Drug Resistance in Clade C HIV Reverse Transcriptase

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Abstract

The human immunodeficiency virus (HIV) is the etiologic agent of acquired immunodeficiency syndrome (AIDS), a significant global cause of human mortality. HIV reverse transcriptase (RT) is a virally encoded RNA-based DNA polymerase that is necessary for HIV viral replication within the body. As such, HIV RT is a popular mechanistic target for anti-HIV drugs. HIV comprises several clades (i.e. subgroups) of viruses, of which clade C is by far the most prevalent and currently the least well characterized/understood. The purpose of the present study is to characterize the drug susceptibility profile of selected clade C HIV mutant RTs for application in continued anti-HIV drug treatments. This purpose is being achieved through the following steps: (1) Point-directed PCR mutagenesis of clade C HIV RT cDNA (cDNA obtained from collaborator). (2) Restriction and ligation of amplified sequence into vectors designed for over-expression, followed by transformation into E. Coli cells. (3) Over-expression of mutant clade C RT followed by collection and purification. (4) Biochemical enzymatic analysis of collected mutant clade C RT in the presence and absence of various selected anti-HIV compounds, as well as crystalization-based characterization of said interaction. We anticipate that our findings will elicit important information regarding clade C RT drug susceptibility and resistance. We anticipate that this information will help direct the future of treatment options for individuals suffering from clade C HIV infections.

Introduction

HIV reverse transcriptase (HIV RT) is an HIV-encoded enzyme that mediates reverse transcription of the HIV ssRNA genome into dsDNA. This is a required step in enabling integration of the HIV genome into the genome of infected host cells and is thus key to the HIV replication cycle. HIV RT operates as a heterodimer of p66 and p51 subunits, the former of which contains catalytic sites for two separate enzymatic functions needed for reverse transcription: (1) both RNA- and DNA-based DNA polymerization and (2) RNase H cleavage (see diagram for details). Due to its importance in HIV replication, HIV RT is a popular target for anti-HIV drugs. Such drugs fall under two broad categories, (1) nucleoside reverse transcriptase inhibitors (NRTIs) and (2) non-nucleoside reverse transcriptase inhibitors (NNRTIs). Several clades of HIV are known to exist, each featuring variations in their respective RT proteins (RT variation exists both within and between clades). Clade C is the most prevalent HIV clade world-wide, as well as the least well characterized. The purpose of this ongoing project is to characterize the drug resistance profile of clade C HIV RT mutants for application in future HIV treatment regimens.

Process of reverse transcription of the HIV genome.

Sarafianos S G et al. EMBO J. 2001;20:1449-1461

HIV RT Inhibition

Approximately half of anti-AIDS drugs target the polymerase activity of HIV RT. Such drugs are separated into two classes: nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs). NRTIs are 3'-OH modified nucleosides which, following activation into nucleotides by host kinases, are incorporated by HIV RT into the nascent viral DNA, resulting in chain termination. NNRTIs bind to HIV RT and disrupt its conformation, inhibiting polymerization. HIV resistance to NRTIs and NNRTIs is common and evolves due to incomplete HIV suppression, high HIV replication rates and error prone DNA synthesis by HIV RT. Currently, no approved drugs target the RNase H activity of HIV RT.

Global Distribution of HIV Clades


Structure of HIV Reverse Transcriptase


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