The involvement of HIV-1 RNAse H in resistance to nucleoside analogues

Bénédicte Roquebert1,2 and Anne-Geneviève Marcelin3,4*

1AP-HP, Groupe Hospitalier Bichat-Claude Bernard, Service de Virologie, Paris F-75018, France; 2Université Denis Diderot-Paris 7, Paris, France; 3AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Service de Virologie, Paris F-75013, France; 4UPMC Univ Paris 06, EA 2387, Paris F-75005, France

Some recent studies have underlined the role of the entire reverse transcriptase (RT) and in particular its carboxy-terminal domain from amino acid 427 to amino acid 560 [the ribonuclease H (RNAse H) domain] in resistance to nucleoside RT inhibitors (NRTIs). RNAse H is implicated in catalysing the degradation of the RNA strand during conversion of the viral genome into double-stranded DNA. It has been shown, by site-directed mutagenesis, that amino acid substitutions in the RNAse H domain could affect the binding enzyme/substrate, resulting in a decrease in the RNAse H activity. For example, mutations at positions 478, 539 and 549 led to a slowing down in the degradation of the RNA strand. In vitro, the mutations H539N and D549N decreased the frequency of RT template-switching and, thereby, increased the time for excision of incorporated NRTIs, and thus enhanced NRTI resistance. It has been confirmed in vivo that mutations at position 558 were statistically associated with a number of thymidine analogue mutations in a study including 144 HIV-1 patients, suggesting that it could be an accessory mutation that could reinforce NRTI resistance. This article highlights evidence that mutations in RNAse H can enhance NRTI resistance, suggesting that phenotypic and genotypic analyses of clinical samples including the entire HIV-1 RT and in particular the RNAse H domain are now required to better characterize the in vivo role of the RNAse H mutations on susceptibility and response to NRTIs in HIV-1-infected patients.

Keywords: reverse transcriptase, NRTIs, susceptibility

Introduction

The replication of human immunodeficiency virus type 1 (HIV-1) within permissive cells is mainly dependent on its reverse transcriptase (RT) enzyme. This enzyme catalyses the conversion of the viral single-stranded RNA genome into double-stranded DNA. Two main domains in the RT molecule are necessary for this process: a DNA polymerase domain and a ribonuclease H (RNAse H) domain.1 The latter domain is required at several stages during viral replication. Although the RT is complexed with the RNA primer, its RNA-dependent DNA-polymerase activity synthesizes the DNA minus-strand. Simultaneously, the RNAse H domain degrades the RNA template of the DNA/RNA hybrid between the 13th and 20th nucleotides behind the polymerase catalytic centre. Furthermore, RNAse H removes the primers, the tRNA and the polypurine tract (ppt) RNA.2 These two domains are located in the p66 subunit of the HIV-1 RT heterodimer (composed of the p66 and p51 subunits) and are linked together by the connection domain.

The RNAse H domain occupies the carboxy-terminal part of the p66 subunit, from amino acid Tyr-427 to Leu-560.3 Since the first decade of the AIDS pandemic, drugs to prevent HIV-1 replication were commercialized and the HIV-1 RT was the first target of the drugs called nucleoside RT inhibitors (NRTIs). NRTIs lead to the termination of the DNA synthesis when they are incorporated into the DNA chain instead of the natural dNTP substrate. Two resistance mechanisms to these NRTIs have been described, one involving NRTI excision4 and another NRTI discrimination.5 They are due to mutations in the DNA polymerase domain of RT, such as the thymidine analogue mutations (TAMs) that increase the rate of nucleotide excision conferring resistance to zidovudine. However, it has been shown by mutagenesis studies that polymerase and RNAse H activities, carried by the same enzyme, are highly interdependent6 and that their active sites share a single substrate-binding cleft.7 From these data, some authors have identified and highlighted the role of RNAse H in generating and/or increasing the

*Correspondence address. Service de Virologie, Hôpital Pitié-Salpêtrière, 83 Boulevard de l’Hôpital, 75013 Paris, France. Tel: +33-142177401; Fax: +33-142177411; E-mail: anne-genevieve.marcelin@psl.aphp.fr
RNAse H mutations affect RT activity

As it was shown about 15 years ago, the RNAse H domain belongs to the carboxy-terminus of the HIV-1 RT p66 subunit (66 kDa and 560 amino acids), from amino acid Tyr-427 to Leu-560. By crystallographic analysis, two major sites of the RNAse H domain have been identified. They are the RNAse H active site composed of four amino acids (Asp-443, Glu-478, Asp-498 and Asp-549) and the RNAse H primer grip, a network of amino acids (Thr-473, Ile-505, Lys-476, Gln-475, Tyr-501, Arg-448, Asn-474 and Gln-500) that contacts the DNA primer strand and/or the RNA template near the RNAse H active site.8 To investigate whether RNAse H mutations could affect RT activity, in vitro and in vivo studies were conducted and effects of amino acid substitutions were analysed. They showed that some of the mutations in these sites could affect the specificity of the RNAse H cleavages and decrease the level of RNAse H activity as well as RT activity. In 2002, by site-directed mutagenesis, Julias et al.9 described RNAse H mutation effects. Their data suggested that, in the vicinity of the RNAse H active site, the DNA contacts might be more important for infectivity than the RNA contacts. However, mutations in the RNAse H domain that contact the DNA or RNA strand could perturb the interactions of RT with its nucleic acid substrate, which affect both RNAse H and polymerase activity and impact replication. For example, the mutation T473A abolished HIV-1 replication; mutations N474A, K476A and Q500A reduced the virus titre, whereas mutations in positions 448 and 505 had no effect on virus titre. In vitro, two mutations playing an important role in primer–template binding, E478Q and D549N,10 and a third mutation, H539,11 had remarkable effects on viral replication by reducing the level of RNAse H activity.12 All these data suggest that mutations in the RNAse H domain affect RT activity by significantly perturbing the enzyme/substrate interactions on the RNAse H side as well as on the polymerase side.

RNAse H mutations enhance NRTI resistance in vivo

During the HIV-1 reverse transcription, two steps including a switch from an RNA strand to the other template are required in order to perform the replication; they are called template-switching events.13 A dynamic copy-choice model was proposed by Svarovskaia et al.,14 to explain the interdependence of the polymerase and the RNAse H activities during HIV-1 replication. They showed that an equilibrium exists between the rate of DNA polymerization and the rate of RNA degradation, an equilibrium that determines the frequency of RT template-switching. This steady state can be disturbed by affecting DNA polymerization, as well as RNA degradation. Some mutations in the RT increase the frequency of RT template-switching, whereas others situated in the RNAse H domain decrease it. Mutations likely to reduce the rate of DNA polymerization and then increase the frequency of RT template-switching have been identified in the RT polymerase domain.15 These mutations (K65R, L74V, Q151N and M184I) are known as NRTI resistance mutations.

Concerning the mutations in the RNAse H domain and their effects on the frequency of template-switching, H539N and D549N decrease the frequency of RT template-switching by 2-fold.15 Therefore, as mutations in the RNAse H domain decrease its activity and consequently the HIV-1 RT template-switching, they increase NRTI resistance by increasing the time for excision of the incorporated NRTI monophosphate from terminated primers.12 In conclusion, these mutations increase zidovudine resistance in vitro only during RNA-dependent DNA synthesis and not during DNA-dependent DNA synthesis.

However, recently, it has been shown that the mutation Q509L in the RNAse H domain was selected by zidovudine in addition to TAMs. The mutation Q509L alone did not alter zidovudine susceptibility, but in combination with TAMs, resistance to zidovudine was significantly increased, up to 190-fold compared with 20-fold for TAMs. This mutation is close to the RNAse H primer grip, which contacts the DNA primer strand.16 Thus, it reinforces the idea that mutations in the RNAse H domain perturb RT activity by affecting primer–template binding.

Leading article
that confers dual-class resistance. Its emergence, which can happen early on during therapy, may significantly impact on a patient’s response to antiretroviral therapies containing zidovudine and nevirapine. Another work suggested that another mutation (i.e. A400T) within the connection domain clusters with known RT inhibitor resistance mutations may be associated with nevirapine exposure. Thus, mutations beyond the codons routinely examined in drug resistance testing may be relevant for assessing drug resistance in clinical practice.

Conclusions

The HIV-1 RT, essential during viral replication, has been subjected to antiretroviral therapy pressure for a long time. Its structure and functions have been identified and it has been shown that its two major domains (the DNA polymerase domain and the RNase H domain) are critical during replication and dependent from each other. Studies about the implication of the C-terminal RNase H domain in RTI resistance were reviewed and it seems that mutations in the RNase H domain could impact NRTI sensitivity in association with NRTI mutations and it seems that mutations in the RNAse H domain could impact NRTI sensitivity in association with NRTI mutations already described in the polymerase domain. Whereas in vitro and site-directed mutagenesis data are consistent and argue in favour of a role of the RNase H domain in NRTI resistance, the small number of studies and sequences analysed in vivo limit their significance. However, taken together, all these results are convergent and raise the need for a greater understanding of the molecular mechanisms implicating RNase H in NRTI resistance. Indeed, a better understanding of mechanisms driving evolution of drug resistance is critical for developing more effective antiretroviral agents and successfully managing antiretroviral therapy for individual patients. In addition, phenotypic and genotypic analyses of clinical samples including the entire HIV-1 RT and in particular the RNase H domain are now required to better characterize the in vivo role of the RNase H mutations on susceptibility and response to NRTIs in HIV-1-infected patients.

Transparency declarations

None to declare. We have not accepted any material support or reimbursement for preparing this leading article.

References


