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A chimeric human APOBEC3A protein with a three amino acid insertion confers differential HIV-1 and adeno-associated virus restriction

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ABSTRACT

Old World monkey (OWM) and hominid APOBEC3Aproteins exhibit differential restriction activities against lentiviruses and DNA viruses. Human APOBEC3A(hA3A)has weak restriction activity against HIV-1Δ*vif*but is efficiently restricted by an artificially generated chimeric from mandrills (mndA3A/G). We show that a chimeric hA3Acontaining the "WVS" insertion (hA3A[^{27}WVS^{29}]) conferred potent HIV-1restriction activity. Analysis of each amino acid of the "WVS" motif show that the length and not necessarily the charge or hydrophobicity of the amino acids accounted for restriction activity. Our results suggest that hA3A[^{27}WVS^{29}]restricts HIV-1at the level of reverse transcription in target cells. Finally, our results suggest that insertion of "WVS" into hA3Amodestly reduces restriction of adeno-associated virus 2(AAV-2)while insertion of the AC Loop1region of the mndA3A/G into hA3A abolished AAV-2 restriction, strengthening the role of this molecular interface in the functional evolution of primate A3A.

1. Introduction

Innate restriction factors provide potential antiviral strategies against invading pathogens such human immunodeficiency virus type 1(HIV-1). One such family of restriction factors is the apolipoprotein B mRNA-editing catalytic-polypeptide 3proteins (APOBEC3; A3), which consists of seven family members (A3A, A3B, A3C, A3D, A3F, A3G, A3H) whose genes are tandemly arranged on chromosome 22of humans and chromosome 10of rhesus macaques (Desimmie et al., 2013; Doehle et al., 2005; Feng et al., 2014; Jarmuz et al., 2002; Kim, 2014; Schmitt et al., 2011; Wissing et al., 2010). The A3proteins are cytidine deaminases, which can be further classified into those with one (A3A, A3C, A3H)and two (A3B, A3D, A3F, A3G) canonical cytidine deaminase domains (H-x-E-$x_{23–28}$-P-C-$x_{2–4}$-C) (MacGinnitie et al., 1995). In the absence of the viral protein Vif (HIV-1Δ*vif*), select members of this family can restrict virus replication (Bishop et al., 2004; Dang et al., 2006; Doehle et al., 2005; Li et al., 2010; OhAinle et al., 2006; Santiago and Greene, 2007; Sheehy et al., 2002; Wiegand et al., 2004; Zheng et al., 2004). If incorporated into the nascent virions from producer cells, these proteins can mediate cytidine deamination of the single-stranded DNA during reverse transcription in the subsequent target cell (Harris et al., 2003; Mangeat et al., 2003; Zhang et al.,2003). In addition to the deamination-dependent mechanism of restriction, deamination-independent mechanisms have been reported such as the inhibition of reverse transcriptase, strand transfer during reverse transcription, and inhibition of integrase (Guo et al., 2006, 2007; Iwatani et al., 2007; Li et al., 2007; Luo et al., 2007; Yang et al., 2007). HIV-1 counteracts the actions of the A3 proteins with the Vif protein, which acts as an adaptor protein that interacts with A3 proteins and the Cullin 5/Elongin B-C/rbx2 E3 ligase system (Liu et al., 2004; Mehle et al., 2004, 2006; Xiao et al., 2006; Yu et al., 2003; Yu et al., 2004). These interactions result in the subsequent ubiquitination of the A3 protein and targeting to the proteasome for degradation (Sheehy et al., 2003; Marin et al., 2003).

Human A3A is incorporated into HIV-1 and HIV-1Δ*vif* virions but has minimal activity against HIV-1 (Chen et al., 2006; Goila-Gaur et al., 2007). This is due to the observation that A3A is not

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hA3A            MEASPASGPRHLMDPHIFTSNFNNGI---IGRHKTYLCYEVERLDNGTSVKMDQHRGFLHN 57
hA3A[27WVS29]   ..........................WVS............................... 60
hA3A[27FVS29]   ..........................FVS............................... 60
hA3A[27YVS29]   ..........................YVS............................... 60
hA3A[27HVS29]   ..........................HVS............................... 60
hA3A[27WLS29]   ..........................WLS............................... 60
hA3A[27WTS29]   ..........................WTS............................... 60
hA3A[27WDS29]   ..........................WDS............................... 60
hA3A[27WVT29]   ..........................WVT............................... 60
hA3A[27WTA29]   ..........................WVA............................... 60
hA3A[27WVD29]   ..........................WVD............................... 60
hA3A[27WV28]    ..........................WV-............................... 58
hA3A[27VS28]    ..........................-VS............................... 59
hA3A[27WS28]    ..........................W-S............................... 59
hA3A[27W]       ..........................W--............................... 59
hA3A[27V]       ..........................-V-............................... 58
hA3A[27S]       ..........................--S............................... 58
hA3A[27AAA29]   ..........................AAA............................... 60
hA3A[27GGG29]   ..........................GGG............................... 60
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Fig. 1. Sequence of hA3A, hA3A[^{27}WVS^{29}], and other mutant proteins examined in this study. For each mutant, the amino acid substitution/insertion is bolded.

incorporated into the nucleoprotein complex. However, targeting A3A to the NPC by either fusion to the N-terminal region of A3G or Vpr A3A results in potent restriction (Aguiar et al., 2008; Goila-Gaur et al., 2007). A3A is expressed at higher levels than A3G in monocytes and is active against HIV-1 by restricting incoming virions (Berger et al., 2011; Koning et al., 2011; Peng et al., 2007; Thielen et al., 2010). A3A has several properties that distinguish it from other A3 proteins. It is localized in both the nucleus and the cytoplasm of growing cells, can activate a DNA damage response and cause cell cycle arrest (Landry et al., 2011; Schmitt et al., 2011, 2013). Unlike the highly processive A3G, A3A exhibits low or no processivity when deaminating synthetic ssDNA substrates (Chelico et al., 2006; Love et al., 2012). Finally, A3A has been reported to bind better to RNA than DNA and recent reports have implicated A3A in editing of RNA transcripts (Mitra et al., 2014; Niavarani et al., 2015; Sharma et al., 2015).

Unlike hA3A, the A3A from rhesus macaques (rhA3A) is capable of restricting simian-human immunodeficiency virus (SHIVΔ*vif*) and HIV-1Δ*vif* (Schmitt et al., 2011). Recently, we showed that the A3A proteins from three Old World monkey species, the colobus monkey, DeBrazza's monkey and the mandrill were capable of restricting not only HIVΔ*vif* but also HIV-1 (Katuwal et al., 2014). A unique feature of the reported A3A proteins from these Old World monkeys was the presence of an AC-loop 1 region characterized by the sequence (^{25}KP/LWVSGQR/HE^{33}), which differed from the sequence (^{25}DLSV/IRGRH/RQ^{33}) found in other Old World Monkeys (Henry et al., 2012). Based on the sequence from Henry and colleagues (2012), we constructed a colobus A3A (colA3A) and showed that unlike hA3A, colA3A could restrict HIV-1 (Katuwal et al., 2014). Recently, the sequence of these A3A proteins has been called into question (McLaughlin et al., 2016). These investigators provided genomic evidence that the exon with the sequence of the AC Loop1 region from colobus monkey, mandrill and DeBrazza's monkey is likely an artifact of the PCR oligonucleotides used to the amplify the sequences. These investigators showed that corrected colA3A had no restriction activity against HIV-1 (McLaughlin et al., 2016). Thus, we have renamed these proteins colA3A/G, manA3A/G, and debA3A/G.

Previously, we showed that insertion of the AC Loop 1 region from mndA3A/G into hA3A resulted in a protein with cellular HIV-1 restriction activity (Katuwal et al., 2014). Understanding the molecular determinants for potent HIV-1 restriction by A3A could have important implications in HIV-1 curative approaches that incorporate restriction factors. Unlike A3G, A3F and A3D, A3A is smaller and may be accommodated better in gene therapy vectors. However, one concern is that mndA3A/G may be immunogenic in humans. Thus, adding minimal modifications into hA3A to gain function may be warranted. In this study, we show that a hA3A protein containing the ^{27}WVS^{29} motif from the mndA3A/G chimeric protein could restrict HIV-1. We have further interrogated the amino acid requirements at these three positions in HIV-1 restriction and show that length of this sequence is likely more important than the nature of the amino acids at these positions. Interestingly, insertion of the mndA3A/G AC Loop-1 region into hA3A abrogated restriction of the human parvovirus AAV-2.

2. Results

2.1. The mutant hA3A proteins are stably expressed in cells

We previously reported that hominid and Old World Monkey (OWM) A3A proteins differentially restrict lentiviruses and DNA viruses, respectively (Schmitt et al., 2011). Previously we mapped the differential restriction activity of mndA3A (prior to data showing it is an mndA3A/G chimera) to the AC-Loop1 region (Katuwal et al., 2014), but the specific amino acid residues involved remain unclear. Thus, we constructed an hA3A protein with ^{27}WVS^{29} motif inserted into hA3A (Fig. 1). We analyzed the steady state levels expression of HA-hA3A, HA-hA3A[^{27}WVS^{29}] and the other hA3A proteins analyzed in this study. 293 cells were transfected with either the empty pcDNA3.1(+) vector or vectors expressing each hA3A protein for 48 h. The cells were starved for methionine/cysteine and then radiolabeled with ^{35}S-methionine/cysteine for 6h. The cells were lysed in 1X RIPA buffer and HA-tagged proteins immunoprecipitated with an anti-HA antibody and protein A Sepharose. The results showed that all proteins were expressed well and the steady-state levels of HA-hA3A and HA-hA3A[^{27}WVS^{29}] were similar (Fig. 2).

2.2. The introduction of the ^{27}WVS^{29} motif into hA3A is sufficient to restrict HIV-1

We next analyzed the ability of HA-hA3A[^{27}WVS^{29}] to restrict HIV-1 and HIV-1Δ*vif* virions. As shown in Fig. 3A and we previously reported, neither hA3A nor hA3G restricted HIV-1 infectious titers as measured by the TZM.bl assay. As expected, HA-hA3G but not HA-hA3A restricted HIV-1Δ*vif*. In contrast to HA-hA3A, HA-hA3A[^{27}WVS^{29}] restricted both HIV-1 and HIV-1Δ*vif* (Fig. 3A and B). These results indicated that the insertion of [^{27}WVS^{29}] into hA3A was sufficient to confer hA3A with the ability to restrict HIV-1 infectious titers. To determine if this effect is due to reduction in virus particle production, we next determined the levels of p24 in the supernatant. Interestingly, p24 released from