Virology 468-470 (2014) 532–544

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Cellular HIV-1 inhibition by truncated old world primate APOBEC3A proteins lacking a complete deaminase domain

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ARTICLE INFO

Article history:
Received 9 June 2014
Returned to author for revisions
25 June 2014
Accepted 3 September 2014
Available online 28 September 2014

Keywords:
APOBEC3A
HIV-1
Restriction factor
Structure–function
Deaminase domain
DNA damage

ABSTRACT

The APOBEC3 (A3) deaminases are retrovirus restriction factors that were proposed as inhibitory components of HIV-1 gene therapy vectors. However, A3 mutational activity may induce undesired genomic damage and enable HIV-1 to evade drugs and immune responses. Here, we show that A3A protein from *Colobus guereza* (colA3A) can restrict HIV-1 replication in producer cells in a deaminase-independent manner without inducing DNA damage. Neither HIV-1 reverse transcription nor integration were significantly affected by colA3A, but capsid protein synthesis was inhibited. The determinants for colA3A restriction mapped to the N-terminal region. These properties extend to A3A from mandrills and De Brazza's monkeys. Surprisingly, truncated colA3A proteins expressing only the N-terminal 100 amino acids effectively exclude critical catalytic regions but retained potent cellular restriction activity. These highlight a unique mechanism of cellular HIV-1 restriction by several Old World monkey A3A proteins that may be exploited for functional HIV-1 cure strategies.

Introduction

Humans express several innate host factors that restrict the replication of lentiviruses such as human immunodeficiency virus type 1(HIV-1). These innate restriction factors include the apolipoprotein B mRNA-editing enzyme catalytic peptide 3 (APOBEC3; A3) proteins, bone marrow stromal antigen 2 (BST-2, also known as CD317, HM1.24 or tetherin), and tripartite motif (TRIM) proteins (Neil et al., 2008; Sheehy et al., 2002; Stremlau et al., 2004; Van Damme et al., 2008). These restriction factors have been the focus of intense study during the past decade due to their translational potential as novel drug targets and as antiviral components of gene therapy configurations for functional HIV-1 cure strategies (Ao et al., 2011; Bushman, 2002; Voit et al., 2013).

The A3 family of proteins includes seven members (A3A, A3B, A3C, A3D, A3F, A3G, and A3H) whose genes are tandemly arranged on human chromosome 22 and rhesus macaque chromosome 10 (Jarmuz et al., 2002; Schmitt et al., 2011). The APOBEC3 proteins are cytidine deaminases that also include activation-induced cytidine deaminase (AID), APOBEC1, APOBEC2, and APOBEC4. All A3 proteins have a canonical zinc-coordinating deaminase domain (H-x-E-x $_{23–28}$-PC-x$_{2–4}$-C) that converts cytidine to uracil (C-to-U) in DNA targets (MacGinnitie et al., 1995). The seven A3 proteins can be further divided into those with a single deaminase domain (A3A, A3C, and A3H) and with two deaminase domains (A3B, A3D, A3G, A3F) (Desimmie et al., 2013; Kitamura et al., 2011; Refsland and Harris, 2013). The anti-HIV-1 activity of the human A3G (hA3G) was initially discovered during the characterization of wild type HIV-1 and HIV-1Δ*vif* viruses produced from permissive (non-A3G expressing) and non-permissive (A3G expressing) cells (Sheehy et al., 2002). A3G has been shown to restrict HIV-1Δ*vif* by both deaminase-dependent and independent mechanisms. Restriction requires its incorporation into the viral nucleocapsid during virion egress from producer cells. The Vif protein counteracts A3G incorporation by acting as an adapter that interacts with both A3G and members of the Cullin 5 E3 ligase complex (Mehle et al., 2004, 2006; Xiao et al., 2006). This results in the ubiquitination both Vif and the A3G and subsequent degradation by the proteasome, thus limiting the amount of A3G that could be incorporated into virions (Conticello et al., 2003; Iwatani et al., 2007; Liu et al., 2004; Marin et al., 2003; Mehle et al., 2004; Sheehy et al., 2002, 2003;Yu et al., 2003). The mechanism for how hA3G inhibits HIV-1Δ*vif* replication is controversial, with different groups preferring either a deaminase-dependent or deaminase-independent mechanism (Santiago and Greene, 2007). When A3G containing

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http://dx.doi.org/10.1016/j.virol.2014.09.001
0042-6822/

viruses infect a target cell, the incorporated A3G causes cytidine deamination of minus strand viral DNA during reverse transcription (Chelico et al., 2006; Yu et al., 2004). The end result of the action of this cytidine deaminase is G-to-A mutations in the sense strand of the viral DNA incorporated into the host chromosome. Deaminase-independent mechanisms of restriction involve inhibition of reverse transcription by preventing: (a) tRNA annealing to the viral RNA; (b) DNA elongation; and (c) strand transfer (Guo et al., 2006, 2007; Iwatani et al., 2007; Li et al., 2007; Yang et al., 2007). Another report showed that A3G and A3F can interact with integrase and prevent integration (Luo et al., 2007).

Previous studies have shown that hA3A does not restrict the replication of HIV-1Δ*vif* in CD4$^+$ T cells but restricts HIV-1Δ*vif* in macrophages (Berger et al., 2011; Bishop et al., 2004; Koning et al., 2011; Thielen et al., 2010). The inability of hA3A to restrict HIV-1Δ*vif* has been linked to its poor incorporation into the nucleocapsid complex during maturation. Targeting hA3A to the nucleoprotein complex by either fusion to the N-terminal domain of A3G or fusion to Vpr results in enhanced incorporation and restriction activity (Aguiar et al., 2008; Goila-Gaur et al., 2007). Human A3A has been shown to inhibit retrotransposition, to induce DNA damage responses, and inhibit the replication of adeno-associated viruses and autonomously replicating parvoviruses (Bogerd et al., 2006; Bulliard et al., 2011; Chen et al., 2006; Landry et al., 2011; Muckenfuss et al., 2006; Narvaiza et al., 2009). Finally, hA3A can restrict HTLV-I and can cause G-to-A hypermutation of human papillomavirus, herpes simplex 1 and Epstein Barr virus genomes (Ooms et al., 2012; Suspène et al., 2011b; Vartanian et al., 2008).

Until recently, all APOBEC3 proteins shown to restrict HIV-1Δ*vif* replication do so in the target cell, not the producer cell. In an effort to understand APOBEC3-mediated lentivirus restriction from an evolutionary perspective, we examined the virus restriction activity of several Old World monkey A3A proteins and showed that the A3A protein from the black and white colobus monkey (mantled guereza; *Colobus guereza*; colA3A) potently inhibited not only HIV-1Δ*vif* replication but also wild-type HIV-1 in producer cells (Schmitt et al., 2013). In this report, we extend these findings by showing that the N-terminal domain of colA3A is critical for cellular HIV-1 restriction, that the A3A proteins from other OWM can also restrict the replication of wild type HIV-1 and that colA3A affects HIV-1 replication at a post-integration step. The results could have important implications in designing gene-therapy approaches using on the unique HIV-1 restriction properties of colA3A, mndA3A or debA3A.

Results

Cells expressing HA–colA3A inhibit HIV-1 production

TZM-bl cells were transfected with a vector expressing HA–colA3A and selected for G-418 resistance for 2 weeks. The resulting G-418 resistant cells were analyzed for the expression of HA–colA3A by immunoprecipitation using an antibody against the HA-tag. The immunoprecipitation analysis revealed that the TZM-bl cells transfected with the vector expressing HA–colA3A expressed HA–colA3A while the parental TZM-bl cell line was negative for HA–colA3A expression (Fig. 1A). We used these HA–colA3A expressing cells to determine if they would restrict the replication of HIV-1. TZM-bl cells expressing HA-colA3A were inoculated with HIV-1 at an MOI of 1.0 and at 48 h post-inoculation, the culture supernatants were assessed for Gag p24 release (Fig. 1B). The results indicate that HIV-1 production was significantly inhibited in TZM-bl cells expressing HA–colA3A, analogous to our previous co-transfection studies (Schmitt et al., 2013).

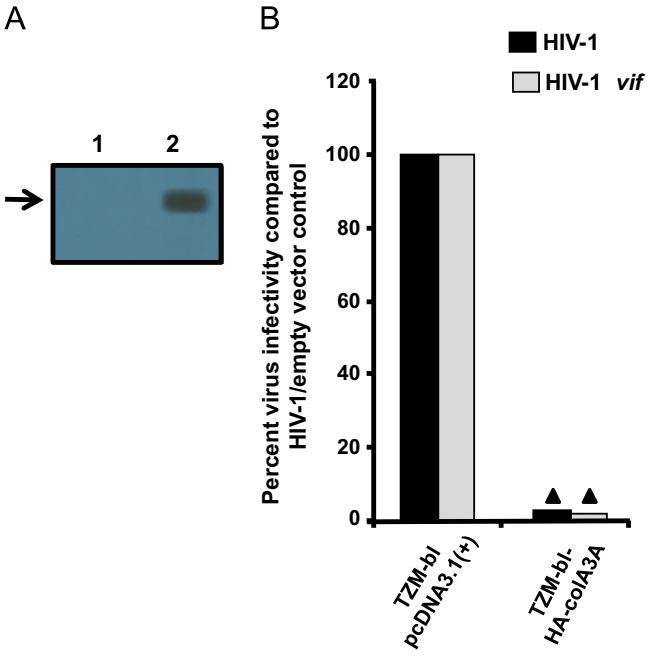

Fig. 1. TZM-bl cells expressing HA–colA3A restrict HIV-1 replication. Panel A. TZM-bl cells were transfected with pcDNA3.1(+) expressing HA–colA3A and selected with G-418 for two weeks. These cultures were starved for methionine/cysteine and then radiolabeled with 200 μCi of ^{35}S-methionine/cysteine for 6 h. Cell lysates were prepared and HA-containing proteins immunoprecipitated using an anti-HA antibody. The proteins were separated by SDS-PAGE (12% gel) and visualized by standard autoradiography techniques. Lane 1. HA-containing proteins immunoprecipitated from TZM-bl–colA3A cells using a control rabbit antibody. Lane 2. HA-containing proteins immunoprecipitated from TZM-bl–colA3A cells using a rabbit anti-HA antibody. Panel B. TZM-bl cells expressing HA–colA3A cells restrict HIV-1 replication. TZM-bl and TZM-bl cells expressing HA–colA3A in 6-well plates were inoculated with 10^3 $TCID_{50}$ of HIV-1 or HIV-1Δ*vif* for 4 h. The inoculum was removed, washed three times and fresh medium added to cultures. At 48 h, the culture medium was harvested and virion infectivity measured using TZM-bl cells. The experiments were performed at least three times. Shown is the mean percentage virion infectivity of virus inoculated onto TZM-bl cells normalized to 100%. Statistical differences with the wild-type control were evaluated using a two-tailed Student's *t*-test, with $p < 0.05$ (Δ) considered significant.

ColA3A does not affect HIV-1 reverse transcription

We determined if colA3A inhibited reverse transcription of HIV-1. For these experiments, 293 cells were transfected with either the empty pcDNA3.1(+) vector or one expressing HA–colA3A. At 24 h post-transfection, cultures were inoculated with VSV-G pseudotyped envelope glycoprotein defective HIV-1 (HIV-1ΔE-/VSV-G) and incubated for 0 or 24 h. Total DNA was isolated, RNase A-treated and used in PCR reactions to detect late (U5-*gag*) reverse transcription products. While the results indicate that the nucleoside reverse transcriptase inhibitor AZT inhibited U5-*gag* products, the levels of late reverse transcription products were not statistically different between cells transfected with the empty vector or one expressing HA–colA3A (Fig. 2). These findings indicate that colA3A does not inhibit HIV-1 replication at the step of reverse transcription.

ColA3A does not affect HIV-1 integration

One of the consequences of inefficient integration is an increase in the number of 2-LTR circles (Butler et al., 2001). 293 cells were transfected with a vector expressing colA3A for 24 h followed by inoculation of cultures HIV-1ΔE/VSV-G. The cells were incubated for an additional 48 h and real time DNA PCR used to quantify the levels of 2-LTR circles (Butler et al., 2001). Controls for 2-LTR circle formation included 293 cells inoculated with HIV-1ΔE/VSV-G in