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Lethal mutagenesis of HIV

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Abstract

HIV-1 and other retroviruses exhibit mutation rates that are 1,000,000-fold greater than their host organisms. Error-prone viral replication may place retroviruses and other RNA viruses near the threshold of "error catastrophe" or extinction due to an intolerable load of deleterious mutations. Strategies designed to drive viruses to error catastrophe have been applied to HIV-1 and a number of RNA viruses. Here, we review the concept of extinguishing HIV infection by "lethal mutagenesis" and consider the utility of this new approach in combination with conventional antiretroviral strategies.

Keywords: HIV-1; Retroviruses; Lethal mutagenesis

1. Introduction

Variation in HIV populations results from the error-prone nature of retroviral replication and the rapid turnover of virus in infected individuals (Coffin, 1995). Mutations in viral genomes provide the genetic potential for immune escape, changes in cellular and species tropism, and the development of antiviral drug resistance (Domingo and Holland, 1997; Rambaut et al., 2004). However, the ability of HIV to adapt to environmental pressures is not without cost; the available evidence suggests that error-prone replication imposes a substantial genetic load on retroviral populations, as discussed in this review. Thus, it has been suggested that the mutation rates of retroviruses and other RNA viruses approach the maximal value that is compatible with sustained production of infectious progeny (Holland et al., 1990). Violation of this theoretical threshold is predicted to result in a sudden and irreversible collapse of the population structure due to an intolerable number of deleterious mutations (Eigen, 1971). The ensuing loss of replicative potential is referred to as "error catastrophe" (Eigen, 2002).

In theory, proximity to the threshold of error catastrophe should render HIV susceptible to extinction due to slight increases in the mutation rate. This concept is the basis of an antiviral strategy designed to specifically increase the error rate of retroviral replication. Here, we review the theoretical and experimental grounds for this strategy, termed "lethal mutagenesis" (Loeb et al., 1999). We begin by examining the sources of spontaneous mutations in retroviral genomes and the rate at which these errors are formed during viral replication. Next, we review data from in vitro studies suggesting that mutagenic compounds can increase the mutation rate of HIV-1 replication beyond the error threshold. We also address recent findings suggesting that specific cellular enzymes can induce a natural form of error catastrophe by directly altering the sequence of the HIV-1 genome. Finally, we examine the possibility of using virus-specific mutagens in combination with conventional antiretroviral drugs, and discuss potential challenges to this new therapeutic approach.

2. Retroviral mutagenesis

Genetic diversity and phenotypic variation are intrinsic properties of retroviral populations. This fundamental aspect of retroviral biology was appreciated as early as 1913, when Rous and Murphy demonstrated that chickens infected with

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serially-passaged strains of Rous sarcoma virus (RSV) often developed tumor types that differed from those produced by the parental virus isolate (Rous and Murphy, 1913). Phenotypic variation was also documented in pioneering studies by Howard Temin, who observed heritable differences in the morphology of cells infected with different strains of RSV (Temin, 1960). As other retrovirus species were isolated and characterized, it became apparent that independent isolates of the same species often varied greatly in tumorigenic and cytopathic potential, cell tropism, and drug sensitivity (Vogt, 1997). This inherent instability placed retroviruses among other RNA viruses, which exhibit similar propensities for phenotypic change (Temin, 1989).

DNA sequence analyses later demonstrated that retroviruses are subject to substantial genotypic variation, as extensively documented in HIV-1 (Coffin, 1986, 1995; Desai et al., 1986; Goodenow et al., 1989; Hahn et al., 1986). Estimates of the average number of nucleotide differences in pairwise comparisons of patient isolates range from 15 to 25% for portions of the *env* gene (Buonaguro et al., 1995; Learn et al., 1996; Murphy et al., 1993; Wang et al., 1995). Clonal analyses demonstrate that multiple subclasses of variants simultaneously coexist in HIV-1-infected individuals and that the relative frequencies of these genotypes often fluctuate during the course of natural infection (Goodenow et al., 1989; Liu et al., 2002; Meyerhans et al., 1989; Shankarappa et al., 1999). These findings have contributed to the widely-accepted view that retroviruses and RNA viruses exist as complex mixtures of related but genetically-distinct subtypes, frequently referred to as "swarms" (Temin, 1989) or "quasispecies" (Domingo et al., 1985; Domingo, 2003; Eigen, 1993).

2.1. Sources of mutations in retroviral genomes

The diversity of retroviral populations is a direct result of the error-prone nature of retroviral replication. Mutations in HIV-1 genomes primarily arise during three distinct polymerization steps in the retroviral life cycle (Fig. 1):

1) RNA-templated, minus-strand DNA synthesis by the viral reverse transcriptase (RT).
2) DNA-templated, plus-strand DNA synthesis by RT.
3) DNA-templated, plus-strand RNA synthesis by host-encoded RNA polymerase II (RNA pol II).

Measurements of the fidelity of RT-catalyzed DNA synthesis in vitro (reviewed in Menendez-Arias, 2002; Preston and Garvey, 1992; Preston and Dougherty, 1996; Svarovskaia et al., 2003) indicate that reverse transcriptases are substantially less accurate than cellular replicative DNA polymerases. Much of the difference in fidelity is due to the lack of an associated $3' \rightarrow 5'$ exonucleolytic proofreading activity in RT (Battula and Loeb, 1976; Roberts et al., 1988). The disparity in the error rates of RT and cellular replicative DNA polymerases suggests that mutations produced during copying of integrated proviral DNA are relatively rare and contribute little to the genetic diversity of actively-replicating retroviruses (Gojobori and Yokoyama, 1985). This view is supported by data showing that a large proportion of the mutations formed during a single cycle of viral replication in culture can be attributed to RT (Kim et al., 1996; O'Neil et al., 2002; Zhang, 2004). In contrast, much less is known about the role of RNA polymerase II in retroviral mutation. Experiments with prokaryotic and plant RNA polymerases suggest that transcription is a relatively error-prone process (Blank et al., 1986; de Mercoyrol et al., 1992; Libby and Gallant, 1991), though the fidelity of RNA synthesis can be modulated by other components of the cellular transcription machinery (Erie et al., 1993; Jeon and Agarwal, 1996; Koyama et al., 2003; Lange and Hausner, 2004; Shaw et al., 2002; Thomas et al., 1998). Analyses of mutations produced during retroviral vector replication also suggest that RNA pol II fidelity contributes to viral variation (Kim et al., 1996; O'Neil et al., 2002). However, the magnitude of this contribution remains unclear.

Other sources of errors are also likely to generate diversity in viral populations. Fluctuations in nucleotide pool levels (Julias and Pathak, 1998; Vartanian et al., 1994) and/or incorporation of dUTP (Chen et al., 2002; Lerner et al., 1995) may generate mutations during viral DNA synthesis. Spontaneous chemical decay of viral RNA or DNA produces aberrant bases that miscode during transcription or reverse transcription (Lindahl, 1993). RT incorporates damaged nucleotides during DNA synthesis in vitro (Bebenek et al., 1999; Feig et al., 1994; Furge and Guengerich, 1997; Hizi et al., 1997; Kamath-Loeb et al., 1997a; Preston et al., 1986) and inserts incorrect nucleotides across from damaged bases in RNA and DNA templates (Furge and Guengerich, 1997). These findings supported the suggestion that exposure of HIV-infected cells to damaged nucleosides might increase the viral mutation rate, thereby driving HIV to error catastrophe (Preston et al., 1988; see below).

Modification of bases in viral RNA or DNA by host-encoded enzymes also represents a potential source of mutations. Studies in both RNA viruses and retroviruses suggest that host enzymes other than DNA polymerases produce 'hypermutations', which occur as clusters of specific base substitutions in the viral genome (Cattaneo et al., 1988; Pathak and Temin, 1990a). For example, the pattern of $A \rightarrow G$ hypermutations observed in avian retroviruses suggests that these mutations result from the editing activity of double-stranded RNA deaminases (Bass, 1997; Felder et al., 1994; Hajjar and Linial, 1995; Pathak and Temin, 1990a). More recently, the cellular enzyme APOBEC3G has been shown to generate $G \rightarrow A$ hypermutations in HIV-1, (Bhagwat, 2004; Goff, 2003; KewalRamani and Coffin, 2003; Vartanian et al., 2003; see below). Thus, as with other RNA viruses (Cattaneo et al., 1988; Macnaughton et al., 2003; Murphy et al., 1991; O'Hara et al., 1984; Polson et al., 1996; Rueda et al., 1994), host-encoded enzymes influence the biological properties of retroviral genomes by directly altering the viral coding sequence.