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APPLICATION OF CLONING IN THE DETECTION OF HIV-1 DRUG RESISTANT MINORITY POPULATIONS

BY

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ABSTRACT

Human immunodeficiency virus (HIV) is the causative agent of acquired immunodeficiency syndrome (AIDS). The virus is genetically highly diversified with different genetic strains infecting different populations worldwide. Highly active antiretroviral therapy (HAART) reduces the morbidity and mortality due to AIDS. Unfortunately, the efficiency of these drugs is limited by the development of drug resistance usually caused by mutations in the protease and reverse transcriptase genes which complicates patient management.

Sequencing direct PCR products reveals the properties of major viral populations but has the potential to miss minority viral populations. This clearly means that resistant strains of minority populations may not be detected in direct sequencing of PCR products. Furthermore, if multiple drug resistance mutations are detected as mixtures in a given sample, it is not possible to determine whether the individual drug resistance mutations are present together on a single virus or exist in different viral sub-populations. Therefore, the general objective of this study was to design and evaluate a single genome sequencing protocol for the detection of HIV-1 drug resistance mutations in the protease and reverse transcriptase genes.

In this study the HIV Pol gene (for protease and reverse transcriptase genes respectively) was amplified from 8 samples of HIV-1 infected individuals (7 treatment experienced and 1 treatment naïve). Subsequent cloning was done on all 8 samples using the Topo TA cloning vector, 35 and 33 clones were obtained for protease and reverse transcriptase respectively. Sequencing of purified PCR products and viral clones was done using a BigDye terminator sequencer. A comparison of sequences obtained from direct sequencing of PCR products and cloned PCR sequences was done. Phylogenetic analysis confirmed that all sequences were of HIV-1 subtype C and amino acid determination helped revealed amino acid substitutions in comparison to the HIV-1 subtype B and C global consensus.

Using the Stanford drug resistance interpretation program, sequences obtained by direct sequencing of PCR products revealed mutations V11I (a minor resistant mutation for the protease region) and M184V (a NRTI resistant mutation), K101E and Y181C (NNRTI resistant mutations) from samples MARBB14 and MARBB73. Viral clones of samples MARBB14 and MARBB73 revealed the same mutations as those observed by direct sequencing of PCR products. In addition viral clones of sample MARBB73 also revealed reverse transcriptase mutations T215Y (a NRTI resistant mutation) and Y181V (a NNRTI resistant Mutation).

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Polymorphisms such as K20R, M36I, M36V, L63P, H69K, V77I and V82I selected by protease inhibitors were also observed in the protease region from both direct sequences of PCR products and cloned PCR sequences. Using the student t-test, p values of 0.03 and 0.0002 for protease and reverse transcriptase respectively were obtained from comparisons of resistant mutations observed in sequences of PCR products and clone sequences for both protease and reverse transcriptase genes and the difference was considered significant.

The cloning technique was used as an approach to detect minority variants. Important resistant mutations were detected in clone sequences and not in sequences of PCR products. This suggests that mutations of interest could be missed in the direct sequencing of PCR products which is currently the norm in many settings.

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