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Poster presentation

Isolation and purification of HIV-1 LTR circles from PBMCs for the analysis of episomal env sequences

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Background

The source of the HIV-1 virions that emerge during suppressive HAART can be identified by a phylogenetic analysis that compares the virions present in plasma with those in cell reservoirs. However, the difficulty found in the characterization of very low plasma RNA could be addressed by the analyses of LTR circles.

Materials and methods

We developed a protocol using an ATP dependent plasmid safe DNAse that selectively digests linear DNA to purify LTR circles from chromosomal DNA. The β-actin gene was amplified to detect contamination from linear DNA. A phylogenetic analysis of the env HIV regions of the LTR circles, PBMCs and free virus in plasma from three patients undergoing HAART within 27 to 852 viral copies/ml viral load range was undertaken to determine if LTR episomes are labile in vivo and suitable for analysis.

Results

The phylogenetic trees and Kimura 2-parameter mean distances show a higher homogeneity within the plasma RNA and LTR episomal DNA groups of each patient. In addition, Wilcoxon's signed ranks tests ($p < 0.05$) from 101 clones, demonstrated that the nucleotide distances between the episomes vs. plasma and episomes vs. PBMCs were significantly different for the three patients.

Conclusion

These findings support our hypothesis showing that episomal DNA is apparently reflecting the viral quasispecies present in plasma RNA, especially in the patient with the lowest viral load. This method is a rapid and economic alternative to selectively amplify HIV-1 regions from LTR circles.