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N348I IN HIV-1 REVERSE TRANSCRIPTASE CAN COUNTERACT THE NEVIRAPINE MEDIATED BIAS TOWARD RNASE H CLEAVAGE DURING PLUS-STRAND INITIATION

Mia J. Biondi, Greg L. Beilhartz, Suzanne McCormick, and Matthias Götte

Dept. of Microbiology and Immunology, McGill University, Montreal, Quebec H3A 2B4

C-terminal mutations in HIV-1 reverse transcriptase (RT) have been associated with decreased susceptibility to AZT. Several groups have provided evidence to show that certain mutations can affect the balance between polymerase and ribonuclease H (RNase H) activities of HIV-1 RT. We have recently demonstrated that the connection domain mutation N348I in HIV-1 RT can cause selective dissociation from RNase H-competent complexes, while the interaction in the context of the polymerase-competent complex remains largely unaffected. In addition, N348I has been associated with decreased susceptibility to the non-nucleoside reverse transcriptase inhibitor (NNRTI), nevirapine (NVP); however, the underlying mechanism remains elusive. To address this problem, we consider recent findings suggesting that NNRTIs can act potently at the level of initiation of plus-strand DNA synthesis. Specifically, independent lines of evidence suggest that both NVP and efavirenz (EFV) are able to change the orientation of RT on its nucleic acid substrate. Under these conditions, the enzyme favors the RNase H-competent orientation, which, in turn, diminishes DNA synthesis.

Here, we demonstrate that N348I can counteract the NNRTI-mediated effects, i.e. the mutant enzyme is severely compromised with respect to the RNase H-mediated primer removal reaction. This reversal is not as pronounced with classic NNRTI-associated mutations Y181C and K103N. Using a substrate which mimics the initiation of plus-strand DNA synthesis we demonstrate that the removal of the RNA primer follows the order WT > Y181C > K103N > N348I. Interestingly, only EFV is able to re-establish RNase H activity to near wild type levels in the presence of N348I. Consequently we are able to conclude that this mutation reverses the increase in RNase H activity associated with the inhibitor solely in the presence of NVP. The properties of EFV as a tight-binding inhibitor appear to be dominant and restrict the influence of the mutation. Overall, the data show that N348I can partially neutralize the NNRTI-mediated bias toward RT binding in an RNase H-competent complex, which provides a novel mechanism for resistance to NVP. Our findings are consistent with the notion that NNRTIs can exert major inhibitory effects specifically during the initiation of plus-strand DNA.