Plasma microRNA profiling predicts HIV-Associated Neurocognitive Disorder

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ABSTRACT

Background: HIV-associated neurocognitive disorder (HAND) is common, affecting 30-50% of HIV-infected patients despite the availability of effective antiretroviral therapy. The development of HAND is influenced by several factors including altered host and viral gene expression. Host-encoded microRNAs (miRNAs) regulate both host and viral gene expression. Thus, host miRNAs could contribute to the pathogenesis of HAND but also serve as biomarkers of diagnosis and prognosis as well as indicators of underlying disease mechanisms of HAND. Herein, we investigated plasma microRNA profiles among HIV/AIDS patients with and without HAND in two independent patients cohorts.

Results: Analyses of comparative expression identified 13 miRNAs in Cohort 1 (n=47) that were up-regulated with a fold change (FC) ≥2 and a p<0.05 in the HAND group compared to the nonHAND group. Analysis of Cohort 2 (n=24) confirmed up-regulation of 3 miRNAs also identified in Cohort 1. In a univariate logistic regression analysis level of education, CD4 and nadir CD4 T cell levels and these three miRNAs predicted HAND status. Prediction of HAND status by the individual miRNAs was more robust than either CD4 nadir or CD4 T cell levels. ROC curves showed that area under the curve (AUC) ranged from 0.78 to 0.86 depending on the individual miRNA.

Conclusions: Our findings revealed differential expression of plasma-derived miRNAs in HAND versus nonHAND patients that were conserved across different patient cohorts. These results suggest that plasma miRNAs might be used as biomarkers for HAND and also provide insights into the underlying disease mechanisms.

BACKGROUND

- Human immunodeficiency virus type 1 (HIV-1) infects and establishes a viral reservoir within the brain early in the course of the disease, in all individuals infected with the virus.
- This infection is associated with a neurodegenerative syndrome, collectively termed HIV-associated neurological disorder (HAND) that is common in 30-50% of HIV-1 infected patients under treatment.
- The development of HAND among HIV infected patients is a multifactorial process that is influenced by both viral and host gene expression, host immune responses as well as highly active antiretroviral therapy (HAART).
- Host-encoded microRNAs represent a potent means to regulating both host and viral gene expression while their profiling can also yield insights into underlying disease mechanisms.
- Recent studies have reported that microRNA (miRNA) profiles in brain differ between HIV infected individuals with HAND and nonHAND; and in HIV infected elite controllers and viremic patients.
- **Thus, we hypothesized that differential expression of plasma miRNAs in HIV/AIDS patients with HAND compared to nonHAND might contribute to neuropathogenesis as well as serving biomarkers for HAND diagnosis.**

METHODS AND MATERIALS

- **Sample preparation:** 5ml of anticoagulated blood was collected in EDTA tubes from HIV/AIDS patients at the Southern Alberta Clinic, Calgary AB with consent. Patients were diagnosed with or without HAND based on established criteria. Cohort 1 samples were derived from archived HAND and nonHAND patients but Cohort 2 samples were obtained from a prospective ongoing cohort. Plasma was obtained by spinning the tubes at 1500 RPM for 10 min within 4 hours of blood collection. Aliquots were prepared and stored at -80°C.
- **RNA Extraction:** RNA was extracted using the Qiagen serum/plasma kit. A total volume of 200 µl plasma was used for extraction according to manufacturer instruction with some modification.
- **miRNA Microarray Hybridization:** Total RNA was labeled using the FlashTag Biotin HSR kit followed by array hybridization of Affymetrix 3.0 miRNA genechip according to manufacturer protocol. The Affymetrix 3.0 miRNA genechip contains 19724 probesets and 1733 human mature miRNAs.
- **Statistical and Bioinformation analyses:** Two different softwares (Affymetrix expression console and GeneSpring) were used to normalize the data and determine differentially expressed miRNAs**.** The normalization in both software was based on Robust Multi-array Average (RMA) algorithm, in which raw data is background corrected, log2 transformed and quartile normalized. The median of each probeset in the HAND or nonHAND was calculated and a non-parametric Mann-Whitney *U* test was used to identify differentially expressed miRNAs. Patients variables and miRNA of interest were compared by unpaired Student *t* test, or Spearman rank sum test (correction). The level of significance was defined as $p<0.05$.

RESULTS

Table 1 :Sociodemographic and clinical features of Cohort 1

Variables	Non-HAND (n=25)		HAND (n=22)		*p* Value	
Age [mean, SD]	40.2	12.1	44.2	9.7	0.21	
Gender [n,%]	19	76.0%	20	91.0%	0.001	Male
	6	24.0%	2	9.0%		Female
Ethnicity [n,%]	20	80.0%	18	82.0%	0.87	Caucasian
	5	20.0%	4	18.0%		Other
Education [n,%]	6	24.0%	10	45.0%	0.12	Grade 12 or less
	19	76.0%	12	55.0%		Higher than Grade 12
HIV Viral Load (log copies/mL) [mean, SD]	3.0	1.43	4.0	1.63	0.03	
Peak HIV Viral Load (log copies/mL)[mean, SD]	4.4	1.17	4.8	0.96	0.19	
CD4 T cells (cells/mm3) [mean, SD]	396.6	250.2	278.7	342.1	0.19	
Nadir CD4 T cells (cells/mm3) [mean, SD]	176.3	146.4	101.7	104.32	0.04	
HIV Subtype [n,%]	13	52.0%	13	59.0%	0.63	Clade B
	12	48.0%	9	41.0%		Other /Unknown
Duration of Infection (years) [mean, SD]	5.25	4.72	4.04	5.05	0.40	

* Student t test and Chi-Squared tests were used to evaluate significance; p<0.05

Table 2:Sociodemographic and clinical features of Cohort 2

Variables	Non-HAND (n=12)		HAND (n=12)		p Value*	
Age [mean, SD]	45.40	10.00	44.92	8.3	0.90	
Gender [n,%]	9.00	0.75	9.00	75.0%	1.00	Male
	3.00	0.25	3.00	25.0%		Female
Ethnicity [n,%]	12.00	1.00	4.00	33.3%	≤ 0.001	Caucasian
	0.00	0.00	8.00	66.7%		Other
Education [n,%]	1.00	0.08	10.00	83.3%	≤ 0.001	Grade 12 or less
	11.00	0.92	2.00	16.7%		Higher than Grade 12
HIV Viral Load (log copies/mL) [mean, SD]	2.04	1.03	2.84	1.5	0.14	
Peak HIV Viral Load (log copies/mL)[mean, SD]	4.78	3.66	5.06	1.0	0.40	
	845.3	263.7	293.6			
CD4 T cells (cells/mm³) [mean, SD]	0	6	0	222.7	≤ 0.001	
	271.4	104.9	109.7			
Nadir CD4 T cells (cells/mm³) [mean, SD]	1	8	5	110.4	≤ 0.001	
HIV Subtype [n,%]	7.00	0.58	4.00	33.3%	0.22	Clade B
	5.00	0.42	8.00	66.7%		Other /Unknown
Duration of Infection (years) [mean, SD]	14.07	8.30	8.48	8.02	0.11	

* Student t test and Chi-Squared tests were used to evaluate significance p<0.05

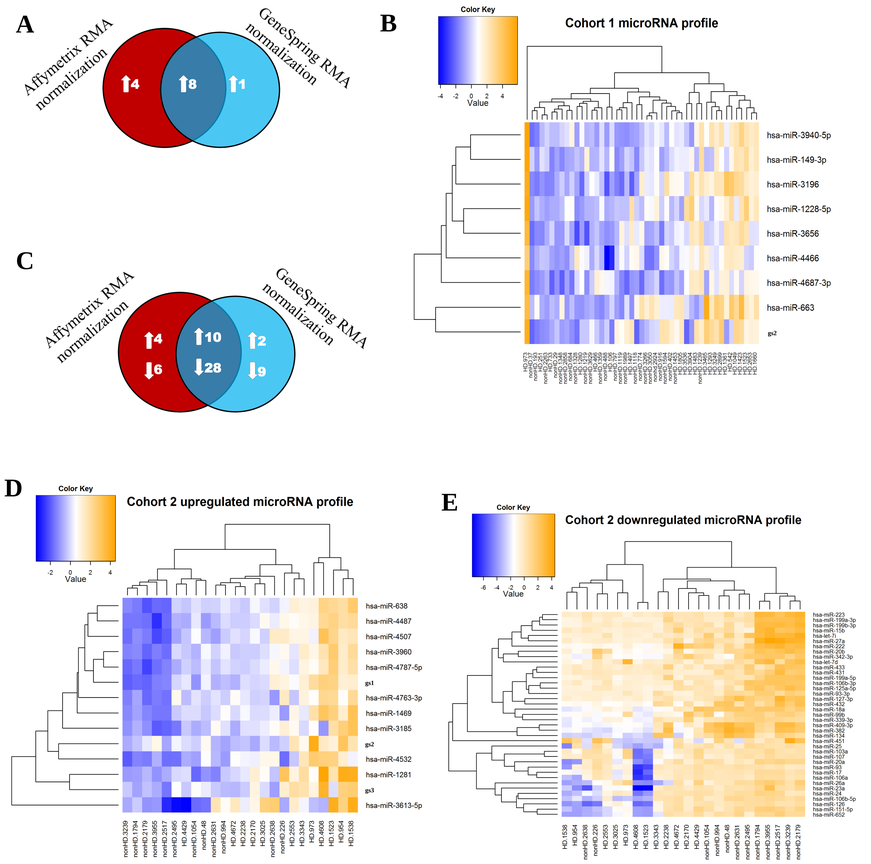


Figure 1: Differentially Expressed miRNAs in HAND compared to nonHAND patients. A) Number of up-regulated miRNAs in HAND compared to nonHAND in Cohort 1 based on Affymetrix and GeneSpring analyses. B) Heat map of up-regulated miRNAs in blood in Cohort 1 based on GeneSpring analyses. C) Number of miRNAs up-regulated and down-regulated in HAND compared to nonHAND in Cohort 2 based on Affymetrix and GeneSpring analyses. D) Heat map of up-regulated miRNAs in blood in Cohort 2 based on GeneSpring analyses. E) Heat map of down-regulated miRNAs in Cohort 2 based on gene spring analyses.

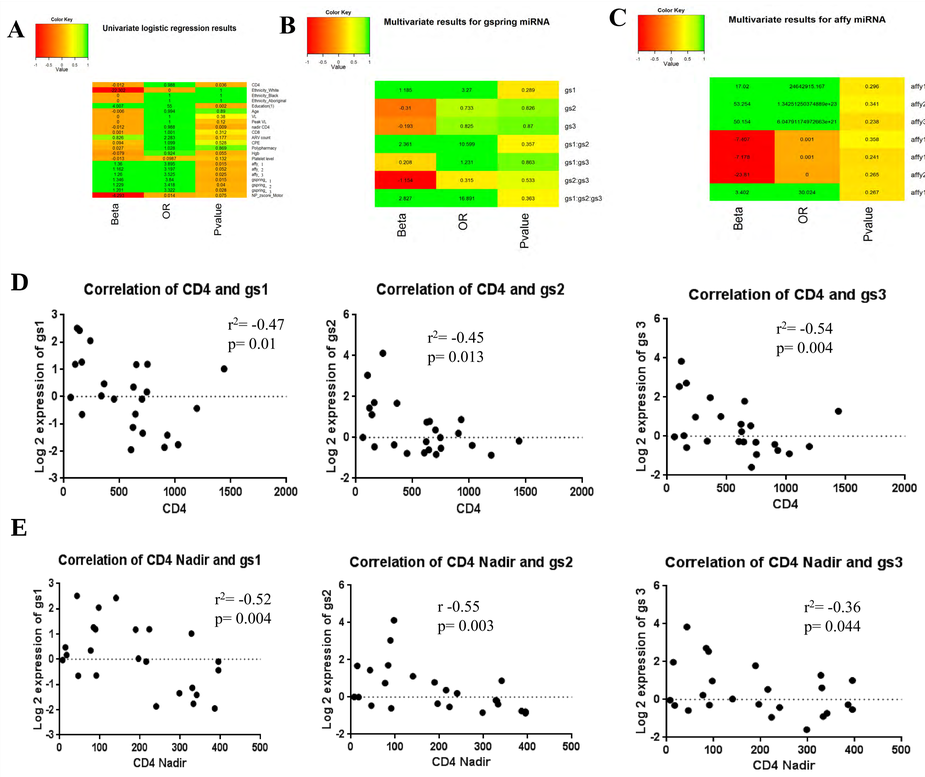


Figure 2: Predictive value of three miRNAs and correlation of individual miRNAs with CD4 and CD4 Nadir. A) Individual predictive value of each miRNA and other variables. B) Synergistic predictive value of a single or a combination of miRNAs based on genespring analyses C) Synergistic predictive value of a combination of miRNAs based on Affymetrix analyses. D) Correlation of individual miRNAs with CD4. E) Correlation of individual miRNAs with CD4 Nadir.

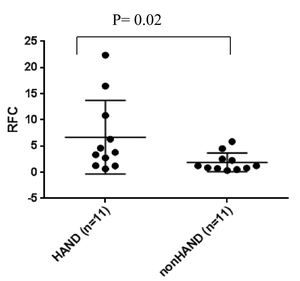


Figure 3: Validation of gs2 by qPCR. We confirmed up-regulation of gs2 using cohort 2 samples.

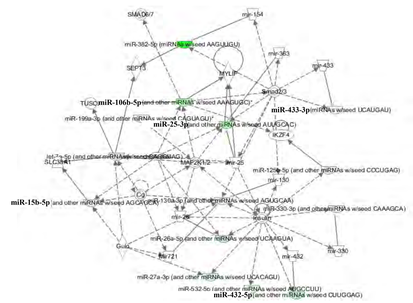


Figure 4: Pathway analysis and networking of Cohort 2 miRNAs target genes.

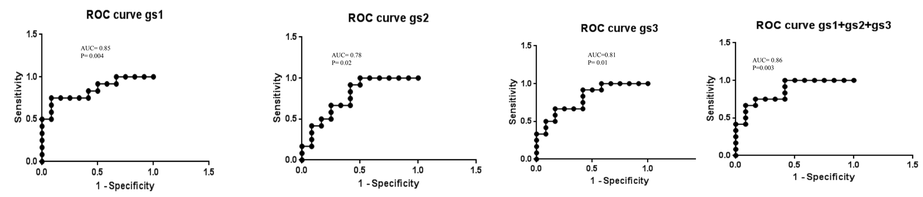


Figure 5: Receiver-operating characteristic (ROC) curve analyses of individual miRNAs in the prediction of HAND. Each miRNA (gs1-3) was predictive of HAND ($p<0.05$) as was the mean values for all 3 miRNAs in Cohort 2.

CONCLUSIONS

- We have identified a human miRNA profile in plasma that distinguishes HAND and nonHAND patients using two cohorts.
- Three miRNAs were identified in both cohorts individually or in combination that predicted HAND but also showed strong correlations with CD4+ T cell at the time of HAND diagnosis and nadir CD4+ T cell in blood.
- The up-regulation of one of these miRNAs was confirmed by qPCR.
- This study demonstrate that plasma miRNAs might contribute to development of HAND and also serve as a biomarkers.

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