Early selection of resistance-associated mutations in HIV-1 RT C-terminal domains across different subtypes: role of the genetic barrier to resistance

Cláudia P. Muniz¹, Marcelo A. Soares¹,² and André F. Santos¹*

¹Departamento de Genética, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; ²Programa de Genética, Instituto Nacional de Câncer, Rio de Janeiro, Brazil

*Corresponding author. CCS – Bloco A – sala A2-120, Cidade Universitária, Ilha do Fundão, 21949-570 – Rio de Janeiro, RJ, Brazil. Tel: +55-21-2562-6383; Fax: +55-21-2562-6396; E-mail: andre20@globo.com

Received 20 March 2014; returned 15 April 2014; revised 14 May 2014; accepted 20 May 2014

Objectives: Interpretation of drug resistance mutation (DRM) has been based solely on HIV-1 subtype B. Reverse transcriptase (RT) C-terminal domains have been disregarded in resistance interpretation, as their clinical relevance is still controversial. We determined the emergence of DRM in RT C-terminal domains of different HIV-1 subtypes, the genetic barrier for the acquisition of these DRM and their temporal appearance with ‘classical’ RT inhibitor (RTI) mutations.

Methods: HIV-1 RT sequences were obtained from information from 6087 treatment-naive and 3795 RTI-treated patients deposited in the Stanford HIV Resistance Database, including all major subtypes. DRM emergence was evaluated for subtype B, and was correlated with the number of DRM in the polymerase domain. Genetic barrier was calculated for each DRM studied and in each subtype.

Results: N348I, T369I and A360V were found at low prevalence in treatment-naive isolates of all subtypes. A371V was common to treatment-naive isolates. N348I was observed in all subtypes, while T369I was only selected in subtype C. A360V and T369V were selected by RTI treatment in several subtypes. A371V was selected in subtypes B and C, but is a signature in subtype A. RT C-terminal mutations were correlated with early drug resistance in subtype B. All subtypes have a low calculated genetic barrier towards C-terminal DRM acquisition, despite a few disparities having been observed.

Conclusions: C-terminal mutations were selected in all HIV-1 subtypes, while some represent subtype-specific signatures. The selection of C-terminal DRMs occurs early in RTI resistance failure in subtype B.

Keywords: connection, RNase H, genetic barrier, subtype, drug resistance

Introduction

Human immunodeficiency virus type 1 (HIV-1) genetic diversity has allowed virus classification into types, groups, subtypes, sub-subtypes and recombinant forms.¹ The design of antiretroviral drugs, the mapping of drug resistance mutations (DRMs) and the development of genotype interpretation algorithms have been based on HIV-1 subtype B.² However, this subtype represented only 10% of novel infections in 2007 and is prevalent only in developed countries.³ Recent interest in anti-HIV therapy efficacy and drug resistance development in patients infected with non-B subtypes is therefore paramount. Genetic differences between HIV-1 strains modify viral protein structures and therefore can impair drug binding and efficacy.² Reverse transcriptase (RT) inhibitors (RTIs) of the non-nucleoside class, for example, are ineffective against HIV-2 and HIV-1 group O.⁴,⁵

HIV RT is a heterodimer with two catalytic activities: polymerization of double-stranded DNA, conferred by its polymerase (POL) domain (codons 1–300), and cleavage of viral template RNA, catalysed by its RNase H (RNH) domain (codons 441–560). POL has been the target of two major classes of antiretroviral drugs,⁶ and therefore various DRMs have emerged in this domain.⁶ RT C-terminal domains were disregarded in genotyping assays until 2005, when mutations in RNH were shown to impair template RNA degradation during reverse transcription, conferring additional time for RTI excision.⁷ The importance of the connection domain (CN, codons 301–440) in resistance was evident with the demonstration that N348I and T369I/V confer resistance to
both RTI classes. Other DRM in CN and RNH do not directly decrease drug susceptibility, but increase resistance conferred by thymidine analogue mutations.

The limited number of entire viral RT sequences from treated patients has prevented an assessment of the clinical relevance of DRM in RT C-terminal domains. Some studies showed reduced susceptibility to RTIs and/or treatment failure associated with definite CN DRM. One study suggested no correlation between therapeutic failure and CN DRM, while another showed a minor effect of those mutations in etravirine failure. Whether CN and RNH DRMs emerge early in treatment failure or only in multiply failed patients remains unknown.

Some RT CN DRMs appear to be polymorphic in non-B subtypes, further preventing elucidation of their role in resistance. Studies with non-B subtypes were performed with a limited number of viral isolates. Our objective was to determine the emergence of DRMs in RT C-terminal domains of different HIV-1 subtypes, estimating the genetic barrier to the acquisition of mutations and assessing their temporal emergence combined with POL mutations.

Materials and methods

HIV-1 RT C-terminal sequences were obtained from information from 6087 treatment-naive and 3795 RTI-treated patients deposited in the Stanford HIV Drug Resistance Database (accessed June 2013). Sequences of five HIV-1 subtypes and two major recombinant forms (A, n = 394 naive and 82 treated; B, n = 3055 and 3118; C, n = 1302 and 195; F, n = 154 and 48; G, n = 232; CRF01_AE, n = 281 and 182; and CRF02_AG, n = 669 and 170) were retrieved. All sequences contained entire or partial CN and/or RNH regions and included the POL domain.

The DRMs previously characterized in HIV-1 CN and RNH domains G335D, N348I, A360V, T369I/V, A371V, A376S, A400T, D488E, Q509L and Q547K were determined. Differences in the proportions of mutations for each subtype relative to HIV-1 subtype B were evaluated by 2 tests with Yate’s correction, and P < 0.05 was considered significant. Amino acid substitutions were considered polymorphisms when their frequency varied between 1% and 95% and genetic signatures had a frequency > 95%.

HIV-1 sequences derived from patients under RTI-based treatment of two groups, B62 without non-nucleoside RTI (NNRTI)-based regimens (1–7 NRTI and 0 NNRTI; group 1) and 2933 with both NRTI and NNRTI exposure (1–7 NRTI and 1–4 NNRTI; group 2), had their CN and RNH amino acid sequences deduced from nucleotide sequences. Major DRMs N348I and T369I/V and the other compensatory/secondary DRMs were compiled. Comparison of DRM proportions in treatment-naive versus RTI-treated groups per subtype was performed with 2 tests with Yate’s correction.

The emergence of RT C-terminal DRM with respect to RT POL DRM was exclusively analysed in subtype B due to the availability of a large number of sequences exposed to RTIs: 682 in group 1 and 2436 in group 2. Sequences of both groups were categorized according to the number of major DRM in the POL domain as defined in Johnson et al. and the number of RT C-terminal mutations. The 2 tests for trend were performed to correlate the emergence of mutations in both domains.

To verify whether individual HIV-1 subtypes harbour genetic signatures that could modulate the acquisition of specific DRM in RT C-terminal domains, sequences from treatment-naive patients were grouped by subtype and the composition of each codon associated with DRM was determined as previously described. The HXB2 sequence (subotype B; GenBank no. K03455) was used as wild-type. Comparison of polymorphism frequencies for each subtype with respect to HXB2 was performed by 2 tests with Yate’s correction.

Discussion

In the present work we showed for the first time that HIV-1 RT CN DRM can be selected in a wide range of HIV-1 genetic forms. Mutations in this domain have been previously reported, but have been restricted to individual HIV-1 subtypes. HIV-1 non-B subtypes carry genomic polymorphisms that correspond to compensatory DRMs in subtype B. Herein, A371V was pointed out as a genetic signature of CRF01_AE and was present in a large proportion of subtype A and CRF02_AG isolates, as previously shown. However, it was rare in treatment-naive
Table 1. Prevalence of individual DRMs at HIV-1 C-terminal RT domains in different subtypes according to patient treatment status

<table>
<thead>
<tr>
<th>DRM</th>
<th>A (1)</th>
<th>(2)</th>
<th>B (1)</th>
<th>(2)</th>
<th>C (1)</th>
<th>(2)</th>
<th>D (1)</th>
<th>(2)</th>
<th>Subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>G335D</td>
<td>67/3055</td>
<td>57/2436</td>
<td>117/232</td>
<td>2/48</td>
<td>62/63</td>
<td>103/137</td>
<td>47/189</td>
<td>21/92</td>
<td>C</td>
</tr>
<tr>
<td>N348I</td>
<td>2/322</td>
<td>2/36</td>
<td>5/1612</td>
<td>21/628</td>
<td>3/1177</td>
<td>19/149</td>
<td>13/124</td>
<td>1/115</td>
<td>C</td>
</tr>
<tr>
<td>T369V</td>
<td>2/36</td>
<td>10/31</td>
<td>10/31</td>
<td>0/46</td>
<td>0/46</td>
<td>0/46</td>
<td>0/46</td>
<td>0/46</td>
<td>C</td>
</tr>
<tr>
<td>A400T</td>
<td>19/27</td>
<td>4/31</td>
<td>21/628</td>
<td>273/456</td>
<td>0/258</td>
<td>0/258</td>
<td>0/258</td>
<td>0/258</td>
<td>C</td>
</tr>
<tr>
<td>Q547K</td>
<td>1/140</td>
<td>12/695</td>
<td>3/236</td>
<td>0/258</td>
<td>0/258</td>
<td>0/258</td>
<td>0/258</td>
<td>0/258</td>
<td>C</td>
</tr>
</tbody>
</table>

*dUnderlined values correspond to frequency of DRM that differs significantly (P < 0.05) between drug-naive sequences of each non-B subtype compared with subtype B.

**Bold type indicates statistical significance between treatment naive versus treated for a given subtype (P < 0.05).
subtype B and C isolates. G335D and A400T also appeared to be polymorphisms, while six other DRM were rare in all HIV-1 forms studied: N348I, A360V, T369I, D488E, I509L and Q547K.

The estimated genetic barrier could partly explain differences in DRM emergence among distinct HIV-1 subtypes, but some disparities were observed. For example, 369V should be more easily selected in subtype A compared with other subtypes. Indeed, one-third of subtype A isolates exposed to both RTI classes presented 369V. In agreement with that, subtype B accumulated 369I, but subtype C and CRF01_AE selected for 369V, different from the expected outcome. A possible explanation for this phenomenon is the impact of 369V on the replicative capacity of distinct subtypes. Further studies are necessary to confirm this hypothesis.

Fitness cost could explain why N348I (score of 2.5) was more selected than T369I/V (score of 1 or 2) in subtype B independent of the RTI class used. Seven CN and RNH codons need only one ts to acquire the respective DRM, including G335D, A360V, T369I,
A369V and A371V. However, our analysis of treatment-experienced patients revealed that G335D was not selected in subtype B, while 369I/V were rare in most subtypes; 400T was selected in subtype B under NRTI treatment and in CRF02_AG under NRTI/NNRTI treatment. Interestingly, the proportion of this mutation decreased in subtype B under NRTI/NNRTI treatment, a fact that requires further assessment. We also observed that three RNH DRM previously reported in vivo—D488E, Q509L and Q547K—were absent in our sequences.

N348I was shown to arise early in drug therapy failure, while A360V and A371V correlated with the number of thymidine analogue mutations. Here, we further extend the observation of early emergence to A360V, A371V, A376S and A400T in subtype B isolates under RTI exposure. Such early emergence can influence treatment efficacy and durability, and deserves attention.

In conclusion, we showed that some HIV-1 RT connection mutations are selected in all HIV-1 genetic forms, while others are present as genetic signatures of specific subtypes. The calculated genetic barrier highlighted, in general, a low barrier to resistance acquisition in the RT C-terminal domains and an early selection of these mutations during RTI failure.

Funding
This work was funded by Brazilian Research Council (CNPq) grant no.s 304416/2010-0 (M. A. S.) and 305085/2013-1 (A. F. S.). and by the Rio de Janeiro State Science Foundation (FAPERJ) grant no. E-26/103.059-2011 (M. A. S.). C. P. M. is recipient of a PhD scholarship granted by the Brazilian Ministry of Education (CAPES).

Transparency declarations
None to declare.

References