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The ADAR1 editing enzyme is encapsidated into HIV-1 virions

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ABSTRACT

Adenosine deaminase acting on RNA1 (ADAR1) was previously reported to affect HIV-1 replication. We report data showing that ADAR1 interacts with the HIV-1 p55 Gag protein, the major structural protein of the immature virus capsid. Furthermore, we found that the endogenous ADAR1 is incorporated into virions purified from the supernatant of primary HIV-1-infected $CD4^+$ T lymphocytes. Additional experiments demonstrated that the expression of the p55 Gag protein is sufficient for ADAR1 incorporation into virus-like particles (VLPs).

Overall, our data originally support the evidence that ADAR1 can be part of the cell protein array uploaded in HIV-1 particles.

Introduction

Adenosine deaminases that act on RNA (ADARs) catalyze the conversion of adenosine (A) to inosine (I) within a double-stranded RNA (RNA editing A to I) (George et al., 2014). Three ADAR enzymes were described in mammalian cells (ADAR1-3), but only ADAR1 and ADAR2 appear catalytically active (Valente and Nishikura, 2005).

There are two ADAR1 isoforms generated by the use of alternative promoters and splicing: the full length p150 ADAR1 form that is interferon-inducible, and the short p110 ADAR1 form that is constitutively expressed (Patterson and Samuel, 1995). ADAR1 proteins have a catalytic domain at the C-terminal, three double-stranded RNA binding motifs in the central region (dsRBD), and Z-DNA binding motifs at the N-terminal (Zβ in p110; Zα and Zβ in p150) (George et al., 2014). Since I is recognized as guanosine (G) by the cellular machinery, RNA editing by ADARs can lead to the formation of an altered protein when occurring within the coding sequence of a mRNA (Valente and Nishikura, 2005; Savva et al., 2012). Nonetheless, it has been recently demonstrated that most A-to-I RNA editing events occur within non-coding regions, introns, and untranslated regions (UTRs), affecting different aspects of RNA metabolism (Valente and Nishikura, 2005; Savva et al., 2012).

Increasing lines of evidence indicate that ADAR1 plays an important role in the replication process of different viruses, although there is no general consensus on the mechanism involved (i.e. dependent or not on the RNA editing activity) and on the outcome (either proviral or antiviral), possibly due to differences in the nature of the virus and the host cell being investigated (Samuel, 2011). Concerning HIV-1, most of the published data strongly indicates that ADAR1 exerts a positive effect on viral replication (Phuphuakrat et al., 2008; Clerzius et al., 2009; Doria et al., 2009; Clerzius et al., 2013), although some contrasting results have been reported (Biswas et al., 2012; Weiden et al., 2014). We and others showed that over-expressed ADAR1 increases the accumulation of HIV-1 proteins with a mechanism that is editing-independent probably by inhibiting the interferon (IFN)-inducible Protein Kinase RNA-activated (PKR) (Clerzius et al., 2009; 2013; Doria et al., 2009) that is a key modulator of translation initiation of viral and cellular mRNAs (Burugu et al., 2014). Moreover, we demonstrated that virions produced in the presence of over-expressed wt ADAR1, but not of an editing-inactive ADAR1 mutant, are released more efficiently and display enhanced infectivity (Doria et al., 2009). Furthermore, binding and editing of viral transcripts mediated by ADAR1 was clearly demonstrated (Phuphuakrat et al., 2008; Doria et al., 2009). In particular, A-to-I editing was identified in the 5′ untranslated region (5′UTR) and within the Tat and Rev sequences and in the vicinity of Rev responsive element (RRE) RNA within the *Env* gene (Phuphuakrat et al., 2008; Doria et al., 2009). Finally, silencing of ADAR1 expression in Jurkat T cells causes impairment of HIV-1 replication (Clerzius et al., 2013).

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Here, we address the question whether ADAR1 associates with viral proteins during HIV-1 replication.

We show that ADAR1 interacts with the HIV-1 p55 Gag protein, the major structural protein of the immature virus capsid and that the endogenous ADAR1 is incorporated into virions purified from the supernatant of primary HIV-1-infected $CD4^+$ T lymphocytes. Furthermore, we report that the sole expression of the p55 Gag protein is sufficient for ADAR1 incorporation into virus-like particles (VLPs). Taken together, our results shed a new light on the role of ADAR1 in HIV-1 replication.

Material and methods

Cells and transfection

293 GPR and 293T cells were grown in Dulbecco's modified Eagle's medium, 10% fetal calf serum and transfected by using Lipofectamine 2000 (Invitrogen).

$CD4^+$ T lymphocytes were isolated from the peripheral blood mononuclear cells of healthy donors by immunomagnetic-based negative selection.

Virion purification

CD4+ T lymphocytes were infected with VSV-G pseudotyped HIV-1 (NL4-3 strain), which was prepared as previously described (Federico et al., 2001) and used at 200 ng CAp24 equivalent/10^6 cells. After 24 h, the cell cultures were extensively washed, and after additional 24 h, the percentage of HIV-1-expressing cells was scored by FACS analysis using the anti-CAp24 KC57 mAb as described (Federico et al., 2001). In the presence of $>50\%$ infected cells, the supernatants were harvested, filtered through 0.45 μM pore size, loaded on 20% sucrose cushion, and ultracentrifuged at 100,000*g* for 2.5 h at 4 °C. Iodixanol gradient purification was performed as previously described (Dettenhofer and Yu, 1999). Briefly, concentrated vesicles were ultracentrifuged at 200,000 × g for 1.5 h at 4 °C in an SW41 Ti rotor (Beckman) through a 6–18% iodixanol density gradient formed by layering iodixanol in 1.2% increments. Then, 0.7 ml fractions were collected starting from the top and titrated by measuring HIV-1 CAp24 contents by quantitative ELISA (Innogenetics).

VLP production

293 GPR cells were used as HIV-1 packaging cells for VLPs production, as previously reported (Sparacio et al., 2001). In 293 GPR cells, the *gag-pol* genes are under the control of an ecdysone-inducible promoter, so that the lentiviral particle production requires cell stimulation with the ecdysone analog ponasterone A (PonA). It was previously shown (Sparacio et al., 2001) that adding to the cell medium butyrate (general inducer of gene expression) together with PonA results in a stimulation of HIV Gag/Pol particle expression to remarkably high levels compared to the stimulation with the PonA alone. Therefore, transfected 293 GPR cells were induced with 5 mM sodium butyrate and 2 μM PonA 8 h post-transfection. After 24 h, supernatants were replaced with fresh medium containing both inducers. VLP containing supernatants were finally harvested from 24 to 48 h later and clarified, and concentrated by ultracentrifugation on 20% sucrose cushion at 100,000 × *g*, for 2 h at 4 °C. VLP preparations were titrated by measuring HIV-1 CAp24 contents by quantitative ELISA (Innogenetics) and by reverse transcriptase assay processed as previously reported (Di Bonito et al., 2009).

Plasmids

The following vectors were previously described: pADAR1-p150-V5 (expressing the full length p150 ADAR1 enzyme fused with a C-terminal V5/6xHis double tag; Clerzius et al., 2009), pV5 vector (pcDNA3.1/V5 empty vector), pBabe-BCL2 (Sgadari et al., 2011), pEGFP-Gag (a vector expressing p55 Gag fused with the EGFP protein to its N-terminus NIH, AIDS Reagent Program catalog number 11468), hGag-Flag (a vector expressing the p55 Gag fused with the Flag sequence to its C-terminus) or derivative Gag mutants D378-Flag, that lacks the NC-p6 domains, and D133-Flag, that lacks the CA-NC-p6 domains, as previously described (Ma et al., 2008), pNL4-3 (HIV-1 proviral DNA).

Dual tag affinity purification

10×10^6 293T cells were co-trasfected with 8 μg of the proviral DNA (pNL4-3) together with 12 μg of either pADAR1-p150-V5 (expressing the full length p150 ADAR1 enzyme fused with a C-terminal V5/6xHis double tag; Clerzius et al., 2009) or pV5 vector (pcDNA3.1/V5 empty vector).

Transfected 293T cells were lysed in NP40 buffer (0.5% NP40, 50 mM Tris–HCl, 150 mM NaCl, 0.05% Tween 20, 50 mM NaH_2PO_4, pH 8, cocktails of protease and phosphates inhibitors from Roche and SIGMA, respectively) supplemented with 10 mM imidazole for 30 min on ice. Two milligrams of cell extracts were incubated with 40 μl of the NiNTA Magnetic Beads (Qiagen) for 3 h at 4 °C followed by several washes (using NP40 buffer with 20 mM imidazole) and elution of His-tagged native protein complex from the beads (using NP40 buffer with 250 mM imidazole). The eluted protein complex was then subjected to a second step of immunoprecipitation (Ip) using the anti-V5-tag magnetic beads (MBL) for 2 h at 4 °C followed by several washes with NP40 buffer. When indicated, RNase V1 (0.002 U/μl, Life Technologies) and RNase A (0.1 μg/μl, Sigma-Aldrich) were added to the cell extract and incubated at room temperature for 30 min before the Ip. The complete degradation of total RNA was assayed by loading a fraction (10 μg) of the treated and untreated cell extract on 1% agarose gel followed by electrophoresis and ethidium bromide staining. The Ip fraction and 20 μg of the total cell extract (input) were resolved by SDS-PAGE and transferred onto Nitrocellulose Membrane, and immunoblotted using specific antibodies.

Protein immunoprecipitation

293T cells transfected with 5 μg pADAR1-p150-V5 together with 5 μg of either pEGFP-Gag or pGFP or hGag-Flag or the truncated versions of hGag-Flag (D378-Flag and D133-Flag) expression vectors were lysed in NP40 buffer for 30 min on ice. Two milligrams of cell extracts were pre-cleared on IgG/dynabeads protein G (Invitrogen) and then co-Ip for 3 h at 4 °C with rotation using mAb either anti-V5 (Invitrogen) or anti-GFP (Invitrogen) or anti-Flag (Sigma-Aldrich) or control IgG (Santa Cruz Biotechnology) followed by incubation with dynabeads protein G (Invitrogen) for 3 h at 4 °C with rotation. The Ip fraction and 20 μg of the total cell extract (input) were resolved by SDS-PAGE and transferred onto Nitrocellulose Membrane and blot analyzed using specific antibodies.

Antibodies

The antibodies used were: anti-V5 (Invitrogen), anti-GFP (Invitrogen), AG3.0 anti-capsid antigen (CA) p24 antibody (NIH AIDS Research and Reference Program), anti-BCL2 (Santa Cruz Biotechnology), anti-Flag (Sigma-Aldrich), anti-APOBEC3G (ImmunoDiagnostics) and anti-ADAR1 (Santa Cruz Biotechnology).