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Short communication

Performance comparison of an in-house integrase genotyping assay versus the ViroSeq™ Integra48, and study of HIV-1 integrase polymorphisms in Hong Kong

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ABSTRACT

Background: Integrase inhibitors are recently prescribed to multi-class drug resistant HIV-1 patients in Hong Kong. Unlike *pol* gene, there are no FDA-approved genotypic resistance tests available for *int*. Limited studies compared the performance between an in-house and commercial integrase genotyping system. Information on baseline polymorphisms was also insufficient in our region.
Objectives: To compare integrase genotyping data obtained from an in-house and ViroSeq™ Integra48 assay, and to illustrate integrase polymorphisms on HIV-1 B and non-B subtypes in Hong Kong.
Study design: A total of 283 HIV-1 patients were recruited during 2006–2012 in Hong Kong. All samples were genotyped by an in-house assay for *pol* and *int* separately, and 46 of them were further genotyped by ViroSeq™ Integra48. Polymorphisms and resistance mutations were analyzed by Stanford HIV Drug Resistance Database.
Results: The included patients were mainly infected by HIV-1 subtype B (38.9%) and CRF01_AE (43.1%), followed by CRF07_BC (5.3%), C (3.9%), CRF02_AG (2.8%), D (1.4%), CRF08_BC (1.1%) or others (3.5%). Of 46 samples genotyped by ViroSeq™ and the in-house assays, all major and minor resistance mutations were concordant. Integrase major resistance mutations were identified in two CRF01_AE raltegravir-treated patients. Integrase minor resistance mutations were observed in subtypes B and CRF01_AE.
Conclusions: With 25% of the commercial cost, the in-house integrase genotyping assay managed to regenerate over 96% concordant results as good as the RUO ViroSeq™ assay. Further investigations are required to understand the effect on integrase minor mutations, which are present in many subtype B and CRF01_AE samples.

1. Background

Raltegravir (RAL) is the first integrase inhibitor (INI) approved by the US Food and Drug Administration in 2007. Since then, RAL has become one of the alternative options added to the antiretroviral therapy (ART) for both treatment-naïve and -experienced patients. In Hong Kong, RAL is practically used as a second line regimen reserving for salvage treatment or patients with severe intolerance to protease inhibitor (PI) or reverse transcriptase inhibitor (RTI).

RAL primary resistance is mainly based on 3 distinct mutations, N155H, Q148K/R/H and Y143R/C/H,[1–3] which are located near the catalytic site of the HIV-1 integrase. The occurrence of any of these mutations could cause >15-fold phenotypic resistant to RAL.[1] Both integrase activity and viral fitness were reported to be associated to these mutations.[1,4] Recent studies indicated that the major and minor mutations profile and INI susceptibilities might be varied in different genotypes.[5–7]

From our previous surveillance studies, an extensive assortment of HIV-1 subtypes was found in Hong Kong. Almost 90% of our HIV-1 patients were infected by either subtype B or CRF01_AE. Other genotypes such as subtypes C, D, CRF02_AG, CRF07_BC and CRF08_BC accounted for the remaining 10% of the infections.[8] With the increasing prescription of INI, it is therefore vital to adopt a proficient and cost-effective integrase genotyping assay to detect the enormous variety of different subtypes.

2. Objectives

To compare the integrase genotyping data obtained from an in-house and ViroSeq™ Integra48 assay, and to illustrate the baseline

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Table 1
HIV-1 patients treatment experiences and subtypes distribution.

	Total (n = 283)	ART-naïve (n = 198)	ART-exp (n = 85)		PIs resistance	RTIs resistance	RAL resistance
			RAL-naive	RAL-exp			
B	110 (38.9%)	77 (38.9%)	29 (34.1%)	4 (4.7%)	4 (4.7%)	12 (14.1%)	–
CRF01_AE	122 (43.1%)	77 (38.9%)	42 (49.4%)	3 (3.5%)	7 (8.2%)	17 (20%)	2 (2.4%)
C	11 (3.9%)	8 (4.0%)	3 (3.5%)	–	–	1 (1.2%)	–
CRF07_BC	15 (5.3%)	14 (7.1%)	1 (1.2%)	–	–	–	–
CRF08_BC	3 (1.1%)	3 (1.5%)	–	–	–	–	–
CRF02_AG	8 (2.8%)	6 (3.0%)	1 (1.2%)	1 (1.2%)	–	1 (1.2%)	–
D	4 (1.4%)	3 (1.5%)	1 (1.2%)	–	–	–	
Others	10 (3.5%)	10 (5.1%)	–	–	–	–	–

ART, antiretrovirals; exp, experienced; RAL, raltegravir; PIs, protease inhibitors; RTIs, reverse transcriptase inhibitors.

integrase genotyping and polymorphisms among HIV-1 B and non-B subtypes in our region.

3. Study design

A total of 198 ART-naïve and 85 ART-experienced HIV-1 patients were recruited at the Hong Kong Government Integrated Treatment Centre during 2006–2012. Plasma samples were collected after patient consent. HIV-1 genotypes were defined by using our in-house *pol* genotyping assay and REGA Genotyping Tool (version 2.0).[9,10] Drug resistance mutations (DRMs) in the integrase region of all 283 samples were first screened by a previously published one-step RT-PCR plus nested PCR in-house integrase genotyping assay.[11] Furthermore, 46 samples including several HIV-1 genotypes circulating in our locality were analyzed by the commercial ViroSeq™ Integra48 Genotyping System (Celera Corporation, USA). The sequences were assembled and manually proof-read by Staden Packages, followed by submitting to the Stanford HIVdb (http://www.hivdb.stanford.edu) in determining the mutation pattern and RAL susceptibility.[12,13] Sequence homologies were determined by NCBI nucleotide BLAST.

4. Results

In this study, nearly 85% of the enrolled patients were male, with an overall median age at 38 (range 20–75 years). All samples had viral load over 1000 copies/mL at collection. REGA genotyping analysis demonstrated that the 283 patients were infected by subtypes B (38.9%), CRF01_AE (43.1%), CRF07_BC (5.3%), subtype C (3.9%), CRF02_AG (2.8%), subtype D (1.4%), CRF08_BC (1.1%) and other subtypes (3.5%). There was no significant difference on subtype distribution between ART-naïve and ART-experienced patients (p = 0.2988). The details on treatment status and subtype distribution for all patients had been summarized in Table 1.

Among the 283 samples of various genotypes, 46 samples (17 CRF01_AE, 10 subtype B, 7 CRF02_AG, 6 subtype C, 3 CRF07_BC, 1 subtype D and 1 subtype G) were further genotyped by the ViroSeq™ Integra48 System. The in-house assay failed to amplify one CRF01_AE strain while the ViroSeq™ Integra48 system failed to amplify another CRF01_AE sample. Of all 44 samples successfully amplified by both assays, over 96% sequence homology was achieved. The detected integrase major and minor DRMs were identical, suggesting both assays were highly concordant.

A wide range of integrase minor DRMs were observed in 22.5% INI-naïve patients, at positions H51, Q95, T97, A128, E138, G140, V151, M154, E157, G163, I203, S230 and R263. According to the Stanford interpretation and other studies, some of the minor DRMs are polymorphic accessory mutations that occur in combination with major mutations.[12,14] These mutations do not reduce INI susceptibility on their own. Three exceptions at positions E138, E157 and G163, were interpreted as low-level resistance or potential low-level resistance. It is unknown whether these accessory mutations will increase the probability of the incidence of the major DRMs or not.

Over 40% of the CRF01_AE samples, including INI-naïve and -experienced patients, had polymorphisms at positions K14, A21, V31, S39, I72, T112, T124, T125, G134, I135, K136, D167, V201, L234 and S283 (Table 2). Other positions at E11, A23, V32, L101, V126 and V165 were also about 10–30% polymorphic.

Eight of our ART-experienced patients were RAL-experienced and 2 of the CRF01_AE infected patients responded failure to RAL. The first patient developed Q148R after 17 months of HAART with didanosine, lopinavir/ritonavir and RAL while the second patient developed N155HN mutation after 11 weeks of zidovudine, tenofovir, emtricitabine and RAL treatment. Previous studies showed that RAL DRMs often appeared after 24–48 weeks of treatment due to the low genetic barrier.[15,16] In our study, we demonstrated not only slow but also rapid raltegravir-resistance development in CRF01_AE patients.

5. Discussion

When HIV-1 patients become resistant to PI or RTI, INI provides an alternative option in salvage therapy especially when there are active background drugs. Both subtypes B and CRF01_AE are the co-prevalent strains affecting Hong Kong, and local clusters were observed within the city.[8,17] We adopted an in-house assay and compared its performance against ViroSeq™ Integra48 assay.[11] At only 25% of the cost, the in-house assay showed highly concordant data towards ViroSeq™ and managed to amplify a wide range of subtypes circulating in our region. As both assays failed to sequence certain CRF01_AE strains, ViroSeq™ might possibly act as a supplementary test in routine practice.

Integrase genotyping on 275 INI-naïve patients demonstrated no major INI DRMs yet 62 of them had minor INI DRMs. One CRF01_AE strain carried E138EK mutation that could cause low INI resistance.[18] E138EK does not reduce INI susceptibility on its own but leads to a decrease of >100-fold of RAL susceptibility with the presence of Q148 mutation. Besides, 4 subtypes B and 1 CRF01_AE strains developed the E157Q/EQ mutation, which was estimated to have potentially low INI resistance.[19] This mutation was suggested to be a rare natural polymorphism for CRF01_AE.[20] The relatively high prevalence of the observed minor INI DRMs highlighted the necessity to further investigate their impact on INI susceptibility.

Several Southeast Asian studies reported the integrase polymorphisms on CRF01_AE patients.[20,21] Our samples observed similar polymorphisms patterns that were reported by Thailand, Vietnam and Cambodia.[21] Other natural polymorphisms in the integrase region of subtype B and CRF01_AE were compared (Table 2). There were no association between ART experiences and the frequency of polymorphisms observed in our data cohort.

Among the RAL-treated patients, 2 CRF01_AE patients developed INI DRMs after a short (11 weeks) and prolonged (17 months) RAL treatment respectively. Some previous studies reported that