Drug Resistance in Clade C HIV Reverse Transcriptase

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Abstract

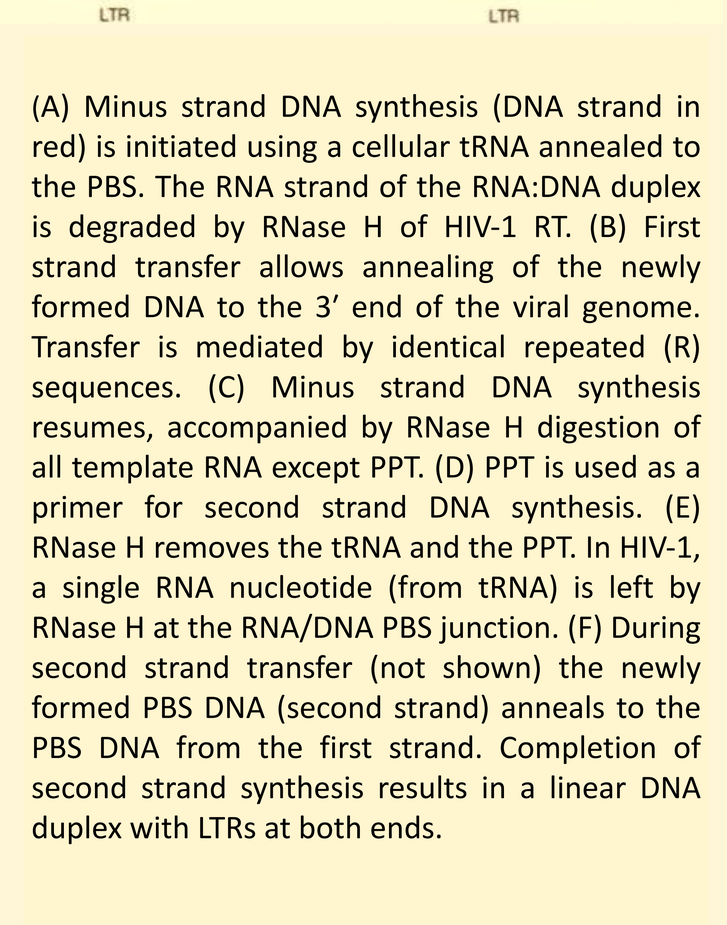
The human immunodeficiency virus (HIV) is the etiologic agent of acquired immunodeficiency syndrome (AIDS), a significant global cause of human mortality. HIV reverse transcriptase (RT) is a virally encoded RNA-based DNA polymerase that is necessary for HIV viral replication within the body. As such, 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 crystallization-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 it's 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 between and within 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.

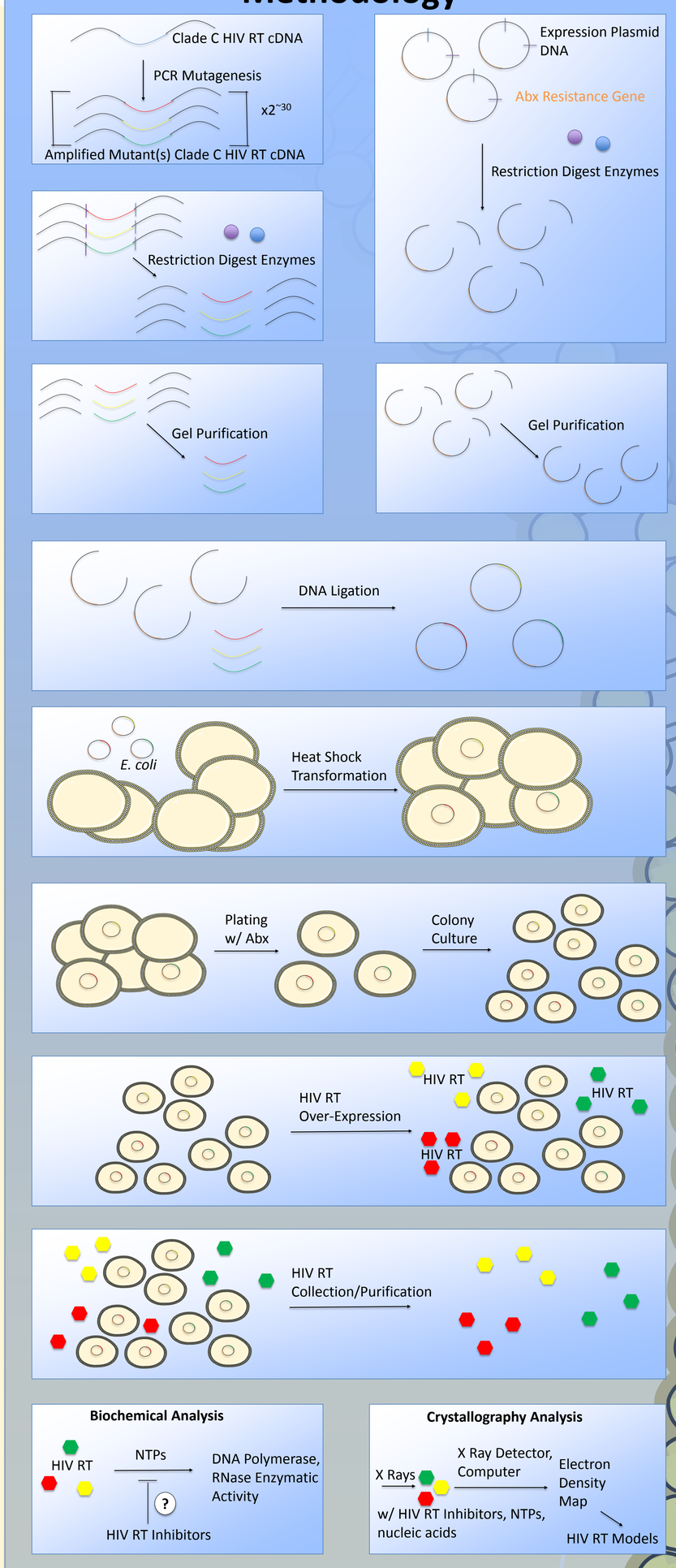
Process of reverse transcription of the HIV genome.

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



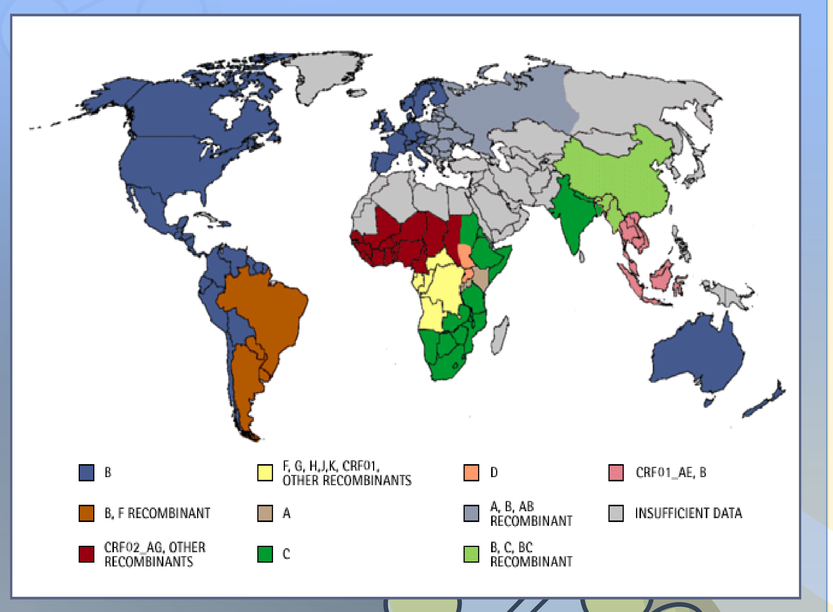
(A) Minus strand DNA synthesis (DNA strand in red) is initiated using a cellular tRNA annealed to the PBS. The RNA strand of the RNA:DNA duplex is degraded by RNase H of HIV-1 RT. (B) First strand transfer allows annealing of the newly formed DNA to the 3′ end of the viral genome. Transfer is mediated by identical repeated (R) sequences. (C) Minus strand DNA synthesis resumes, accompanied by RNase H digestion of all template RNA except PPT. (D) PPT is used as a primer for second strand DNA synthesis. (E) RNase H removes the tRNA and the PPT. In HIV-1, a single RNA nucleotide (from tRNA) is left by RNase H at the RNA/DNA PBS junction. (F) During second strand transfer (not shown) the newly formed PBS DNA (second strand) anneals to the PBS DNA from the first strand. Completion of second strand synthesis results in a linear DNA duplex with LTRs at both ends.

Methodology



Global Distribution of HIV Clades

http://www.pbs.org/wgbh/pages/frontline/aids/atlas/clade.html

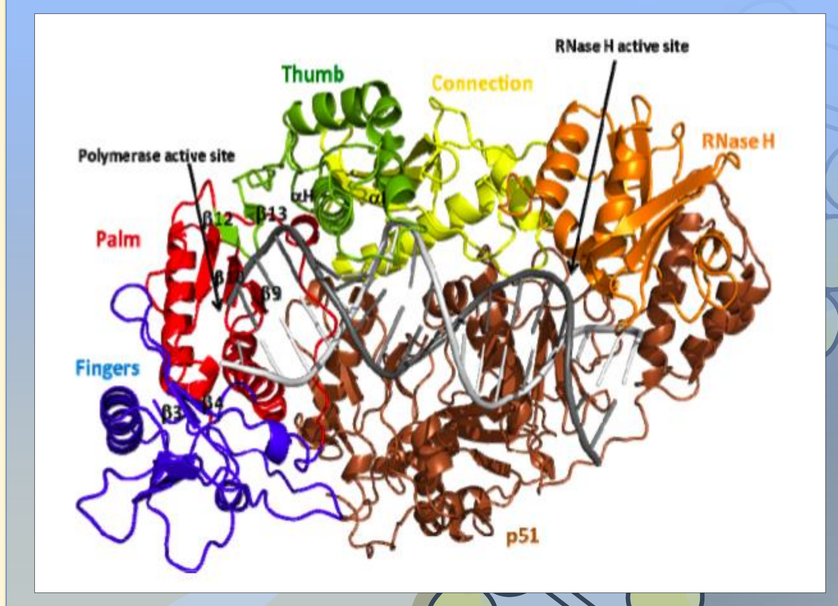


HIV RT Inhibition

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.

Structure of HIV Reverse Transcriptase

Singh K et al. Viruses. 2010; 2:606-638



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