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Co-variation between HERV-K RNA Expression and CD4 Cell Counts in Untreated HIV-1 Infected Individuals

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Abstract

Human endogenous retroviruses (HERVs) have been implicated in the etiology of cancer, chronic inflammation, and other diseases, and emerging data supports the regulation and functional interaction between K type HERV (HERV-K) and HIV-1. In this study, we performed a reverse transcription-polymerase chain reaction (RT-PCR) assay using DNase I digestion to remove "contaminating" HERV-K genomic DNA and examined HERV-K RNA expression in plasma samples from 54 untreated, newly diagnosed, HIV-1 infected individuals. We found that, HERV-K expression did not correlate with HIV-1 viral load. However, the mean CD4 cell count was significantly lower in HERV-K RNA positive specimens. Further studies are necessary to examine the regulatory relationship between HERV-K and CD4 cell counts in HIV-1 infected individuals.

Keywords: HERV-K expression; HIV-1 viral load; CD4 cell count; DNase I digestion

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Introduction

The human genome has approximately 400,000 genetic loci, half of which are composed of elements that consist of DNA transposons and retroelements[1,2]. Human endogenous retroviral (HERV) sequences are categorized as types of retro elements that comprise approximately 8.3% of the human genetic sequence[3,4]. According to a previous hypothesis, HERVs were once exogenous retroviruses that were incorporated into the human germ-line resulting in vertical transmission and becoming endogenous[5]. HERVs share sequence homology with animal retroviruses such as the mouse mammary tumour virus and the murine leukemia virus; however, they are found exclusively in catarrhines (old world monkeys, apes, and humans)[6]. Moreover, there are specific HERV loci that are exclusive to humans, such as the AF001550 LTR cluster, the AC003023 LTR cluster, and others[7,8]. The genetic composition of HERV elements is similar to that of HIV-1, HIV-2, HTLV 1 and HTLV 2. A complete HERV strain consists of genes that code for a group-specific antigen (GAG) a retroviral capsid protein, protease (PRO), a reverse transcriptase domain (POL) and an envelope protein (ENV), flanked by regulatory sequences called long terminal repeats (LTRs). The majority of HERV elements found within the genome are degenerated and defective due to a number of mutations within their sequences; that said, some are believed to have biological activities and retain relatively complete genetic sequences. According to studies regarding HERV expression, when present, it can be related to an active role in the pathogenesis of cancer, chronic inflammation, and other diseases[9]. In addition, these elements have been associated with playing a functional role in the regulation of innate immune pathways, as well as in the regulation of host gene encoding immunity factors[10].

Retroelements can be divided in two different categories: one with LTR and one without LTR; with HERVs falling in the category that contains LTRs in their genetic structure[9]. The discovery of HERVs results from various experimental approaches. One approach focused on using probes derived from animal retroviruses with conserved POL-regions to screen human genomic libraries under low-stringency conditions[11,12]. Other approaches used oligonucleotides with homology to viral primer binding sites[13], and the use of cross-reacting antibodies to detect highly conserved Gag retroviral proteins[14]. Still, some HERV families are detected by chance[15-17]. Asides from the presence of LTRs, HERVs are grouped into three different classes: Class I, most closely related to gamma retroviruses, including HERV-W and HERV-H; Class II, most closely related to beta retroviruses, including various strains of HERV-K (one of the most complete and active HERVs in the human genome); and Class III, most closely related to spuma retroviruses, including HERV-K and HERV-S[2-7]. Asides from such classifications, in regards to protein biosynthesis, HERVs are classified within that category based on their tRNA primer binding site if they were to be replicating viruses, HERV-K would use lysine, and HERV-W would use tryptophan[18].

The HERV-K strand is then classified in 10 different families, ranging from HERV-K human MMTV-like (HML)-1 to HML-10[19,20]. Among HERV-K families, HERV-K HML-2 has been the focus of recent investigations due to its open reading frames for all major retroviral proteins: Gag, Pro, Pol, and Env. HERV-K HML-2 also carries a sequence specific nuclear RNA export factor, Rec, that is functionally analogous to the HIV-1 Rev protein and the HTLV Rex protein[21].

HERV expression is mostly negative, however, factors such as UV radiation, cytokines or hormones have been reported to activate HERV expression[22-25]. It has been well established that HERV-K mRNA and proteins are expressed in many tissues. As mentioned prior, enhanced expression is present in a wide range of pathological conditions including: viral infections, multiple sclerosis, HIV-1, and melanomas[9,26,27]. The focus of this study was to examine the possible correlation of HERV-K RNA expression in relation to HIV-1 clinical phenotypes, in this case viral load and CD4 cell count.

Materials and Methods

Patient specimen

HERV-K expression was measured in plasma from 54 untreated, newly diagnosed, HIV-1 infected individuals submitted for HIV-1 clinical testing in the Molecular Diagnostics Laboratory at The University of Texas Medical Branch (UTMB) between May and August 2013. The study was approved by the UTMB Institutional Review Board.

HIV-1 viral load

Plasma HIV-1 RNA assay with a lower limit of quantifica¬tion of 75 copies/mL was performed by the VERSANT HIV-1 RNA 3.0 Assay (bDNA) using the standard procedure according to manufacturer instructions (http://www.fda.gov/downloads/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/PremarketApprovalsPMAs/UCM091276.pdf, Siemens Healthcare Diagnostics, Washington, DC).

CD4 cell count

CD4 cell count was quantified by flow cytometric method using BD FACSCanto II Flow Cytometer following standard manufacturer instructions (http://www.uni-regensburg.de/Fakultaeten/Medizin/Pathologie/pdf/CantoManual.pdf, BD Biosciences, San Jose, CA).

RNA extraction and DNase I digestion

RNA was extracted from patient samples with the use of HIV-1 ViroSeq RNA preparation method (Abbott Molecular, Des Plaines, IL)[13,28]. 500 µL plasma were extracted for every patient sample, with final RNA diluents of 100 µl for the samples. Samples were then stored at -80^{0}C. A DNase I Digestion Kit (New England Biolabs, Ipswich MA) was used to eliminate residual DNA from RNA diluents. 4 µl of DNase buffer, 1 µl of DNase I and 15 µl of ddH_2O were combined with 20 µl of extracted RNA samples prior to the digestion process. A sample totaling 40 µl was placed in a GeneAmp 9700 Thermocycler for 1 hr at 37^{0}C. Sample was then removed and left for 1 hr at room temperature (15 - 25^{0}C). At 50 minutes, 0.4 µl EDTA was added to prevent RNA degradation. Tubes were then placed in 75^{0}C heat block for heat inactivation. Tubes were then allowed to cool to room temperature prior to storage at -80^{0}C.

HERV-K PCR and HERV-K reverse transcriptase-PCR

Proceeding DNase I digestion, two separate reaction protocols were followed in order to assure no residual DNA remained in the sample and to confirm positive HERV expression: HERV-K PCR and reverse transcriptase-PCR (RT-PCR).

PCR: 5 µl PCR buffer, 1 µl forward primer, 1 µl reverse primer, 2 µl dNTPs, 0.25 µl Taq polymerase, and 13.75 µl ddH_2O were combined with 2 µl of digested sample. The following standard PCR reaction protocol was used on a GeneAmp 9700 Thermocycler: 95^{0}C (5 min); 95^{0}C, 56^{0}C, 72^{0}C (35 cycles at 1 min each); 72^{0}C (10 min), 40^{0}C (sample preservation temperature).

Reverse transcriptase-PCR: 5 µl of RT-PCR buffer, 1 µl forward primer, 1 µl reverse primer, 2 µl dNTPs, 1 µl Qiagen One Step RT-PCR enzyme mix, 13 µl ddH_2O were combined with 2 µl of digested sample. The following standard RT-PCR protocol was used on a GeneAmp 9700 Thermocycler: 50^{0}C (30 min); 95^{0}C (5 min); 95^{0}C, 56^{0}C, 72^{0}C (35 cycles at 1 minute each); 72^{0}C (10 min), 4^{0}C (sample preservation temperature).

Gel Electrophoresis: PCR and RT-PCR products were analyzed by fractionation in 1% agarose gel and visualized by GelRed DNA stain (Phenix Research Products, Candler, NC). Images were then captured using Fotodyne FOTO/Analyst Workstation (Fotodyne Incorporated, Hartland, WI). HERV-K expression was compared to 100 bp DNA Ladder (Promega, Madison, WI) with designated parameters: -, +/-, +, ++, +++, ++++.

Statistical analysis: The relationships among HERV-K, HIV-1 viral load, and CD4 cell counts were examined by pooled t-test and Pearson correlation coefficients using SAS 9.4 (SAS Institute Inc., Cary, NC).

Results and Discussion

After sample analysis, we detected HERV-K RNA positivity expression following RT-PCR in 35% (19 of 54) of plasma specimens from newly diagnosed, HIV-1 infected individuals (Table 1). Of note, patients in this study were previously untreated HIV-1 infected individuals. This is in contrast to previous reports where patients absence of antiretroviral treatment history was not considered[29].

Table 1: HERV-K RNA detection in 54 untreated HIV-1 infected individuals.

Sample	HERV-K Interpretation			HIV-1 Viral Load (copies/ml)	CD4 Count (cells/µl)
	PCR	RT-PCR	HERV-K RNA		
47	-	(++++)	Positive	209881	4
43	-	(++)	Positive	57133	36
18	-	(+/-)	Positive	11037	232
84	-	(++)	Positive	205104	247
55	-	(+)	Positive	31301	261
41	(+)	(++)	Positive	2522	271
52	-	(+++)	Positive	26172	294
64	-	(+)	Positive	34242	304
49	-	(+)	Positive	1788	331
57	-	(+)	Positive	1930	346
17	-	(+++)	Positive	1106	351
63	-	(+)	Positive	8356	351
46	-	(+++)	Positive	14505	440
50	(++)	(++++)	Positive	13237	454
19	-	(+)	Positive	9773	499
83	-	(++)	Positive	4282	508
20	-	(+)	Positive	10691	510
44	-	(++)	Positive	11375	621
51	(+)	(++++)	Positive	2126	650
72		-	Negative	41397	8
54		-	Negative	1696	42
24	(+/-)	-	Negative	8597	138
76		-	Negative	20352	142
32	(+/-)	-	Negative	2413	200
28	(+)	-	Negative	40885	224
71		-	Negative	14671	344
5	(+)	-	Negative	108	345
14	(+++)	(++)	Negative	26870	345
2	-	-	Negative	44075	349
85		-	Negative	18154	374
78		-	Negative	93	379
74		-	Negative	33935	386
15	(+)	-	Negative	33071	405
81		-	Negative	2330	426
75		-	Negative	339	429
33	(++++)	(+)	Negative	188623	434

22	(++++)	(+)	Negative	1451	487
21	-	-	Negative	< 75	494
82		-	Negative	1558	577
69		-	Negative	3537	578
61		-	Negative	< 75	583
29	(++)	-	Negative	1390	596
23	(++)	-	Negative	17680	618
70		-	Negative	86238	624
73		-	Negative	14188	643
26	(+/-)	-	Negative	1291	646
31	(+/-)	-	Negative	958	660
77		-	Negative	22468	671
79		-	Negative	1628	693
59		-	Negative	1495	710
27	(+/-)	-	Negative	< 75	747
40	(+)	(+/-)	Negative	1226	776
25	(+/-)	-	Negative	111	919
30	(+)	-	Negative	163	1039

RNA extraction was performed with the use of HIV-1 ViroSeq RNA preparation method (Abbott Molecular, Des Plaines, IL). DNase I digestion was performed utilizing the previously described protocol to remove contaminating cellular DNA. As stated in other reports, positive HERV-K expression is possible due to the presence of circulating cell-free RNA that is active within patient samples[27].

Several factors can contribute to variation in the expression of HERV-K RNA; such as, different HERV-K assay sensitivity, difference in HIV-1 infection progression, exogenous factors as mentioned earlier, variation in patient medical history and background, and sub-population polymorphisms of HERV-K sequences. The positivity rate can also be attributed to the DNase I protocol used to further reduce "contaminating" HERV-K genomic DNA in RNA extractions.

In HERV-K RNA positive subjects, mean CD4 cell count was 358 cells/µl, ranging from 4 to 650 cells/µl, and mean viral load was 34,736 copies/ml, ranging from 1,106 to 209,881 copies/ml (Table 1). We found no significant difference of mean HIV-1 viral load in HERV-K RNA positive and negative specimens ($p < 0.2995$, pooled t-test), consistent with our previous report[29]. Interestingly, mean CD4 cell count was significantly lower in HERV-K RNA positive than negative specimens ($p < 0.0335$, pooled t-test). Further studies are necessary to examine the co variation between HERV-K expression and CD4 cell count. If confirmed, it should warrant investigation of the biological significance and regulatory relationship between HERV-K and CD4 cell counts in HIV-1 infected individuals.

The pathogenic involvement of HERV-K remains unknown and warrants further study. HERV-K activation has been associated with malignancies, autoimmune disorders, and neuropathological conditions[9]. Methodology optimization is a key to assess HERV-K positive expression to properly understand its role in disease pathogenesis. Further research focusing on patient's stage of HIV-1 infection progression, age, race, and presence of comorbidities should be conducted in order to better understand whether HERV-K positivity has an impact in HIV-1 viral load, CD4 cell count, and other clinical phenotypes.

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