tRNA as diagnostic biomarkers for HIV-1 and HIV-2 infection

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Background

HIV-1 and HIV-2 are comprised of closely related but not identical viruses. Accurate laboratory diagnosis of HIV is essential to reduce the risk of HIV positive individuals transmitting HIV infection. With increasing use of PrEP, incident and acute cases may be negative for viral markers in plasma or serum. Identification of HIV-1 and HIV-2 specific non-viral host biomarkers would be useful as surrogate markers to accurately identify new HIV infections where viral markers are absent or undetectable by current HIV assays. Transfer RNAs (tRNAs), the most abundant noncoding-RNA in cells, are also under stringent control in HIV infection and could serve as potential prognostic and diagnostic biomarkers to discriminate between HIV-1 and HIV-2 infection.

Purpose

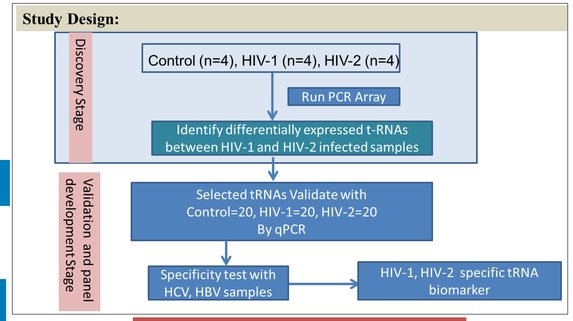
The goal of this study is to elucidate the differential expression profiles of circulating tRNAa in samples from HIV-1 and HIV-2 infected patients .

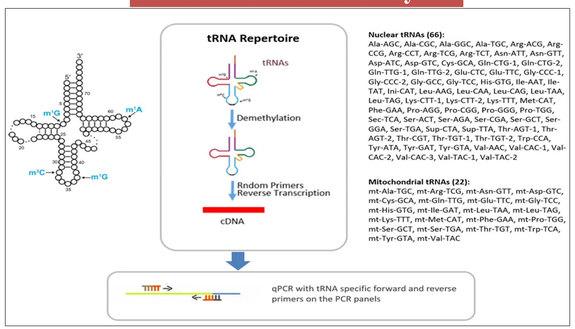
Introduction

The Human Immunodeficiency Virus (HIV) is an RNA virus in the genus of lentiviruses, a family of retrovirus that is characterized by its chronic and persistent infection. HIV is characterized by a high degree of genetic variation, and includes two major types, HIV-1 and HIV-2. HIV-1 is the predominant cause of AIDS worldwide, with approximately 38 million people infected. HIV-2, mainly found in West Africa, can also cause AIDS, and between 1 and 2 million people are infected worldwide. HIV-1 and HIV-2 are closely related viruses that have many characteristics in common, including viral replication, pathology, and methods of transmission. Although there are significant differences, HIV-2 is less virulent than HIV-1, has lower viral loads, and progresses more slowly to opportunistic infections. HIV-2 patients typically survive longer after developing AIDS and have a higher CD4 cell count at the time of the disease. Current treatment strategies for HIV-2 infected patients are limited, as some antiretroviral drugs especially nonnucleoside reverse transcriptase inhibitors and some protease inhibitors are ineffective against HIV-2. In addition to accurate laboratory diagnosis of HIV there is a need for developing diagnostic tests that can accurately discriminate between HIV-1 and HIV-2 infection. Thus, there is a need to add host derived prognostic and predictive biomarkers to the current diagnostic strategy which could be achieved with a panel of validated host transfer RNA (tRNA) biomarkers described in this report. Transfer RNA, also known as transfer ribonucleic acid or tRNA, is a short chain of 70–90 ribonucleotides, folded into a clover form that transfers and carries amino acids. In addition to transporting amino acids and participating in the regulation of biological processes of cells, tRNAs can influence cell proliferation, differentiation, apoptosis, and metabolism. Current research indicates that HIV-1 may have the ability to control the host's tRNA pool. It is yet unclear how HIV-1 and HIV-2 infection and tRNA expression dysregulation are related to one another. In the current work, we studied the modulation of tRNA expression profiles in plasma from individuals infected with HIV-1 and HIV-2 to assess the clinical significance.

Materials and Methods

PCR arrays (Arraystar, Inc.) were used to identify differentially expressed tRNAs in plasma samples from HIV-1 and HIV-2 infected patients. Eighteen tRNAs were selected and validated in 40 plasma samples from HIV-1 and HIV-2 infected individuals and 20 healthy controls.





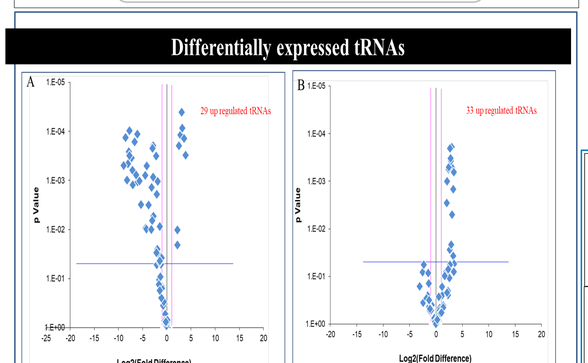


Figure 1. : Volcano plot of differentially expressed tRNAs in (A) HIV-1 infected patient group compared with control and (B) HIV-2 infected patient group compared with control group.

Results and Discussion

Preliminary results indicated that a total of 69 tRNAs (40 down regulated and 29 up regulated) were differentially expressed in HIV-1 infected patient group compared to uninfected control group and 33 tRNAs were up regulated in HIV-2 infected patient group compared to uninfected control group. We selected eighteen differentially expressed circulatory tRNAs for further validation. Among these tRNAs, Asn-ATT, Glu-TTC, Gly-CCC-2, Leu-TAA, Pro-TGG, Ser-GGA, Ser-TGA, Val-AAC, mt-tRNA Lys-TTT and mt-tRNA Met-CAT were associated with the both HIV-1 and HIV-2 infection. mt-tRNA Ala-TGC, mt-tRNA Asp-GTC, mt-tRNA Cys-GCA, mt-Gly-TCC and mt-tRNA Leu-TAG were associated with only HIV-2 infection. We constructed a multivariate model for HIV infection using specific tRNAs. The model based on the tRNA expression signatures can help detect and differentiate between HIV-1 or HIV-2 infection.

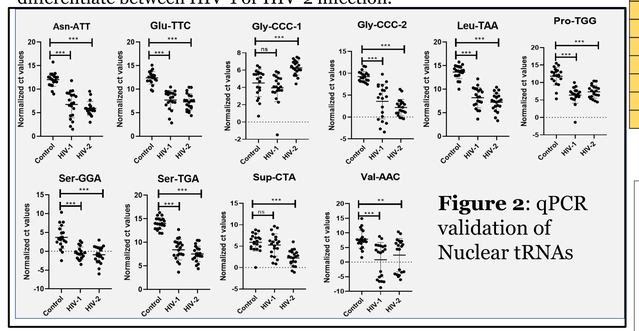


Figure 2: qPCR validation of Nuclear tRNAs

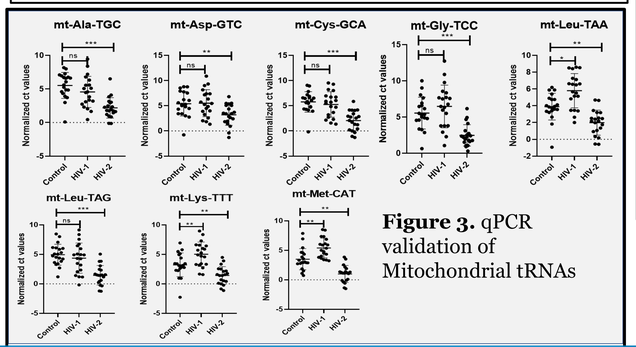


Figure 3. qPCR validation of Mitochondrial tRNAs

Table 1. tRNAs selected for validation

No.	Name of t-RNA	Fold Change		Expression comparison between HIV-1 & HIV-2
		HIV-1	HIV-2	
1	Asn-ATT	4.61	8.13	Both same
2	Gly-CCC-1	0.05	0.02	Both same
3	Gly-CCC-2	4.44	5.12	Both same
4	Leu-TAA	7.04	5.36	Both same
5	Ser-GGA	11.88	10.45	Both same
6	Ser-TGA	5.63	5.36	Both same
7	Val-AAC	14.74	9.78	Both same
8	Pro-TGG	0.40	0.52	Both same
9	mt-Asp-GTC	0.01	0.98	Both same
10	Glu-TTC	0.72	2.57	Difference
11	mt-Ala-TGC	0.002	2.56	Difference
12	mt-Cys-GCA	0.007	7.590	Difference
13	mt-Glu-TTC	0.01	4.78	Difference
14	mt-Gly-TCC	0.01	9.65	Difference
15	mt-Leu-TAA	0.006	6.12	Difference
16	mt-Leu-TAG	0.009	7.48	Difference
17	mt-Lys-TTT	0.003	10.93	Difference
18	mt-Met-CAT	0.004	3.61	Difference

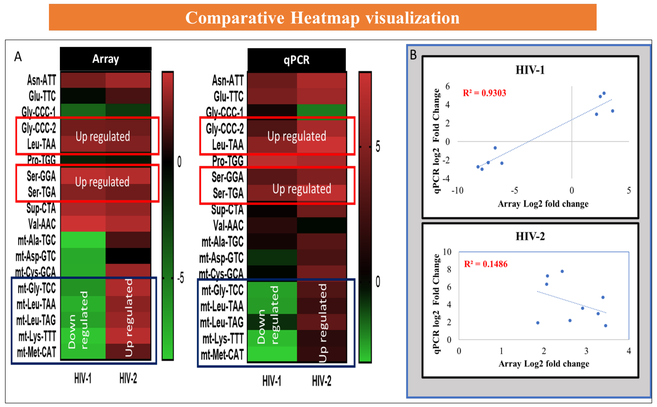


Figure 4. : Comparisons of target tRNA expression by qPCR validation. (A) Heatmap representing log2 FC expression values from array and qPCR. (B) Regression plot displaying the direct correlation between the two values for selected tRNAs.

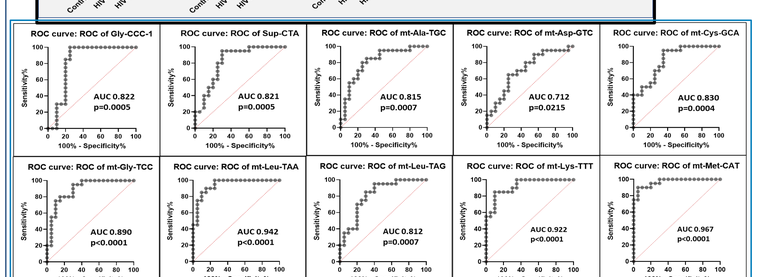


Figure 5. : ROC analysis of the sensitivity and specificity of selected plasma tRNAs. Samples from HIV-2 (n=20), and HIV-1 (n=20) infected patients were subjected to ROC analyses.

Conclusion

Our research has identified new biomarkers that can distinguish HIV-1 from HIV-2 infection. Further investigation of these biomarkers may shed light on the mechanisms underlying the delayed disease progression observed in HIV-2 infected individuals and aid in the prediction of disease severity. Furthermore, these biomarkers have the potential to be used as new diagnostic and therapeutic targets.