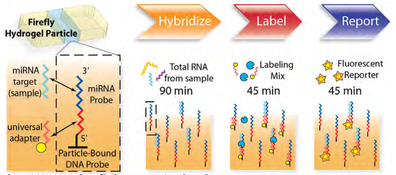
- We analyzed the expression of 192 plasma-derived miRs from 69 HIV-infected individuals and 24 uninfected controls without a history of clinical CVD
  - using Taqman miR Expression Assays and a high throughput RT-PCR instrument (Fluidigm).
  - Global mean normalization was performed.
- Using the above cohort, we also examined the association between 68 circulating miRs and FMD in 12 HIV-infected individuals from the top FMD quartile and 12 HIV-infected individuals from the bottom FMD quartile
  - using Firefly Bioworks hydrogel particles-based miR assays.
  - Data was normalized to geNorm-determined stable reference genes, mir-17-5p, mir-20b-5p, and mir-93- 5p.
- Comparisons were made using Student's t-test, Wilcoxon rank-sum test, ANOVA, and Kruskal-Wallis test where appropriate, and false discovery rate (FDR) control was applied to correct for multiple comparisons.

Results

Baseline Characteristics

	HIV+ Patients (N=69)	HIV- Patients (N=24)
Median age (yrs)	46	44
Female	4%	4%
Race	White:39 African American: 18 Latino:5 Other:7	White: 16 African American:4 Latino:1 Other:3
Average Duration of HIV Infection:	14.3 years	n/a
Off ART at study visit with VL<75 (N)	19	
On ART at study visit with VL>75 (N)	25	
On ART at study visit with VL<75 (N)	25	
Off ART at study visit with VL>75 (N)	0	
Average FMD (%)	4.51	4.90
Smokers	59%	68%
HTN (%)	18%	9%
Diabetes (%)	4%	0%

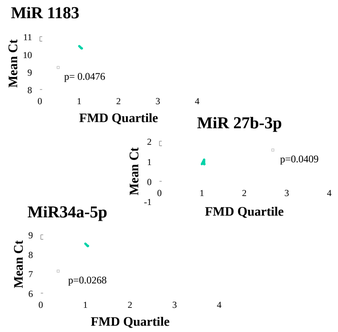
Firefly Bioworks Hydrogel Particles-based MicroRNA Assay



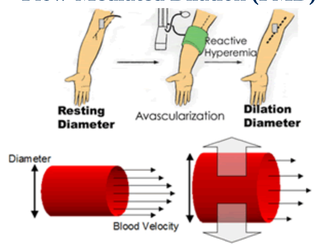
http://www.fireflybio.com/technology

- Uses a unique post-hybridization ligation-based scheme to fluorescently label bound microRNA targets:
- (1) The **Hybridize** step binds your microRNA targets to the target-specific probes attached to the hydrogel Firefly™ Particles.
- (2) The **Label** step ligates a universal biotinylated adapter to the captured targets.
- (3) A fluorescent **reporter** binds to the universal adapter during the Report step.
- (4) This reporter molecule is ultimately used for microRNA detection in a **flow cytometer.**

Levels of miRs-1183, 27b, and 34a varied with different FMD quartiles (p<0.05).



Flow-Mediated Dilation (FMD)



- Blood pressure cuff is applied for 5 minutes to apply suprasystolic forearm occlusion.
- Cuff is then deflated after 5 minutes to cause a reactive hyperemia.
- This increased blood flow produces a shear stress stimulus that induces the endothelium to release nitric oxide.
- FMD is calculated as the increase in the brachial artery diameter from baseline after release of the blood pressure cuff.
- The more the artery dilates in response to shear stress, the better the endothelial function, the higher the FMD and the lower the CV risk.

Circulating miRs that are Differentially Expressed between HIV+ and HIV- Subjects:

These miRs have been reported to have intracellular roles in HIV latency.

These miRs have been shown to be differentially expressed in coronary artery disease among individuals without HIV.

MiRNA	P value	FDR
miR-125a-5p	0.005	0.134
miR-1291	0.006	0.134
miR-31-5p	0.007	0.134
miR-146b-5p	0.007	0.134
miR-1255b-5p	0.008	0.134
miR-1274b	0.011	0.134
miR-223-5p	0.012	0.134
miR-1274a	0.012	0.134
miR-29c-3p	0.014	0.134
miR-342-3p	0.015	0.134
let-7g-5p	0.016	0.134
let-7c	0.018	0.134
miR-145-5p	0.020	0.134
miR-28-5p	0.020	0.134
miR-151a-5p	0.022	0.134
let-7e-5p	0.023	0.134
miR-16-5p	0.026	0.134
miR-409-3p	0.026	0.134
miR-184-1	0.028	0.134
miR-382-5p	0.029	0.134
miR-1271-5p	0.030	0.134
miR-24-3p	0.035	0.142
miR-127-3p	0.038	0.142
miR-126-3p	0.039	0.142
miR-140-3p	0.039	0.142
miR-142-3p	0.041	0.142
miR-106b-5p	0.042	0.142
miR-152	0.042	0.142
let-7d-5p	0.046	0.148

Circulating miRs that are Differentially Expressed In HIV+ Subjects from the low FMD Quartile compared to the High FMD Quartile

miRNA	P value	FDR
miR-133a-3p	0	0.018
miR-133b	0.001	0.018
miR-342-3p	0.001	0.018
miR-485-3p	0.002	0.018
miR-337-5p	0.002	0.018
miR-370-3p	0.002	0.018
miR-433-3p	0.002	0.018
miR-335-5p	0.002	0.018
miR-28-5p	0.003	0.018
miR-181b-5p	0.003	0.018
miR-375	0.003	0.018
miR-20b-5p	0.004	0.018
miR-34a-5p	0.004	0.018
miR-1	0.004	0.018
miR-155-5p	0.004	0.018
miR-31-5p	0.004	0.018
miR-590-5p	0.005	0.018
miR-499a-5p	0.005	0.018
miR-380-3p	0.005	0.018

miRNA	P value	FDR
miR-505-5p	0.006	0.018
miR-125b-5p	0.007	0.02
miR-194-5p	0.007	0.02
miR-145-5p	0.007	0.02
miR-26b-5p	0.009	0.025
miR-125a-5p	0.01	0.027
miR-208a-3p	0.011	0.027
miR-144-5p	0.011	0.027
miR-208b-3p	0.013	0.029
miR-214-3p	0.016	0.037
miR-93-5p	0.019	0.041
miR-142-3p	0.024	0.051
miR-18a-5p	0.025	0.051
let-7e-5p	0.029	0.057
miR-29b-3p	0.029	0.057
miR-374a-5p	0.031	0.059
miR-27b-3p	0.033	0.061
miR-27a-3p	0.035	0.062
miR-199a-3p	0.036	0.063

These miRs have been shown to be differentially expressed in coronary artery disease patients without HIV.

Conclusions

- We demonstrate a unique miR expression profile of 29 miRs in HIV-infected individuals.
- We demonstrate a unique profile of 38 miRs in HIV-infected individuals with high CVD risk and impaired endothelial function.
- We demonstrate three miRNAs whose level of expression varied with different FMD Quartiles.

Future Directions

- These miR profiles may help::
  - identify HIV-infected individuals who are at increased risk for CVD.
  - elucidate the underlying mechanisms of HIV-associated CVD.
  - may be therapeutic targets for new anti-HIV drugs and CVD drugs.

Disclosures

- None

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