Lack of Correlation between HERV-K Expression and HIV-1 Viral Load in Plasma Specimens

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Abstract. HERV-K viral RNA has been reported in plasma specimens of HIV-1 infected individuals. Emerging data support the regulation and functional interaction between HERV-K and HIV-1, which warrant development of accurate HERV-K assays to evaluate HERV-K activation. In this study, we examined HERV-K RNA expression after careful removal of “contaminating” cellular DNA using DNase I. We found that DNase I digestion effectively reduced HERV-K RT-PCR positive signal. We also found that levels of HERV-K expression did not correlate with HIV-1 viral load. Our study is in agreement with the published studies on HERV-K activation in HIV-1 positive plasma specimens, and in addition, calls for careful removal of cellular DNA to accurately evaluate HERV-K RNA expression.

Key words: HERV-K expression, HIV-1 viral load, DNase I digestion

Introduction

HERVs are thought to be germline-integrated genetic remnants of exogenous retroviral infections and comprise approximately 8% of the human genome [1, 2]. HERVs can be classified into over 20 families based on tRNA specificity of the primer binding site used to initiate reverse transcription; thus, HERV-K would use lysine and HERV-W tryptophan if they were replicating viruses [3]. Through millions of years of evolution, HERVs have become indispensible parts of the human genome. For example, syncytin-1, encoded by the envelope (ENV) gene of HERV-W, mediates intercellular fusion of trophoblast cells to form syncytiotrophoblast and prevents maternal immune attack against the developing embryo, thereby facilitating implantation of the embryo [1, 2]. Similar to exogenous retroviruses such as human immunodeficiency viruses (HIV) and human T cell leukemia viruses (HTLV), a complete HERV sequence is composed of GAG, PRO, POL, and ENV genes flanked by two long terminal repeats (LTRs).

Although most HERVs are degenerated with disruptive open reading frames, a few proviruses have retained intact genes, and the corresponding proteins can thus be expressed [1, 4].

HERVs have been implicated in the etiology of cancer, chronic inflammation, and other diseases [2], and emerging data support the regulation and functional interaction between HERV-K activation and HIV-1 viral infection. For example, HERV-K viral RNA is detected in plasma specimens of HIV-1 infected individuals [5-7] and frequently precedes HIV-1 rebounds [8]; HERV-specific T cell responses are observed in HIV-1 infected individuals, and have been associated with control of HIV-1 viremia [9-11]. These studies support a link between HERV-K expression and HIV-1 infection, and warrant development of accurate HERV-K assays to further examine the regulatory and functional interactions between HERV-K and HIV-1.

In this study, we examined HERV-K transcript expression after careful removal of “contaminating” HERV-K DNA using DNase I. Although HERV-K transcripts were detected in some HIV-1 viral positive plasma specimens, levels of HERV-K expression did not correlate with HIV-1 viral load.
Materials and Methods

Specimens and RNA extraction. The study included a total of 41 plasma specimens from HIV-1 infected individuals submitted for routine HIV-1 clinical testing in the Molecular Diagnostics Laboratory at University of Texas Medical Branch (UTMB) between September and December of 2010. HIV-1 viral load was measured using Versant HIV-1 RNA 3.0 branched DNA (bDNA) (Siemens Healthcare Diagnostics, Washington, DC). Three groups of specimens were selected for this study: HIV-1 High, Low, and <75 that corresponded to HIV-1 viral loads of >1,000 copies/ml, 75-1,000 copies/ml, and <75 copies/ml, respectively. RNA was extracted using ViroSeq HIV-1 genotyping protocol according to manufacturer’s instructions (Abbott Molecular, Des Plaines, IL) [12, 13]. 500 µl plasma was extracted per sample with the final RNA diluents of 100 µl and 50 µl when HIV-1 viral loads were >15,000 copies/ml or ≤15,000 copies/ml, respectively. Samples were stored at -80°C before use. The study was approved by the UTMB Institutional Review Board (IRB).

DNase I digestion and HERV-K reverse transcriptase PCR. To remove residual cellular DNA, extracted RNA specimens were digested using DNase I Digestion kit (New England Biolabs, Ipswich, MA). Briefly, 20 µl of extracted RNA sample was treated with 2 units of RNase-free DNase I for 1 hr at 37°C in a total volume of 40 µl. DNase I was subsequently heat inactivated at 75°C for 10 min; 2 µl of DNase I-digested RNA mix was used as RT-PCR template. HERV-K POL PCR forward and reverse primer sequences were: 5’-CCA CTG TAG AGC CTC CTA AAC CC-3’ and 5’-GCT GGT ATA GTA AAG GCA AAT TTT TC-3’ [14]. PCR amplification parameters were as described [14, 15]. RT-PCR was performed using OneStep RT-PCR kit (Qiagen, Valencia, CA) on GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA). PCR products were analyzed by fractionation in 1% (w/v) agarose gel and visualized by GelRed DNA stain (Phenix Research Products, Candler, NC). Images were captured using Fotodyne FOTO/Analyst Workstation (Fotodyne Incorporated, Hartland, WI). HERV-K signal was assigned -, +, ++, ++++, ++++ based on comparison of band intensity with 100 bp DNA Ladder (Promega, Madison, WI).

Results and Discussions

We detected HERV-K RNA expression by RT-PCR in 66% (27 of 41) of plasma specimens from patients infected with HIV-1, which is in agreement with reported detection of HERV-K RNA genomes in HIV-1-infected plasma samples [5-7]. However, the HERV-K RNA positive rate is lower than the reported almost universal (95-100%) positivity in HIV-1 plasma samples [5, 6], whereas is closer to the 70-80% positive rates of HERV-K peptide and anti-HERV-K antibody in the plasma of HIV-1 infected individuals [16].
Several factors may contribute to the variation in the percentage of HERV-K in HIV-1-positive patients; for example, different HERV-K assay sensitivity, various stages of HIV-1 infection, and subpopulation polymorphisms of HERV-K sequences. It is also conceivable that the lower positive rate of our result may be attributed to the careful removal of cellular DNA using DNase I.

We performed RNA extraction using HIV-1 ViroSeq RNA preparation method (Abbott Molecular, Des Plaines, IL). According to the manufacturer, this RNA preparation kit extracts total nucleic acid including RNA plus DNA (Dr. Gavin Cloherty, personal communication) [5,6] used QIAamp Viral RNA Mini kit (Qiagen, Valencia, CA) in their studies. According to Qiagen, “The QIAamp Viral RNA Mini Kit is not designed to separate viral RNA from cellular DNA, and both will be purified in parallel if present in the sample” (page 9, QIAamp Viral RNA Mini Handbook, 04/2010). We used DNase I digestion to effectively remove contaminating cellular DNA in the RNA samples. As demonstrated in Figure 1, without DNase I digestion, HERV-K RT-PCR was uniformly positive in all five HIV-1 positive plasma specimens, whereas only samples #3 and #5 were positive after DNase I digestion. The likely source of the specific DNA for HERV-K in the plasma samples is cell-free genomic DNA, released from cells undergoing apoptosis in circulation or in solid organs [17]. Circulating cell-free DNA has been actively investigated for non-invasive markers of prenatal and cancer diagnosis, as well as other medical indications [17, 18].

We detected varying amounts of HERV-K RNA expression that did not correlate with HIV-1 viral load after cellular DNA was carefully removed from RNA templates (Table 1 listed representative cases). Notably, apart from HIV-1, multiple endogenous and exogenous factors may activate HERV-K. For example, cytomegalovirus and Epstein-Barr virus have been reported to transactivate HERV-K [19]. We have reported the regulation of HERV-K by MEK-ERK and p16-CDK4 pathways in melanoma cells [15]. HERV-K can also be regulated by other factors, for example, UV radiation, CpG methylation, and other transcription modulators [20-23]. Further studies are necessary to understand the roles and interactions of HIV-1 and other factors in modulating HERV-K expression during HIV-1 infection.

The pathogenic impact of HERV-K in HIV-1 infection is still an open question and deserves further study. HERV-K activation has also been associated widely with malignancies, autoimmune disorders, and neuropathological conditions [2]. Optimized methodology in the laboratory is essential to the accurate assessment of HERV-K activation. Further research and development of sensitive and specific HERV-K assays are required to expand the understanding of the role of HERV-K in HIV-1 infection and other pathological conditions.

<table>
<thead>
<tr>
<th>Sample</th>
<th>HERV-K RNA</th>
<th>HIV-1 Viral Load (copies/ml)</th>
</tr>
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<tbody>
<tr>
<td>N3</td>
<td>+</td>
<td>&lt;75</td>
</tr>
<tr>
<td>N5</td>
<td>+++</td>
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<td>N6</td>
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</tr>
</tbody>
</table>

Note: listed are HERV-K RT-PCR results (after DNase I treatment) of 4 cases in three groups of HIV-1 viral load (<75 copies/ml, 75-1,000 copies/ml, and >1,000 copies/ml).

Table 1. No correlation between HERV-K transcript and HIV-1 viral load in plasma samples
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References