Identification of plasma Long non-coding RNA biomarkers to differentiate acute / early infection versus long-term HIV-1 infection

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Background

Individuals with acute / early HIV-1 infection are often unaware that they are infected with HIV-1 and may be involved in high-risk behavior leading to transmission of HIV-1. Identifying individuals with acute / early HIV-1 infection is critical to prevent further HIV-1 transmission, as diagnosis can lead to several effective HIV-1 prevention strategies. With increasing use of PrEP, incident and acute cases may be negative for viral markers in plasma or serum. This will also apply to samples from latently infected individuals who are on long term Antiretroviral therapy (ART). Identification of disease-stage specific non-viral host biomarkers would be useful as surrogate markers to accurately identify new HIV-1 infections in disease stages where viral markers are absent or undetectable by current HIV assays.

Purpose

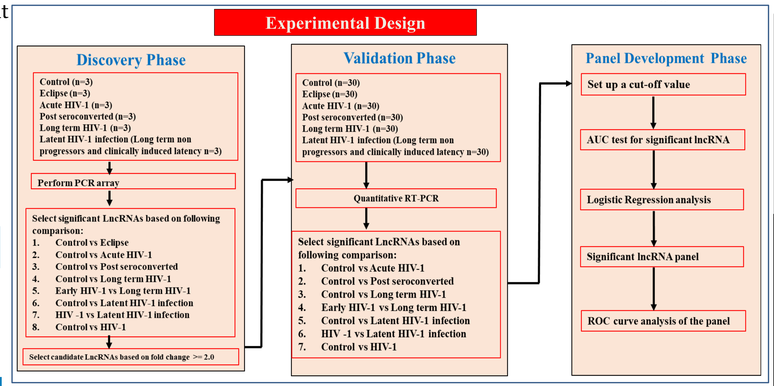
The goal of this study was to identify plasma derived host long non-coding RNAs (lncRNAs) that could serve as prognostic and predictive biomarkers to detect early/acute HIV-1 infection.

Introduction

People with acute/early HIV infection are generally unaware of their infection and can be participating in high-risk behaviors leading to HIV transmission. As a result, early diagnosis is a crucial component of HIV transmission prevention strategy. Antiretroviral therapy (ART) started during the acute stage of HIV infection was found to improve CD4+ T cell recovery, immunity maintenance, and HIV reservoir size decrease. Together with these advantages, ART that is started during acute and very early stages of HIV infection significantly reduces viral load and infectivity, preventing transmission. Non-protein coding RNA transcripts longer than 200 nucleotides are generally referred to as long non-coding RNAs (lncRNAs). Although most studies have been on the intracellular functions of lncRNAs, there is growing interest in the potential use of circulating lncRNAs as diagnostic biomarkers. In this study, we investigated the possibility of using a carefully chosen subset of lncRNAs as non-invasive biomarkers to detect and differentiate acute/early HIV-1 infection from long-term HIV-1 infection. We screened 84 distinct lncRNA candidates using a hypothesis-driven methodology; these candidates are among the most numerous and functionally significant lncRNAs discovered in cells to date.

Materials and Methods

PCR arrays were used to identify differentially expressed lncRNAs in plasma samples from HIV-1 infected patients. The early and long-term HIV-1 infected patients were divided into five groups Eclipse stage of HIV-1+, Acute / Pre seroconverted HIV-1+, Post seroconverted HIV-1+, Long-term HIV-1.



Results

Preliminary results indicate that eleven lncRNAs (7 up and 4 down regulated) were differentially expressed between eclipse vs. control group, 37 lncRNAs (35 up and 2 down regulated) were differentially expressed between acute / pre seroconverted HIV-1+ vs. control group, 4 lncRNAs were up regulated between post seroconverted HIV-1+ vs. control group, 37 lncRNAs (35 up and 2 down regulated) were differentially expressed between long-term HIV-1+ vs. control group and 23 lncRNAs were up regulated between ART-treated HIV-1+ vs. controls.

Table 1. List of selected LncRNAs for validation

Symbol	Fold Change (compared to control group)				
	Eclipse	Acute	Early	Long-term	ART
DANCR	1.18	4.84	4.04	2.73	6.57
DISC2	11.98	123.21	243.79	123.43	0.75
FTX	0.64	13.26	11.50	26.63	3.05
GACAT1	88.62	2320.95	2331.43	1207.03	5.07
H19	266.20	688.83	1140.14	128.07	350.01
HOXA11-AS	36.68	1458.48	1588.28	1293.89	10.15
IPW	1.17	12.29	88.83	392.69	5.00
KRASP1	102.54	342.63	638.26	1234.53	1.67
LINC00853	1.30	14.87	54.70	11.84	0.56
LUCAT1	0.33	0.65	2.03	0.86	1.38
MIAT	347.38	342.63	6325.66	1404.17	27.05
NEAT1	2.00	15.23	9.75	6.56	52.07
OTX2-AS1	190.18	3349.55	3511.20	2363.16	6.32
PANDAR	43.08	463.12	804.53	762.85	3.42
PRINS	43.23	2182.10	184.92	1270.34	9.75
PTCSC3	89.72	856.91	2405.72	977.87	3.91
PTENP1-AS	21.35	341.98	144.51	308.74	0.65
SOX2-OT	31.38	338.15	8819.61	11398.05	2.37
TERC	1.41	40.09	27.14	9.58	0.11
WT1-AS	33.46	190.18	474.41	1220.92	2.11
ZFAS1	0.35	0.09	0.10	0.18	0.76

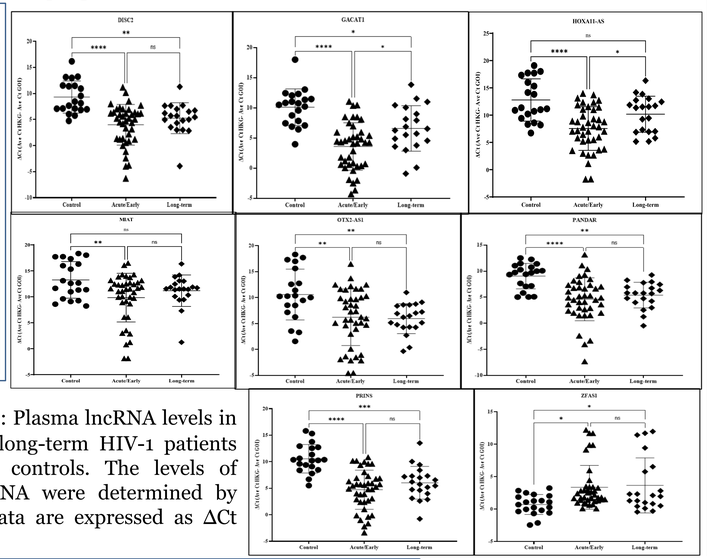


Figure 3 : Plasma lncRNA levels in acute/early, long-term HIV-1 patients and healthy controls. The levels of plasma lncRNA were determined by RT-qPCR. Data are expressed as ΔCt values.

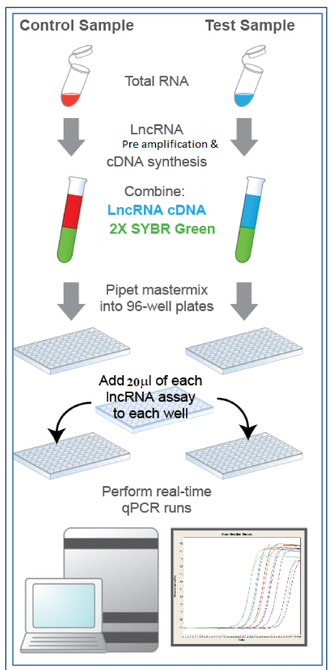


Figure 1: Human lncRNA PCR Array Work flow

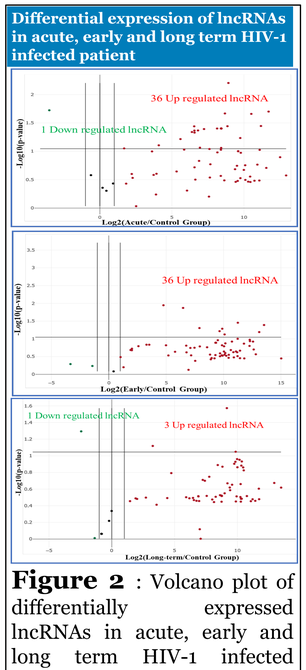


Figure 2 : Volcano plot of differentially expressed lncRNAs in acute, early and long term HIV-1 infected patients compared with healthy controls.

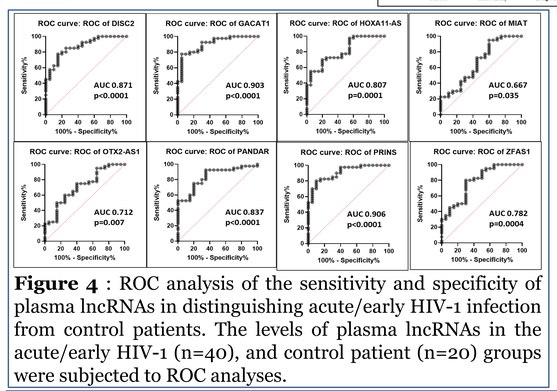


Figure 4 : ROC analysis of the sensitivity and specificity of plasma lncRNAs in distinguishing acute/early HIV-1 infection from control patients. The levels of plasma lncRNAs in the acute/early HIV-1 (n=40), and control patient (n=20) groups were subjected to ROC analyses.

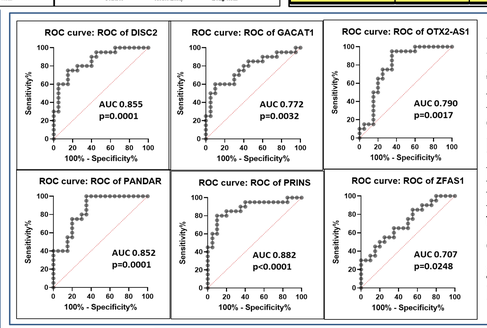


Figure 5 : ROC analysis of sensitivity and specificity of plasma lncRNAs in distinguishing long-term HIV-1 infection from control patients. The levels of plasma lncRNAs in long-term HIV-1 (n=20), and control patient (n=20) groups were subjected to ROC analyses.

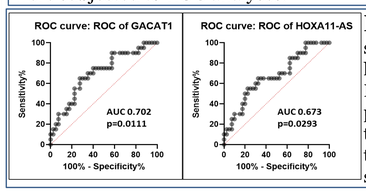


Figure 6 : ROC analysis of the sensitivity and specificity of plasma lncRNAs in distinguishing acute/early HIV-1 from long-term HIV-1 infected patients. The levels of plasma lncRNAs in the acute/early HIV-1 (n=40), and long-term HIV-1 (n=20) groups were subjected to ROC analyses.

Conclusion

In conclusion, lncRNA expression is significantly modulated in response to HIV-1 infection and during ART treatment. Our findings also highlight the potential of circulating lncRNA in the detection of both acute/early stages of HIV-1 infection, which may help to shorten the window period and facilitate early detection and treatment initiation. Initiating ART treatment at this stage would significantly reduce HIV-1 transmission. These differentially expressed lncRNA could be used as prognostic and diagnostic biomarkers for HIV infection, as well as to identify new therapeutic targets.