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HIV-1 reverse transcriptase inhibitor resistance mutations and fitness: A view from the clinic and *ex vivo*

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Abstract

Genetic diversity plays a key role in human immunodeficiency virus (HIV) adaptation, providing a mechanism to escape host immune responses and develop resistance to antiretroviral drugs. This process is driven by the high-mutation rate during DNA synthesis by reverse transcriptase (RT), by the large viral populations, by rapid viral turnover, and by the high-recombination rate. Drugs targeting HIV RT are included in all regimens of highly active antiretroviral therapy (HAART), which helps to reduce the morbidity and mortality of HIV-infected patients. However, the emergence of resistant viruses is a significant obstacle to effective long-term management of HIV infection and AIDS. The increasing complexity of antiretroviral regimens has favored selection of HIV variants harboring multiple drug resistance mutations. Evolution of drug resistance is characterized by severe fitness losses when the drug is not present, which can be partially overcome by compensatory mutations or other adaptive changes that restore replication capacity. Here, we review the impact of mutations conferring resistance to nucleoside and nonnucleoside RT inhibitors on *in vitro* and *in vivo* fitness, their involvement in pathogenesis, persistence upon withdrawal of treatment, and transmission. We describe the techniques used to estimate viral fitness, the molecular mechanisms that help to improve the viral fitness of drug-resistant variants, and the clinical implications of viral fitness data, by exploring the potential relationship between plasma viral load, drug resistance, and disease progression.

Keywords: Human immunodeficiency virus; HIV-1; AIDS; Antiretroviral therapy; Reverse transcriptase; Replication capacity; Drug resistance; Viral fitness; Nucleoside analogues; Nonnucleoside analogues

1. Introduction

In a human immunodeficiency virus (HIV)-infected individual, the rapid turnover, high-mutation rate, and high frequency of recombination result in a diverse viral population. Unlike eukaryotic DNA polymerases, HIV-1 reverse transcriptase (RT) has no proofreading ability, and its error rate has been estimated to be between 10^{-4} and 10^{-5} mutations per nucleotide and replication cycle (reviewed in Menéndez-Arias, 2002; Svarovskaia et al., 2003). If one assumes that 10^9 to 10^{10} viral particles are produced each day in an infected person, then these particles must be the product of at least 10^7 to 10^8 replication cycles. Given the length of the HIV-1 genome (approximately 10,000 nucleotides), it is likely that every possible single point mutation (and probably many double mutations) will occur at least once each day in an infected individual (Coffin, 1995). Although specific combinations of multiple mutations may be rare, it is clear that the degree of potential genetic change drives the diversification of HIV-1 in response to the selective pressure of host immune responses or antiretroviral therapy. When we talk about divergent, rapidly changing HIV-1 variants, we frequently use the term 'quasispecies.' This term was originally coined by Eigen (Eigen, 1971; Eigen and Schuster, 1979) to designate closely related but distinguishable genomes that undergo continuous genetic variation, competition, and selection.

The introduction of potent antiretroviral therapy has made an enormous contribution to the control of HIV infection and AIDS. Current treatments target viral enzymes such as RT and protease (PR), as well as the envelope glycoprotein gp41. During 2007 the United States Food and Drug Administration (FDA) approved a pair of first-in-class antiretrovirals for HIV-infected patients with drug-resistant disease: maraviroc is the first chemokine receptor antagonist, and raltegravir is the first integrase inhibitor. Despite the success of potent combination regimens, the development of

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HIV-1 drug resistance constitutes a major hurdle for the long-term efficacy of current antiretroviral therapy (Menéndez-Arias, 2002). The evolutionary pathways leading to resistance have been widely studied for many antiretroviral drugs. In general, drug resistance mutations confer a fitness cost, in part because the mutations associated with drug resistance may decrease the enzymatic efficiency of the target proteins.

Fitness is a complex parameter that describes the replicative adaptability of an organism to its environment. What Charles Darwin called "natural selection, or the preservation of favored races in the struggle for life" was called "survival of the fittest" by a 19th-century contemporary, Herbert Spencer (Goudsmit, 2004). A specific virus population is fitter than another when it is more able to multiply and spread, given certain conditions. However, those fittest under one set of conditions can quickly be supplanted if a new set of conditions suddenly prevails, such as when the inner environment of an HIV-infected person changes as a result of taking (or changing) antiretroviral drugs. In this case, the drug-resistant virus that prevails might not have survived under the old conditions, but it is now in the right place at the right time, while all drug-sensitive viruses will seemingly not survive (Goudsmit, 2004). Thus, viruses change as fast as conditions change. For HIV (and other RNA viruses), an experimentally useful approach to fitness is its relative ability to produce stable infectious progeny in a given environment (e.g., cell culture, bloodstream) (for reviews, see Domingo and Holland, 1997; Domingo et al., 1999). Evolution of resistance to antiretroviral drugs is characterized by significant fitness costs and subsequent repair strategies that include compensatory mutations in the targeted gene, as well as other molecular mechanisms that improve fitness. Recognition of virological and clinical correlates of viral fitness is becoming more and more important in clinical practice, and in this review we discuss the relevant techniques used to measure fitness, focusing on the effect of RT inhibitor resistance mutations in viral fitness and their implications in the management of HIV infection.

2. Currently used techniques for estimating the fitness of drug-resistant HIV variants

The term viral fitness refers to the ability of a virus to produce infectious progeny under specific environmental circumstances (i.e. a defined host cell system, host, or host population). Viral fitness depends on multiple viral and host factors. First, all events involved in the virus life cycle could have an impact on replication capacity: (i) target cell entry, (ii) reverse transcription, (iii) integration, (iv) gene expression, (v) assembly, (vi) budding, and (vii) maturation. Second, selective factors impose certain constraints on viral replication. These factors include the state of the host cell for optimal replication and virion production, the host immune system, the density of target cells at the site of infection, the number of transmitted virions, and the complex distribution of viral quasispecies (Domingo et al., 1999, 2006). Replication capacity could be considered an specific case of viral fitness, in which the ability to replicate of two or more viral variants is compared in an experimentally defined *in vitro* host cell system. Although fitness and replication capacity may be reported in terms of gains or losses, they are often reported in a relative fashion, as a mutant compared with a wild-type (WT).

Emergence of mutations during treatment with current antiretroviral drugs (mostly RT and PR inhibitors) is likely to affect the catalytic efficiency of the viral enzymes that have a detrimental effect on replication. Enzymatic assays provide good estimates of the effect of drug-resistance mutations on the catalytic efficiency of viral enzymes. The effect of mutations on HIV-1 RT has been clearly shown by monitoring the polymerase or RNase H activities of the enzyme (i.e. its processivity). Amino acid substitutions that diminish the catalytic efficiency of viral enzymes are expected to have a detrimental effect on replication capacity, by producing viruses that are less fit than their WT counterpart.

To approach viral fitness *ex vivo*, researchers use replication kinetics assays that quantify the efficiency of HIV replication in parallel cultures and competitive culture assays in which the proportions of the competing viruses are carefully monitored over time using various techniques (reviewed in Quiñones-Mateu and Arts, 2002). Several studies have extended the definition of relative fitness by comparing (i) virus turnover in HIV-1-infected individuals, (ii) infectious virions/virus particle ratios, and (iii) HIV infectivity in single-cycle replication assays.

The simplest method to obtain a relative fitness value is by comparing the replication kinetics for each virus after infection of cell cultures (Fig. 1) and measuring the amount of p24 antigen or RT activity in the culture supernatants (Maeda et al., 1998; Prado et al., 2004, 2005; Villena et al., 2007). These assays are usually performed with cell lines (e.g. MT-2, MT-4,

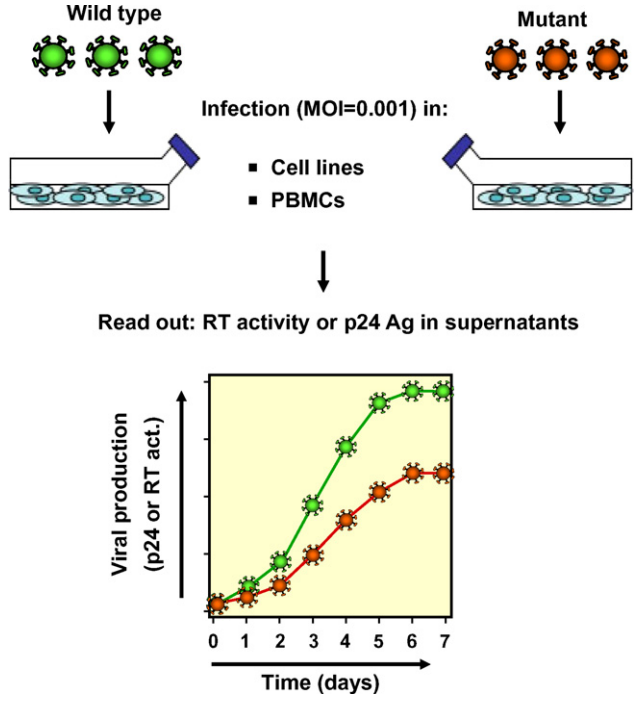

Fig. 1. Replication kinetics of independent virus strains. Normalized amounts of two or more viral variants are used to infect HIV-1-susceptible cell lines or PHA-stimulated PBMC from healthy donors. Viral production in parallel cell cultures is measured by p24 antigen or RT activity over a few days. Results are frequently expressed in slopes. MOI, multiplicity of infection.