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Human APOBEC3F incorporation into human immunodeficiency virus type 1 particles

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ABSTRACT

APOBEC3 proteins are a family of cytidine deaminases that exhibit broad antiretroviral activity. Among APOBEC3 proteins, APOBEC3G (hA3G) and APOBEC3F (hA3F) exhibit the most potent anti-HIV-1 activities. Although the incorporation of hA3F into virions is a prerequisite for exerting its antiviral function, the detail mechanism underlying remains incompletely understood. In this work, we present data showing that the nucleocapsid (NC) domain of HIV-1 Gag and a linker sequence between the two cytidine deaminase domains within hA3F, i.e., 104-156 amino acids, are required for viral packaging of hA3F. A detailed mapping study reveals that the cluster of basic residues surrounding the N-terminal zinc finger (ZF) and the linker region between the ZFs of HIV-1 NC play an important role in A3F incorporation, in addition, at least one of two ZFs is required. A hA3F fragment is able to compete with both hA3G and hA3F for viral incorporation, suggesting a common mechanism underlying virion encapsidation of hA3G and hA3F. Taken together, these results shed a light on the detail mechanism underlying viral incorporation of hA3F.

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1. Introduction

APOBEC superfamily consists of a large group of cytidine deaminases that exhibit RNA or DNA editing activity. The presence of one or two cytidine deaminase domains is a common characteristic of these proteins, which bear a conserved zinc-binding motif (Cys/His)-X-Glu-X23-28-Pro-Cys-X2-4-Cys (Conticello, 2008; Jarmuz et al., 2002; Rogozin et al., 2005). In humans, the APOBEC family of proteins comprises eleven members, including activation-induced deaminase (AID), APOBEC1, APOBEC2, APOBEC3 proteins, and APOBEC4. The human genome encodes up to seven different APOBEC3 proteins (designated hA3A, hA3B, hA3C, hA3DE, hA3F, hA3G, and hA3H, respectively), tandemly located in a genes cluster on human chromosome 22 (LaRue et al., 2009). hA3G and hA3F are best known for their potent antiviral activity against Vif-deficient HIV-1 replication (Chiu and Greene, 2009; Zheng et al., 2004), while others exhibit no or mild activity (Berger et al., 2011; Bourara et al., 2007; Dang et al., 2008, 2006; Doehle et al., 2005).

Both hA3G and hA3F are expressed in human CD4T cells that are principal targets for HIV-1 infection in vivo, suggesting that both enzymes naturally encounter HIV-1 (Ulenga et al., 2008). hA3G and hA3F restrict HIV-1 replication through deaminase activity dependent and independent mechanisms, and HIV-1 Vif counteracts their antiviral activity via proteasome-dependent degradation (Dang et al., 2010; Ebrahimi et al., 2012; Russell et al., 2009; Wang et al., 2007b). hA3F and hA3G exhibit a high degree of homology in amino acid sequence and domain organization, however, hA3F shows several unique characteristics that are different from hA3G in term of the target sequence for editing and the sensitivity to Vif (Bishop et al., 2006; Ebrahimi et al., 2012; Holmes et al., 2007; Lassen et al., 2010; Miyagi et al., 2010).

The encapsidation of hA3F into newly assembled progeny virions is a prerequisite for exerting its antiviral activity (Lassen et al., 2010). Several reports have demonstrated that the encapsidation of

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hA3F into HIV-1 particles is mediated by nucleocapsid (NC) domain of Gag protein (Wang et al., 2008; Yang et al., 2007). Cellular RNAs, such as 7SL RNA, are proposed to facilitate viral incorporation of hA3F (Bach et al., 2008; Zhang et al., 2010). However, much less attention has been paid to understanding how hA3F is packaged into HIV-1, compared with the intensive studies on the mechanism underlying viral incorporation of hA3G. In this report, we present data showing that the incorporation of hA3F into HIV-1 requires sequences between the two zinc coordination motifs of hA3F (amino acids 104-157) and the NC domain in Gag. Mutagenesis studies of Gag NC reveal that the basic amino acid residues flanking the N-terminal zinc finger (ZF) and at least one of two ZF motifs are important determinants for the incorporation of hA3F into HIV-1. This work sheds a light on the detail mechanism underlying viral incorporation of hA3F.

2. Materials and methods

2.1. Plasmid construction

SVC21BH10.P$^-$ is a simian virus 40-based vector that contains full length wild-type HIV-1 proviral DNA containing an inactive viral protease (D25G), and was a gift from E. Cohen, University of Montreal (Cen et al., 2004a). SVC21BH10.FS-contains mutations at the frameshift site, i.e., from 2082-TTTTTT-2087 to 2082-CTTCCT-2087, which prevents frameshifting during the translation of Gag protein, and generates viruses that contain Gag, but not Gag-Pol (Cen et al., 2004a). ZWt-p6 encodes a full-length HIV-1 genome, in which the nucleocapsid sequence has been replaced with a yeast leucine zipper domain (Accola et al., 2000). BH10.P$^-$.Vif$^-$, BH10.FS$^-$.Vif$^-$, and ZWt-p6.Vif$^-$ were generated by introducing a stop codon right after ATG of the Vif reading frame (Cen et al., 2004a). The hGag plasmid, which encodes the HIV-1 Gag sequence, produces mRNA whose codons have been optimized for mammalian codon usage and was a kind gift from G. Nabel (Cen et al., 2004a). All the N- or C-terminally deleted hGag expression plasmids were constructed, as previously described (Cen et al., 2004a). Plasmids coding for mutant HIV-1 Gag NC were generated using site-directed mutagenesis or overlapping PCR-based strategy, namely ZF1/MuLV ZF, ZF1/MMTV ZF1, ZF1/MMTVZF2, NC1-1, NC2-2, NC2-1, C15C18S, C36C39S, C15C18C36C39S, ΔZF1, ΔZF2, ΔZF1ZF2.

The plasmid expressing HA-tagged wild type hA3F was described previously (Wang et al., 2008; Yang et al., 2007). To construct mutant hA3F, this cDNA was PCR-amplified and digested with HindIII and XhoI, whose sites were placed in each of the PCR primers. These fragments were cloned into the HindIII and XhoI sites of the pcDNA3.1 V5/His A vector. We used the following primers: hA3F(104-374), 5′-AGA AAA GCT TAT GGG CCG AAT TCC TGT CTG AGC AC-3′; hA3F(157-374), 5′-AGA AAA GCT TAG ATG GAA AAC TTT GTG TAC AGTG-3′; hA3F(1-103), 5′-TAG ACT CGA GTC AAG CGT AAT CTG GAA CAT CGT ATG GAT ACA GCT TCG CCA CAC AGT CC-3′; hA3F(1-156), 5′-TAG ACT CGA GTC AAG CGT AAT CTG GAA CAT CGT ATG GAT ACC AGC AGT ATG CAA ATT CT-3′; hA3F(1-237), 5′-TAG ACT CGA GTC AAG CGT AAT CTG GAA CAT CGT ATG GAT ACT GCC TGA CTC AGC CTG CA-3′; hA3F(1-299), 5′-TAG ACT CGA GTC AAG CGT AAT CTG GAA CAT CGT ATG GAT AGA GAT TCA CGT TGC TGT GCC T-3′. The resulting constructs expressing HA-tagged mutant hA3F were transfected into 293T cells.

2.2. Cells, transfections, and viruses purification

293T cells were grown in complete Dulbecco's modified Eagle's medium plus 10% fetal calf serum, 100 units of penicillin, and 100 g of streptomycin/ml. For the production of viruses, HEK-293T cells were transfected using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Supernatant was collected 48 h post-transfection. Viruses were pelleted from culture medium by centrifugation in a Beckman SW-41 rotor at 35,000 rpm for 1 h.

2.3. Protein analysis

Cellular and viral proteins were extracted with radioimmune precipitation assay buffer (10 mM Tris, pH 7.4, 100 mM NaCl, 1% sodium deoxycholate, 0.1% SDS, 1% Nonidet P-40, 2 mg/ml aprotinin, 2 mg/ml leupeptin, 1 mg/ml pepstatin A, 100 mg/ml phenylmethylsulfonyl fluoride). The cell and viral lysates were analyzed by SDS-PAGE (10% acrylamide), followed by blotting onto nitrocellulose membranes (Millipore). Western blots were probed with monoclonal antibodies that are specifically reactive with HIV-1 capsid (Zepto Metrocs Inc.), HA (Santa Cruz Biotechnology Inc.), and β-actin (Abcom). Detection of proteins was performed by enhanced chemiluminescence (Millipore), using as secondary antibodies anti-mouse (for capsid and β-actin) and anti-rabbit (for HA), both obtained from Santa Cruz Biotechnology Inc. Bands in western blots were quantitated using ChemiDoc^{TM} MP (Bio-Rad) automated digitizing system.

2.4. Immunoprecipitation assay

293T cells from 100-mm plates were collected 48 h post-transfection and lysed in 500 μl of TNT buffer (20 mM Tris–HCl, pH 7.5, 200 mM NaCl, 1% Triton X-100). Insoluble material was pelleted at 1800 g for 30 min. The supernatant was used as the source of immunoprecipitated Gag/hA3F complexes. Equal amounts of protein were incubated with 30 μl of HA-specific antibody for 16 h at 4 °C, followed by the addition of protein A-Sepharose (Santa Cruz Biotechnology Inc.) for 2 h. For a Western blot of different cell lysates, 500 μg of lysate protein was used for immunoprecipitation from each lysate, while for different nuclease experiments on the same lysate sample, 200 μg of lysate protein was used for immunoprecipitation. Lysate protein was determined by the Bio-Rad assay. The immunoprecipitate was then washed three times with TNT buffer and twice with phosphate-buffered saline. After the final supernatant was removed, 30 μl of 2× sample buffer (120 mM Tris–HCl, pH 6.8, 20% glycerol, 4% SDS, 2% β-mercapto ethanol, and 0.02% bromphenol blue) was added and was analyzed using Western blots. In the nucleases treatment assay, the cell lysates were pretreated by 100 μg DNase or RNase for 20 min at 37 °C before the immunoprecipitation assay.

3. Results

3.1. The NC domain of Gag is necessary for the packaging of hA3F into HIV-1

We first examined whether hA3F is able to be packaged into viral like particles (VLPs). The plasmid coding for HA-tagged hA3F was co-transfected into 293T cells with the plasmids BH10.P$^-$.Vif$^-$ or hGag. BH10.P$^-$.Vif$^-$ encodes a mutant HIV-1 proviral DNA with inactive viral protease and Vif protein. The plasmid hGag contains a codon usage optimized HIV-1 Gag gene for translation in mammalian cells, and only produces Gag protein and VLP (Cen et al., 2004a). The cell and viral lysates were subjected to Western blot analysis using anti-HA and anti-CAp24, respectively. The results show that a similar amount of hA3F was found in immature virions and Gag VLP (Fig. 1A), suggesting that, among viral proteins, HIV-1 Gag alone is sufficient for viral incorporation of hA3F. This also indicates that HIV-1 genome is dispensable for the incorporation of hA3F into virions, due to the lack of genome RNA in Gag VLP (Cen et al., 2004a).

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