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HIV-1 Evolution under Pressure of Protease Inhibitors: Climbing the Stairs of Viral Fitness

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Key Words

Human immunodeficiency virus · Protease inhibitors ·
Drug resistance · Virus evolution · Viral fitness ·
Second-site suppressor · Translational frameshift ·
Gag-Pol protein processing

Abstract

The human immunodeficiency virus (HIV-1) has evolved into a viral quasispecies with a high replication capacity or fitness. Antiretroviral drugs potently inhibit replication of the wild-type virus, but HIV-1 responds by selection of drug-resistant variants. Here we review, in brief, the evolution of resistance to protease inhibitors that is characterized by severe fitness losses and an abundance of subsequent repair strategies. The possibility to restrict HIV-1 fitness is discussed in relation to the control of HIV-1 pathogenesis.

Introduction

Potent antiviral drugs have been developed against HIV-1, targeting either the reverse transcriptase (RT) or the protease enzyme. But even in the context of the potent multidrug combination therapy now widely in use in

some countries, the development of drug resistance remains a major problem in the treatment of HIV-1 infection and AIDS. The evolution of drug resistance has been analyzed both in patients and in tissue culture replication studies. For RT inhibitors, the picture that emerges is relatively simple, with one or a few amino acid substitutions in the RT enzyme that provide the drug-resistant phenotype. A more complex picture emerges in the evolution of resistance to protease inhibitors. The initial changes that provide resistance are observed in the protease gene, but there is continued evolution both in the protease gene and in secondary loci of the HIV-1 genome. This continued evolution is caused by the damaging side effects of the initial drug resistance mutations on protease enzyme function and virus replication. This loss of replication capacity or viral fitness is the driving force behind the subsequent cascade of adaptive changes. The multistep evolution of resistance to protease inhibitors will be described and contrasted with the relatively simple evolution of RT inhibitor resistance.

Fitness Loss in Drug-Resistant HIV-1

Fitness losses were first reported for HIV-1 variants with an amino acid substitution at position 184 within the catalytic core of the RT enzyme which provides resistance

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Fig. 1. Organization of the HIV-1 genome and domain structure of the Gag and Gag-Pol precursor proteins. The position of the frameshift site is indicated, used at a 5% frequency during Gag translation to produce the Gag-Pol fusion protein. The 9 cleavage sites recognized by the HIV-1 protease in the Gag and Gag-Pol fusion protein are shown as white bars. The Gag domains are MA (matrix), CA (capsid) and NC (nucleocapsid), and p2 and p1 are spacer peptides. Pol encodes the protease, RT and integrase enzymes. The RTp66 protein forms a homodimer of which a single subunit is cleaved by protease to produce the p66-p51 heterodimer. The initial resistance mutations in protease trigger a multistep repair process that includes adaptive changes in protease itself, mutations in the Gag cleavage sites, the frameshift signal, or the RT processing sites.

to the 3TC nucleoside analog [1]. Polymerase and other defects have been reported for this RT enzyme [27, 43, 55] and RT variants that are resistant to other drugs including zidovudine (AZT) [21, 23, 24, 42, 45]. More recently, impaired enzyme function and reduced viral fitness were reported for HIV-1 isolates with amino acid changes in the protease enzyme that provide resistance to protease inhibitors [12, 18, 25, 30, 38, 58]. The replication defects reported for protease-resistant viruses appear to be severer than the defects of RT-resistant viruses, which may in part explain the elaborate evolution pattern observed for the former variants. I will now provide an overview of the multistep evolution of protease resistance. In brief, the initial resistance mutations affect the enzyme function and proper processing of the viral Gag and Gag-Pol precursor proteins at one or several of the 9 cleavage sites (fig. 1). Compensatory changes are observed at secondary sites within the protease enzyme, and mutations are selected at other loci of the HIV-1 genome,

either to increase the expression of the malfunctioning protease enzyme or to modify the target-processing sites in the Gag and Gag-Pol precursor proteins.

Step-by-Step Evolution of Protease Inhibitor Resistance

Step 1: Potent Inhibition of the Wild-Type Virus

The HIV-1 quasispecies present in an infected individual occupies a narrow segment of total sequence space, which is illustrated in figure 2 as a peak of high fitness in the sequence landscape. Antiviral drugs interfere with replication of the wild-type virus, causing a severe loss of fitness. The potent antiviral effect forces HIV-1 to explore sequence space for variant genomes with improved viral replication properties or fitness. The virus can examine a number of alternative and disparate molecular pathways for correction of the genetic lesion and for fitness gain, but

Fig. 2. HIV-1 evolution pathways following treatment with a potent antiviral. The current HIV-1 quasispecies has evolved from its simian counterparts during natural evolution [22] and has reached a narrow peak of high fitness in the sequence landscape of which only a small, two-dimensional segment is shown. HIV-1 is forced to explore sequence space in response to potent antivirals, and this route is marked by arrows. 1 = The protease inhibitor blocks replication; 2 = appearance of protease resistance mutations; 3 = compensatory mutations in protease; 4 = mutations in the Gag-processing sites; 5 = mutation within the frameshift signal; 6 = mutation of the RT cleavage sites. The protease inhibitor severely inhibits replication of the naive, wild-type virus, resulting in a dramatic fitness loss (step 1).

From this low-fitness valley, the virus recovers approximately wild-type fitness in a multistep pathway (steps 2–6, pathway A). The order of these steps may differ between individual patients, and the magnitude of the fitness gain in distinct steps will also differ depending on the actual mutation and the viral genomic background. Nevertheless, it is likely that the initial response will provide a relatively major fitness benefit (step 2), with minor upward adjustments at the subsequent stages. It remains to be tested whether certain drug regimens can drive HIV-1 towards a mountain of intermediate fitness (pathway B). In this situation, there are no routes towards areas of higher fitness in the direct neighborhood, and HIV-1 therefore cannot easily escape from this suboptimal section of sequence space.

only two possible routes will be illustrated (fig. 2). Route A brings the virus to a new peak of approximate wild-type fitness, but route B stalls at a mountain of intermediate fitness. The process of adaptation of an RNA virus occurs by the competitive rating of mutants that are continuously being generated during error-prone replication [14, 16, 20]. Although viral fitness will largely dominate the evolution, other parameters may influence this process in some situations. For instance, evolution is restricted to those areas of sequence space that are proximate to the starting position of the viral quasispecies. Due to the limited error rate of RT polymerase, evolution is gradual with steps that represent single nucleotide substitutions, and HIV-1 will not easily jump through the sequence space with steps that are greater than two nucleotide substitutions [31].

Step 2: Acquisition of Resistance Mutations

In response to protease inhibitors, the cumulative acquisition of six or more amino acid substitutions has

been described [11]. Each protease inhibitor elicits some unique mutations, but there is also some overlap in resistance mutations, and some mutations provide a certain level of cross-resistance [57]. The exact evolutionary pathway will depend not only on the type of protease inhibitor, but also on the viral genetic background [48] and stochastic events [32]. There is a certain order in the accumulation of mutations, but this pattern may vary significantly within individual patients [40]. Most of the initial resistance mutations in the protease enzyme are located proximal to the drug-binding site, resulting in direct steric perturbation of the drug-enzyme interaction [46]. Other mutations at more distant residues may affect binding of the drug through conformational changes, and one example is the resistance position 48 that is part of the mobile flap region [26]. Although these initial variants are fitter than the wild-type virus in the presence of the drug, there is accumulating evidence for a resistance-associated loss of enzyme function and virus replication [12, 18, 25, 30].

Reduced replication capacity is due to a reduced cleavage efficiency of the resistant protease at several cleavage sites in the Gag precursor protein (fig. 1), which results in the accumulation of partially cleaved Gag subunits in the virus particle [38, 48, 58]. Interestingly, resistant proteases exhibit distinct processing defects, indicating that specific resistance mutations affect cleavage differentially at distinct sites [38, 47]. The dramatic impact of at least some resistance mutations on the protease enzyme function (e.g., Gly48Val) is confirmed by the rapid loss of these particular mutations upon virus culturing in the absence of the drug [44]. Thus, HIV-1 maintains these resistance mutations only in the presence of the drug, in which case this virus will find alternative strategies for repair of the protease function (step 3) or restoration of virus replication by other means (steps 4–6).

Step 3: Repair of Enzyme Function by Compensatory Protease Mutations

The primary mutation(s) are acquired to provide resistance, but these changes also cause a partial enzyme defect. Repair is possible by second-site changes within the protease that compensate for these negative effects. Such second-site changes restore both enzyme function and virus viability. The idea that the secondary changes are triggered by the initial protease mutations, and not by the protease inhibitor, is demonstrated by the observation that secondary changes can also be selected upon withdrawal of the drug [7]. The type of second-site adaptation is closely linked to the type of first-site change.

For instance, changes at codon 10, 63 or 71 have been proposed to compensate for a reduced viability of HIV-1 variants with the resistance mutation at position 82 [19, 37, 39, 44, 48]. Intragenic second-site suppressor mutations are not unique to the field of drug resistance. In particular, the forced evolution of mutated HIV-1 variants in tissue culture has yielded several revertant viruses with interesting second-site changes within the protein or sequence motif under scrutiny [2, 6, 33, 51, 54, 56].

Step 4: Restoration of Gag Processing by Mutation of the Cleavage Site

The acquisition of protease inhibitor resistance mutations is not restricted to the protease gene itself, and a second locus was described in 1996 within the Gag gene [17, 59]. These changes are located within the protease cleavage site, provide better peptide substrates for the mutated protease, and partially compensate for the resistance-associated loss of viral fitness [17, 38, 58]. In particular, such mutations are observed in the two cleavage sites that

flank the p1 spacer peptide (fig. 1). Because specific protease mutants show distinct cleavage defects at one or multiple cleavage sites of the Gag polyprotein [38, 47], it is not surprising that there is also a correlation between the cleavage site that is modified and the original protease mutation [8]. Extragenic second-site suppressor mutations are also not unique to the field of drug resistance [4, 13, 36, 53].

Step 5: Increased Protease Synthesis by Mutation of the Frameshift Signal

An unexpected evolutionary escape route was described in 1998 for the protease-inhibitor-resistant variants with impaired fitness [18]. These viruses can benefit from an increased level of Gag-Pol frameshifting that would increase the level of protease enzyme in the virus particle, thereby compensating for reduced protease enzyme activity. Alternatively, increased expression of the Pol open reading frame may represent a viral strategy to express more RT enzymes of which the expression is affected by some drug-resistant protease mutants (step 6). Frameshifting is an unusual translational event that occurs at a low efficiency (approximately 5%) during Gag synthesis. The actual frameshift is induced by specific sequence and structure motifs in the viral RNA near the end of the Gag open reading frame (fig. 1), and a single nucleotide transition in the p1/p6 junction was found to create an additional frameshift site [18]. Most strikingly, the nucleotide mutation that facilitates this frameshift is the same as the second locus mutation that improves Gag cleavage by amino acid substitution in the p1/p6 processing site (step 4). Thus, it appears that HIV-1 is able to kill two birds with one stone.

Step 6: Restoration of RT Processing by Mutation of the Cleavage Site?

Another surprising result was reported earlier this year. Some protease-inhibitor-resistant viruses display abnormalities in the processing of the RT enzyme [9]. Although speculative, it is also possible that certain drug-resistant protease variants will exhibit a defect in the autolytic cleavage of the protease itself or the removal of the integrase domain (fig. 1). The RT-processing defect results in reduced levels of RT enzyme in virus particles and is likely to contribute to reduced fitness of these variants [9]. It remains to be analyzed which RT cleavage step is affected and whether RT-processing defects are associated with particular protease mutations, as observed for the Gag cleavage defects. This recent finding should warn us that the continuing story of virus evolution in response to pro-

tease inhibitors is perhaps not yet to end. In particular, HIV-1 may modify the processing sites in the RT substrate that are affected, and it would be of interest to screen patient isolates with damaging protease mutations, for instance the Gly48Val substitution, for possible changes in this third locus. Furthermore, the increased frameshifting frequency discussed in the context of malfunctioning protease may also rescue virus variants with an RT-processing defect. Consistent with this idea is the observation that the frameshift mutation at the p1/p6 junction does increase the level of RT activity in virus particles [Jeeninga and Berkhout, unpubl. data].

Why Is There No Cascade of Mutations in Response to RT Inhibitors?

It was argued that some of the resistance-linked amino acid substitutions near the catalytic core of the protease enzyme have a pronounced effect on enzyme activity, which triggers the subsequent multistep evolution by which HIV-1 restores approximately wild-type replication kinetics. A much different pattern is seen for RT drug resistance, which is usually mediated by a single amino acid change in RT [41]. Apparently, the RT inhibitor resistance mutations do not have such a devastating effect on RT enzyme function, thereby bypassing the requirement for selection of new variants with improved fitness. Thus, the question asked above should be rephrased as 'why are there no severe enzyme defects apparent for HIV-1 variants that are resistant to RT inhibitors?'.

Several possibilities will be discussed. First, it is possible that the RT enzyme is more flexible than the protease in absorbing resistance mutations, but this seems unlikely given the sophisticated structure of the RT enzyme. Second, resistance mutations may be less harmful to RT enzyme function because they are distal to the nucleoside triphosphate binding site. This idea seems correct for the nonnucleoside RT inhibitors. These drugs bind to external RT domains, and the resistance mutations are also located in this domain, thereby providing a simple steric explanation for the mechanism of resistance [35, 49]. The mutations that provide resistance to nucleoside analogs were initially also reported to be distal to the active site of the RT enzyme, at least in the X-ray crystal structure of the 'open' RT conformer [29, 34, 52]. However, there is recent evidence that the catalytically active RT enzyme adopts a 'closed' conformation in which many resistance positions (e.g., 65, 70, 74, 151, and 215) are located much closer to the nucleoside triphosphate binding site [28].

Similar to the nonnucleoside RT inhibitors and the protease inhibitors, steric hindrance is therefore a simple possibility for the mechanism of resistance. Most importantly, these findings negate the simple idea that resistance mutations in RT are less detrimental because they are located in the periphery. In addition, some RT mutations are actually positioned in the catalytic core or even within the well-conserved 'YMDD' motif (e.g., position 184) [50].

The third possibility is that RT resistance mutations represent relatively conservative amino acid substitutions in comparison with nonconservative resistance mutations in protease, with a concomitant differential effect on enzyme function. Although speculative, this idea may relate to the fact that protease inhibitors were designed to fill up the catalytic site, which may necessitate rather blatant amino acid changes to interfere with drug binding. In contrast, most nucleoside RT drugs are simple variants of natural nucleoside triphosphates that lack the 3'-hydroxyl on the sugar ring and were not designed to optimally occupy the nucleoside triphosphate binding site. An exception is the drug AZT which has a bulky azido group that occupies the 3'-pocket of the catalytic site [28]. Interestingly, the evolution of AZT resistance is also rather exceptional among the nucleoside analogs in that multiple RT mutations are acquired over time. This continued evolution resembles the situation described for the evolution of resistance to protease inhibitors and suggests that the initial AZT-resistant RT enzymes exhibit functional defects that are repaired by second-site changes, and there is accumulating evidence for this idea [21, 23, 24, 42, 45]. These arguments prompt further modification of nucleoside analogs with bulky substituents because such drugs may provoke a more debilitating response.

The evolution of RT inhibitor resistance is also restricted in the sense that no compensatory changes are observed in other loci of the HIV-1 genome. One could argue that the involvement of a second locus is unique for the protease function which has an intimate interaction with cleavage sites in the Gag and Gag-Pol precursor protein. Although hypothetical, such second locus changes can be envisaged for the RT enzyme. For instance, RT mutants that show a defect at the level of initiation of reverse transcription, which is a regulated process involving higher-order structure in the retroviral genome, may be compensated by changes in the viral RNA sequences. As mentioned above (step 6), an RT defect may also be compensated for by increased frameshifting at the Gag-Pol junction.

Can One Use This Evolutionary Insight to Gain Clinical Benefit?

The incessant evolution observed during the acquisition of protease resistance is a clear indication that the virus is continuously striving to improve its fitness. This walk in the fitness landscape will continue as long as there are routes that go further uphill. In other words, as long as this evolution is ongoing, it is likely that the virus has not yet reached the approximate fitness of the 'wild-type' virus. But there may be certain drug regimens that can drive the HIV-1 evolution onto mountains of intermediate fitness from which it is impossible to escape in an uphill direction. This situation is depicted in pathway B of figure 2. Because viral fitness is an issue of clinical relevance, it is important to know whether drug-resistant viruses with a permanently restricted fitness can be selected [3]. The recent finding that some resistant proteases exhibit both Gag- and RT-processing defects [9] adds to this prospect, in that certain combination therapy regimens may be able to select for HIV-1 variants with reduced expression of variant RT and protease enzymes that exhibit inferior activity.

Reduced virus replication is expected to result in a decrease in viral load and an increase in the number of CD4+ cells. Such changes are associated with a decreased risk of disease progression, and even a partial reduction of

virus replication may be of considerable clinical benefit. For instance, it has been calculated that a 2-fold reduction in the number of productively infected cells can lead to a 2-fold increase in mean clinical latency [10]. Thus, it is of interest to learn more about drug-induced HIV-1 adaptation, the fitness gains along these evolutionary pathways and the replication fitness of the viral end product of evolution. These studies should indicate whether evolution can be steered by drugs into a direction of sequence space where the virus has lost its ability to regain full replication capacity [3]. Although this scenario may seem unlikely, one should realize that even the natural diversity in human and simian immunodeficiency viruses is likely to represent only a very limited section of total sequence space. The idea that the modern HIV-1 isolates occupy the summit of fitness, reached after a careful evolutionary walk through the whole sequence space, is implausible because the genetic repertoire of natural HIV-SIV isolates is constrained by the success of a predecessor of the contemporary viruses [5, 15].

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