

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, researchers examined HERV-K RNA expression after careful removal of contaminating cellular DNA using DNase I. Researchers found that DNase I digestion effectively reduced HERV-K RT-PCR positive signal. Researchers also found that levels of HERV-K expression did not correlate with HIV-1 viral load. The study is in agreement with the published studies on HERV-K activation in HIV-1 viral 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, specimens infected individuals, DNA, USA

INTRODUCTION

The human endogenous retrovirus is thought to be germline-integrated genetic remnants of exogenous retroviral infections and comprise approximately 8% of the human genome (De Parseval and Heidmann, 2005; Kurth and Bannert, 2009). HERVs can be classified into >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 (Blomberg *et al.*, 2009). Through millions of years of evolution, HERVs have become indispensable 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 as well as preventing maternal immune attack against the developing embryo, thereby facilitating implantation of the embryo (De Parseval and Heidmann, 2005; Kurth and Bannert, 2009).

Similar to exogenous retroviruses such as Human Immunodeficiency Virus (HIV) and Human T cell Leukemia Virus (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 (Ahn and Kim, 2009; De Parseval and Heidmann, 2005).

HERVs have been implicated in the etiology of cancer, chronic inflammation and other diseases (Kurth and Bannert, 2009) 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 (Contreras-Galindo *et al.*, 2006a, b; Laderoute *et al.*, 2007) and frequently precedes HIV-1 rebounds (Contreras-Galindo *et al.*, 2007) and HERV-specific T cell responses are observed in HIV-1 infected individuals and have been associated with control of HIV-1 viremia (Gupta *et al.*, 2011; Sharp *et al.*, 2011; Tandon *et al.*, 2011). 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, researchers examined HERV-K transcript expression after carefully 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 were corresponding to HIV-1 viral load of >1,000, 75-1,000 and <75 copies mL⁻¹, respectively. RNA was extracted using ViroSeq HIV-1 genotyping protocol according to manufacture's instructions (Abbott Molecular, Des Plaines, IL) (Xu *et al.*, 2010; Yang *et al.*, 2008). About 500 µL plasma was extracted per sample with the final RNA diluents of 100 and 50 uL when HIV-1 viral loads were >15,000 or ≤15,000 copies mL⁻¹, 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 treated with DNase I using DNase I Digestion kit (New England Biolabs, Ipswich, MA). Briefly, 20 uL of extracted RNA sample was treated with 2 units of RNase-free DNase I for 1 h at 37°C in a total volume of 40 uL. DNase I was subsequently heat inactivated at 75°C for 10 min. About 2 uL 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' (Serafino *et al.*, 2009).

PCR amplification parameters were as described by Li *et al.* (2010) and Serafino *et al.* (2009). RT-PCR was performed using OneStep RT-PCR kit (Qiagen, Valencia, CA) on GeneAmp PCR System 9700 (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 DISCUSSION

Researchers 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-

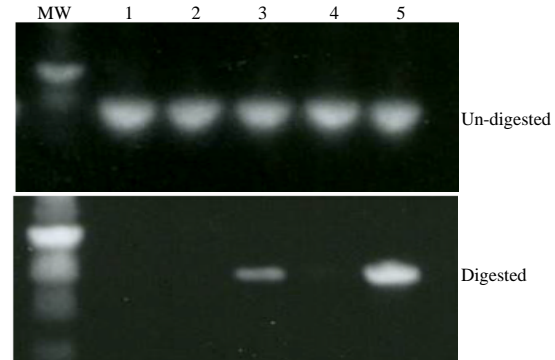


Fig. 1: DNase I digestion affects HERV-K RT-PCR results. Viral RNA was extracted from five HIV-1 positive specimens using HIV-1 ViroSeq kit. Without DNase I digestion, HERV-K RT-PCR was uniformly positive in all the 5 cases whereas only samples #3 and 5 remained positive after DNase I digestion. MW: Molecular Weight marker

infected plasma samples (Contreras-Galindo *et al.*, 2006a, b; Laderoute *et al.*, 2007). However, the HERV-K RNA positive rate is lower than the reported almost universal (95-100%) positivity in HIV-1 plasma samples (Contreras-Galindo *et al.*, 2006a, b) 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 (Lower *et al.*, 1996; Laderoute *et al.*, 2007).

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 sub-population polymorphisms of HERV-K sequences. It is also conceivable that the lower positive rate of the result may be attributed to the careful removal of cellular DNA using DNase I.

Researchers performed RNA extraction using HIV-1 ViroSeq RNA Preparation Method (Abbott Molecular, Des Plaines, IL). According to Dr. Gavin Cloherty (Scientific affairs, Abbott Molecular, personal communications) this RNA preparation method actually extracts total nucleic acid (i.e., RNA plus DNA). Contreras-Galindo *et al.* (2006a, b) 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). Researchers used DNase I digestion to effectively remove contaminating cellular DNA in the RNA samples. As shown in Fig. 1 without DNase I digestion, HERV-K RT-PCR was uniformly positive in all the five HIV-1 positive

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

Samples	HERV-K RNA	HIV-1 viral load (copies mL ⁻¹)
N3	+	<75
N5	++++	<75
N6	+++	<75
N7	+	<75
L2	+	221
L4	+	81
L10	++++	754
L11	++++	112
H2	++	199, 100
H5	+++	33, 620
H7	++	421, 580
H9	+	53, 575

HERV-K RT-PCR results (after DNase I treatment) of 4 cases in three groups of HIV-1 viral load (<75, 75-1,000 and >1,000 copies mL⁻¹)

plasma whereas only samples #3 and 5 were positive after DNase I digestion. Researchers 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).

CONCLUSION

Notably, apart from HIV-1, multiple endogenous and exogenous factors may activate HERV-K. For example, cytomegalovirus and the Epstein-Barr virus have been reported to transactivate HERV-K (Ryan, 2004). Researchers have reported the regulation of HERV-K by MEK-ERK and p16-CDK4 pathways in melanoma cells (Li *et al.*, 2010). HERV-K can also be regulated by other factors, for example, UV radiation, CpG methylation and other transcription modulators (Fuchs *et al.*, 2011; Reiche *et al.*, 2010; Schanab *et al.*, 2011; Stengel *et al.*, 2010).

RECOMMENDATIONS

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 (Ryan, 2004; Kurth and Bannert, 2009). 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.

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