Human endogenous retrovirus-K (HERV-K) reverse transcriptase (RT) displays a lamivudine resistant genotype and phenotype

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Background

Human endogenous retroviruses (HERVs) comprise nearly 8% of the human genome and are derived from ancient integrations of retroviruses into the germline. A significant number of functional proteins are produced by HERV-encoded genes including reverse transcriptases (RTs) that display DNA synthesis activity on DNA and RNA templates. There is accumulating evidence supporting disease association with endogenous retroviruses; hence, anti-retroviral therapy could be explored for the management of these conditions and nucleoside analogue RT inhibitors (NRTIs) have been considered in this regard. To further assess the feasibility of this approach, we compared the inhibitory effects of several NRTIs against purified human immunodeficiency virus type 1 (HIV-1) RT and HERV-K RT.

Backbone superimposition highlights remarkable similarity of HERV-K RT with HIV-1 RT

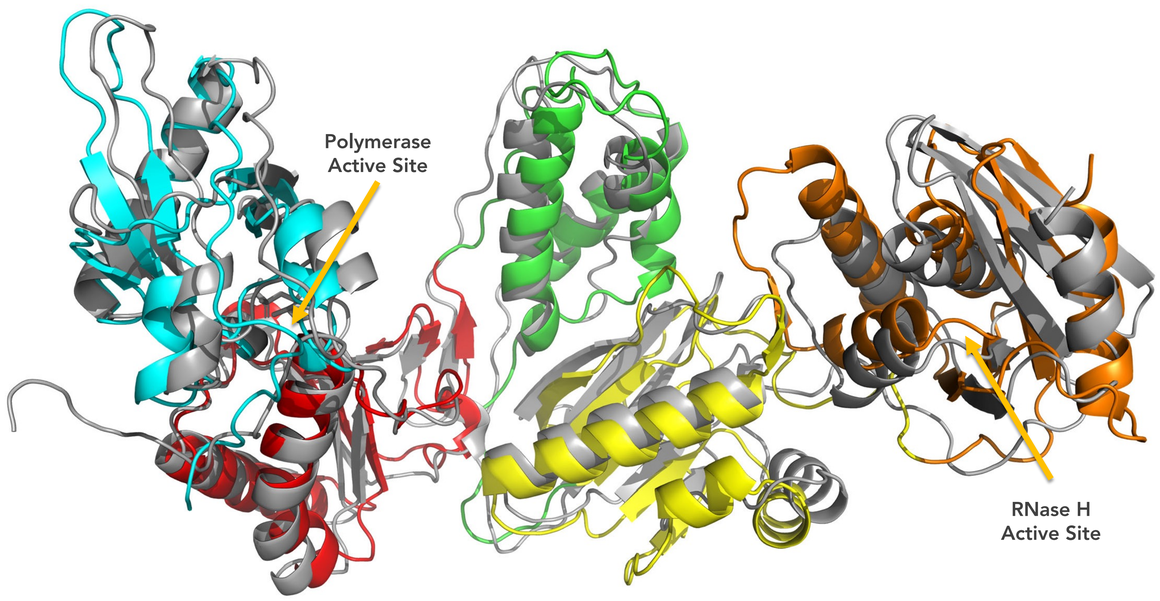

Figure 1.
Superimposition of HIV-1 p66 (5TXL.PDB) [in gray] upon HERV-K RT molA with an RMSD of 2.67 Å for 381 **α** carbon atoms. The distance from the polymerase active site to the RNase H active site is nearly identical between the two enzymes. The polymerase active site is on the left and the RNase H active site on the right.

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Results

We successfully expressed HERV-K RT in insect cells and purified the active enzyme to near homogeneity. The crystal structure of HERV-K RT has been recently solved and revealed striking similarities with HIV-1 RT (Fig. 1). HERV-K RT is an asymmetric homodimer with a subunit arrangement very similar to HIV-1 RT. However, the conserved YM(184)DD motif in HIV-1 RT is equivalent to YI(195)DD in HERV-K RT. M184V/I mutations in HIV-1 RT confer high-level resistance to lamivudine [3TC] (Sarafianos-1999) and low-level of resistance to abacavir, while susceptibility to zidovudine is slightly increased in this context. Here we demonstrate that the triphosphate version of lamivudine (3TC-TP) inhibits HERV-K RT less efficiently than HIV-1 RT and the I195M mutation in HERV-K RT reverses the resistant phenotype (Fig. 2, left panel). These effects are less pronounced with the triphosphate versions of abacavir and zidovudine (Fig. 2, middle and right panels). Using the X-ray structure of HERV-K RT and various HIV-1 RT structures we can rationalize the biochemical results by observing a steric clash between 3TC and isoleucine (YIDD) of loop (Fig. 3).

Sarafianos, S. G., et al. (1999). "Lamivudine (3TC) resistance in HIV-1 reverse transcriptase involves steric hindrance with beta-branched amino acids." Proc Natl Acad Sci U S A 96(18): 10027-10032.

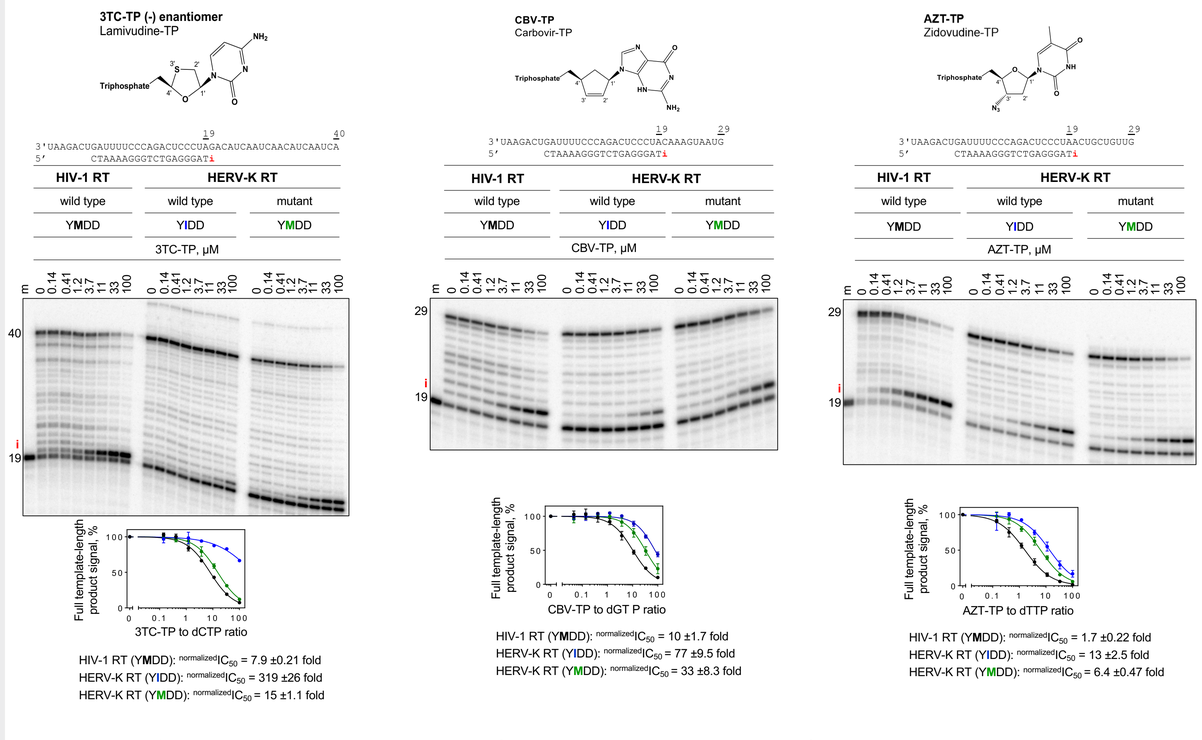

Figure 2. Nucleotide analog-dependent inhibition of the DNA polymerization activity of HIV-1 and HERV-K RTs

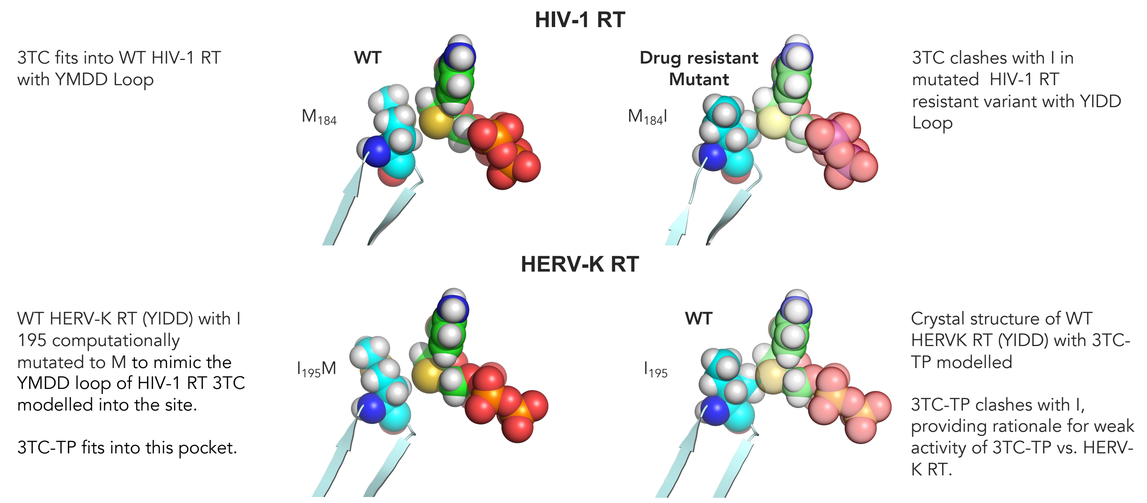

Figure 3.

Top left, Position of 3TC with respect to YMDD loop (cyan) in the crystal structure of wild type HIV-1 RT with 3TC (PDB 6ujy). M184 and 3TC-TP are shown as space-filling models.

Top right, YIDD loop (cyan) in the crystal structure of the M184I mutant of HIV-1 RT (PDB 1j5o). The 3TC-TP position (modelled) from the wild-type structure is shown as transparent spheres.

Bottom left, YMDD loop and docked 3TC-TP in the crystal structure of HERV-K RT with I195 mutated to Met. The I195M mutant was modeled in Maestro (Schrödinger) and 3TC was docked using Glide (Schrödinger).

Bottom right, YIDD loop in the crystal structure of wild-type HERV-K RT with the modelled inhibitor 3TC-TP position from the I195M model shown as transparent spheres.

Conclusion

The results of this study demonstrate that resistance to 3TC can be ascribed to the same genotype in HIV-1 and HERV-K.

Acknowledgments

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