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MUTATIONS IN HIV-1 RNASE H DOMAIN CONFER HIGH-LEVEL RESISTANCE TO NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS AND PROVIDE NOVEL INSIGHTS INTO THE MECHANISM OF NUCLEOTIDE EXCISION-MEDIATED DRUG RESISTANCE

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GN Nikolenko¹, S Palmer¹, F Maldarelli¹, JW Mellors², JM Coffin¹ and VK Pathak¹

¹HIV Drug Resistance Program, NCI-Frederick, Frederick, Md.; and ²University of Pittsburgh, Pittsburgh, Pa., USA

BACKGROUND: Understanding the mechanisms of drug resistance is critical for developing more effective antiretroviral agents and successful management of therapy. We recently observed that 3'-azido-3'- deoxythymidine (AZT) increased the frequency of reverse transcriptase (RT) template switching and recombination. Based on this observation and our previously described dynamic copy-choice mechanism for retroviral recombination, we now propose a novel mechanism for nucleoside reverse transcriptase inhibitor (NRTI)-mediated abrogation of HIV-1 replication. We postulate an equilibrium between NRTI incorporation, excision of NRTI, resumption of DNA synthesis, and RNase H activity; degradation of the RNA template by RNase H before resumption of DNA synthesis, leads to dissociation of the template and primer strands, termination of reverse transcription and abrogation of HIV-1 replication. In these studies, we tested a prediction of our model that mutations reducing the rate of RNA degradation will confer NRTI resistance by increasing the time period for excision of incorporated NRTIs from terminated primers.

METHODS: We determined the sensitivity to AZT, 2,3-didehydro-2,3-dideoxythymidine (d4T), 2',3'- dideoxyinosine (ddI), 2',3'-dideoxy-3'-thiacytidine (3TC), and efavirenz of wild-type RT, RT containing a cluster of thymidine analogue-associated mutations (TAMs) and two RNase H mutations (H539N and D549N). The sensitivities to antiretroviral agents were determined using a single cycle of infection and the firefly luciferase reporter gene.

RESULTS: The D549N mutation increased resistance to AZT and d4T 10- and 2.6-fold, respectively, similar to the increase in resistance observed by the TAMs. Even more

dramatically, the H539N substitution increased resistance to AZT and d4T by 180- and 10- fold, respectively, which was nine- and four-fold higher relative to the TAMs. The RNase H mutations resulted in a modest increase in resistance to ddI but did not alter the sensitivity to 3TC or efavirenz. Interestingly, one clone isolated from a patient (Genbank No. 13095143) possessed the D549N substitution in association with other TAMs, suggesting that it may have contributed to drug resistance.

CONCLUSIONS: These results support our proposed mechanism for NRTI-mediated abrogation of HIV-1 replication and indicate that mutations in that RNase H domain can confer a high level of resistance to AZT and d4T. Our results strongly suggest that mutations in RNase H could be selected during antiviral therapy and significantly contribute to drug resistance either alone or in combination with NRTI resistance mutations in RT.

PRESENTING AUTHOR: VK Pathak

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