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Mechanistic interplay among the M184I HIV-1 reverse transcriptase mutant, the central polypurine tract, cellular dNTP concentrations and drug sensitivity

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ABSTRACT

We recently reported that the M184I 3TC resistant mutation reduces RT binding affinity to dNTP substrates. First, the HIV-1 M184I mutant vector displays reduced transduction efficiency compared to wild type (WT) RT vector, which could be rescued by both elevating the cellular dNTP concentration and incorporating WT RT molecules into the M184I vector particles. Second, the central polypurine tract (cPPT) mutation and M184I mutation additively reduced the vector transduction to almost undetectable levels, particularly in nondividing cells. Third, the M184I (−) cPPT vector became significantly more sensitive to 3TC than the M184I (+) cPPT vector, but not to AZT or Nevirapine in the dividing cells. Finally, this 3TC sensitizing effect of the cPPT inactivation of the M184I vector was reversed by elevating the dCTP level, but not by the other three dNTPs. These data support a mechanistic interaction between cPPT and M184I RT with respect to viral replication and sensitivity to 3TC.

Introduction

The ability of lentiviruses to infect terminally differentiated/nondividing macrophages is unique among retroviruses (Lewis et al., 1992; Lewis and Emerman, 1994; Weinberg et al., 1991) and also important for their utility as gene delivery tools for nondividing cell types such as neurons (Naldini et al., 1996). The infection of macrophages by human immunodeficiency virus type 1 (HIV-1) produces a landmark viral phenotype in the early phase of HIV-1 pathogenesis (Crowe et al., 2003; Verani et al., 2005). Various viral elements have been identified that enable lentiviruses to infect nondividing cells, such as Vpr, the central polypurine tract (cPPT)-DNA flap, and reverse transcriptase (RT) (as reviewed by Yamashita and Emerman) (De Rijck et al., 2007; Yamashita and Emerman, 2006). We have also previously reported that lentiviral RTs are able to aid in the infection of non-diving cells by efficiently synthesizing proviral DNA in limited dNTP pools, such as those found in macrophage, due to their unique high affinity to dNTP substrates (Diamond et al., 2004).

The cPPT sequence, which is found among lentiviruses (Charneau et al., 1992), lies at the exact center of the ~9.6 kb long HIV-1 RNA genome within the coding region of the integrase gene. A key function of the cPPT is to generate an additional RNA primer to initiate second (+) strand viral DNA synthesis (Charneau et al., 1992). The cPPT-central termination sequence (CTS) has also been proposed to create a flap at the end of (+) strand DNA synthesis, which is recognized by unknown host/viral factors (Arhel et al., 2007). This flap is believed to enhance the nuclear import of the pre-integration complex (PIC) in nondividing cells (Zennou et al., 2000). However, since the flap does not have to be generated at the exact middle in order to be recognized by host or viral factors (De Rijck et al., 2005), the reason why the cPPT lies at the center of the genome still remains unclear. Furthermore, the role of the cPPT in nuclear import also remains controversial, since it has been shown that HIV-1 lacking the cPPT still efficiently replicates in macrophages and CD4+ T cells (Dvorin et al., 2002; Marsden and Zack, 2007).

We have recently reported that the addition of the cPPT can compensate for the delayed proviral DNA synthesis in a 3-vector system under the control of a cytomegalovirus promoter. The HIV-1 vectors harbored the RT mutants, V148I and Q151N, which are defective in binding to dNTPs and thus fail to synthesize proviral DNA in cells with low dNTP concentrations, such as macrophages (Skasko and Kim, 2008). This finding suggested that the cPPT is necessary for HIV-1 to accelerate (+) strand DNA synthesis by creating an additional priming site, which can shorten the length of (+) strand synthesis replicated by one primer from 9.6 kb to ~5 kb. Thus, it would be reasonable to assume that the cPPT becomes more critical for HIV-1 replication in macrophages where the viral replication kinetics are slow due to limited dNTP pools (Diamond et al., 2004), and during the delayed replication of HIV-1 vector containing partially defective RT mutations such as Q151N and V148I (Diamond et al., 2004; Jamburuthugoda et al., 2006, 2008).

The HIV-1 M184I RT mutation is clinically important due to its 3TC resistance, which can be generated through the deamination of cytosine at this residue by APOBEC3G (Mulder et al., 2008). This RT

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mutation appears consistently in 3TC therapy, and ultimately results in a M184V mutation (Frost et al., 2000). The transient nature of the M184I mutation is likely due to its limited replicational fitness, compared to the M184V mutation (Domaoal et al., 2008; Frost et al., 2000). In fact several biochemical defects in the M184I mutant RT have been reported (Gao et al., 2008; Jamburuthugoda et al., 2008; Sarafianos et al., 1999). The β-branched side chain of isoleucine in M184I reduces the binding affinity of RT for dNTP substrates and raises its K_d approximately 50-fold (from 1 μM of WT RT K_d to 56 μM) (Jamburuthugoda et al., 2008). Thus M184I RT exhibits a severely decreased activity at low dNTP concentrations (Jamburuthugoda et al., 2008), whereas M184V RT, which displays WT levels of dNTP binding affinity (Domaoal et al., 2008), remains enzymatically active at both the low and high dNTP concentrations found in macrophages and activated CD4+ T cells, respectively (Aquaro et al., 2005; Diamond et al., 2004). Structural studies also demonstrated that the M184I mutation alters the template–primer interactions of RT, which explains its reduced processivity (Gao et al., 2008).

In this report, we investigated the mechanistic interplay between host dNTP pools, HIV-1 RT dNTP binding affinity and cPPT with respect to viral sensitivity and resistance to RT inhibitors. We have identified a specific compensatory relationship between the viral resistance of M184I HIV-1 RT and its dependence on the cPPT and cellular dNTP concentrations.

Results

The effect of the cPPT and cellular dNTP concentrations on HIV-1 replication

First, we investigated the effects of the cPPT mutation and the cellular dNTP concentrations on the transduction efficiency of a HIV-1 vector in primary human lung fibroblasts (HLF). HLFs have been used as an ideal model system for both dividing and nondividing cells because their cell cycles can be easily manipulated (Jamburuthugoda et al., 2008; Skasko and Kim, 2008). In addition, the cellular dNTP concentrations of HLFs can be modulated by both deoxynucleoside (dN) treatment and serum starvation (Jamburuthugoda et al., 2006). Dividing HLFs cultured in 10% serum have an intracellular dNTP concentration of approximately 150−300 nM (Jamburuthugoda et al., 2006), which is higher than the dNTP concentration found in human macrophages (~50 nM), but lower than activated CD4+ T cells (2−4 μM) (Charneau et al., 1992; Diamond et al., 2004). Treating dividing and nondividing HLFs with deoxynucleosides (dNs) elevates their cellular dNTP concentration to 50 μM and ~1 μM, respectively (Jamburuthugoda et al., 2008). In this study, we employed a VSV-G pseudotyped D3HIV-GFP vector which encodes a ~9.6 kb long HIV-1 NL4-3 based RNA genome similar to the length of an infectious HIV genome, and expresses all HIV-1 proteins except for Env and Nef, which was replaced with GFP. The cPPT sequence of this vector was inactivated by mutating a series of purine bases to pyrimidine bases without altering the amino acid sequence of the integrase gene (Fig. 1A), as described previously (Charneau et al., 1992).

First, we transduced HLFs with an equivalent p24 level (2.77×10^5 pg) of the (+) and (−) cPPT WT RT HIV-1 vectors and cultured them in three separate media conditions; 10% serum that allows for cellular division, 0.2% serum (nondividing), and also 0.2% serum supplemented with 1 mM of dNs. GFP expression was determined through FACS analysis at 48 h post transduction. As shown in Fig. 1B, the cPPT mutation had only a 1.8-fold decrease in the WT RT vector transduction in dividing HLFs, while a 7.7-fold decrease was observed by the cPPT inactivation in nondividing HLFs. Combining cPPT inactivation and nondividing culture conditions led to a 9-fold decrease of the WT RT vector transduction. This large decrease in transduction with nondividing HLFs could be due to loss of the cPPT functions in both nuclear import and the replication enhancement during (+) strand DNA synthesis. However, as seen in Fig. 1B, the treatment with dNs only slightly (1.5×) compensated for the 9-fold transduction decrease seen in the nondividing HLFs. This minimal compensation by the dN treatment supports the idea that a major factor for the cPPT inactivation, which induced a 9-fold decrease of the WT RT vector transduction in nondividing cells, is the loss of the nuclear import function of cPPT which cannot be compensated by the elevation of cellular dNTP concentrations. In addition, the minimal effect of the dN treatment is predictable because WT RT alone is sufficient for the efficient proviral DNA synthesis even at very low concentrations found in nondividing cells without the dNTP level elevation.

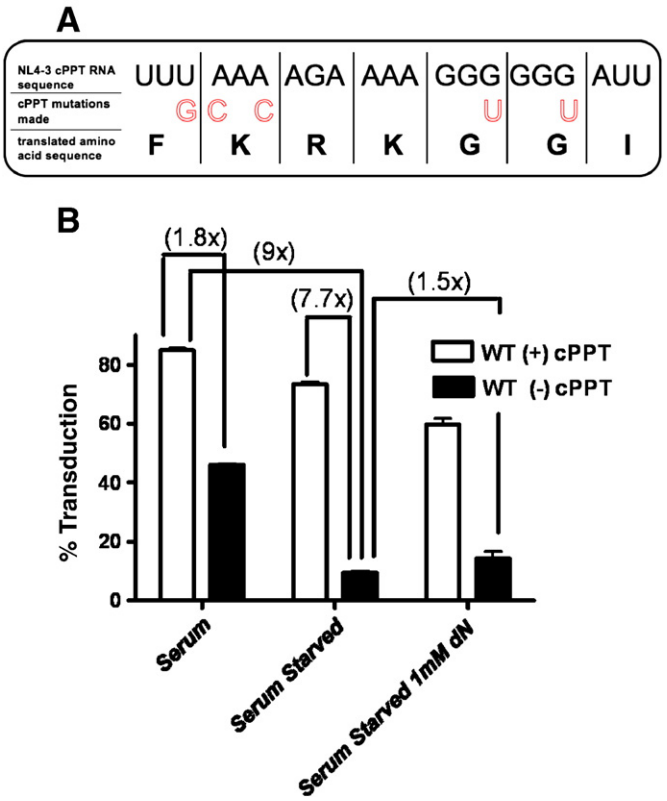

Fig. 1. Effect of cPPT mutation and cellular dNTP level on transduction efficiency of HIV-1 vector. (A) Nucleotide sequences of wild type and mutant NL4-3 cPPTs used in this study. The translated amino acid sequence of the cPPT is a part of HIV-1 integrase. (B) Effect of the cPPT mutation on the transduction of the WT RT vector in dividing (serum) and nondividing primary human lung fibroblasts (HLFs) with (serum-starved with 1 mM dN) and without (serum-starved) the elevation of cellular dNTP levels. Primary HLFs were plated, 2.5×10^5, in media containing 10% serum and then in media containing 0.2% serum for 48 h in the presence and absence of 1 mM dNs. HLFs cultured under these conditions were transduced with 2.77×10^5 pg of WT RT HIV-1 vector with (+) or without (−) the cPPT. The transduced cells were analyzed by FACS for GFP expression at 48 h post transduction and the transduction efficiency was plotted. The experiments presented in this figure were performed at least in triplicate.

Roles of the cPPT and cellular dNTP pools in a HIV-1 vector harboring the 3TC resistant M184I RT mutant

Next, we investigated the effects of the cPPT mutation and cellular dNTP concentrations on the transduction of the HIV-1 vector bearing the M184I RT variant. M184I RT displays reduced polymerase activity and is clinically relevant due to its 3TC resistance (Jamburuthugoda et al., 2008; Li et al., 2009). This mutant appears transiently but consistently during 3TC treatment and ultimately results in M184V (Frost et al., 2000). Importantly, we have previously reported that the M184I RT displays a significantly reduced dNTP binding affinity when compared to the wild type (WT) RT. However, the reduced activity of