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GANC Interacts with APOBEC3G and Facilitates Its Encapsulation into the Virions To Reduce HIV-1 Infectivity

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The ssDNA-dependent deoxycytidine deaminase apolipoprotein B mRNA-editing, enzyme-catalytic, polypeptide-like 3G (A3G) is a potent restrictive factor against HIV-1 virus lacking viral-encoded infectivity factor (Vif) in CD4+ T cells. A3G antiretroviral activity requires its encapsulation into HIV-1 virions. In this study, we show that germinal center–associated nuclear protein (GANC) is induced in activated CD4+ T cells and physically interacts with A3G. Overexpression of GANC augments the A3G activity requires its encapsulation into HIV-1 virions. In this study, we show that GANC is a cellular factor that facilitates A3G encapsidation into HIV-1 virions to inhibit viral infectivity. The Journal of Immunology, 2013, 191: 6030–6039.

Activation-induced cytidine deaminase (AID)/apolipoprotein B mRNA-editing, enzyme-catalytic, polypeptide-like (APOBEC) protein family is composed of 11 cytidine deaminases that catalyze C→U on ssDNA or RNA to regulate Ab diversification during adaptive immune response and to provide innate resistance against retroviruses and exogenously introduced genetic materials (1–3). AID has been well characterized as a critical initiator of Ig somatic hypermutation (SHM) and class-switch recombination in germlinal center (GC) B cells during the immune responses to T cell–dependent Ags (4–8). AID initiates SHM and CRS by catalyzing C→U conversion at actively transcribed V region and switch (S) region of Ig genes (4–8). In contrast to AID that acts preferentially at Ig loci in B cell genomic DNA, proteins of a subgroup APOBEC3 (A, B, C, DE, F, G, H) primarily target retroviral cDNA in the cytoplasm to cause lethal hypermutation in viral genomes (9). In particular, APOBEC3G (A3G) possesses a potent antiretroviral activity that restricts HIV-1 replication in T cells in the absence of viral infectivity factor (Vif) (10). A3G antiviral activity requires its encapsidation into the HIV-1 virions. Upon entry into the secondary infected target cells, A3G catalyzes deamination of C→U, preferentially at many 5′-YCC(Y = C or T) motifs located on the nascent minus-strand cDNA reverse transcribed from HIV-1 genomic RNA (gRNA) (9). Subsequently, this creates G→A hypermutation in the HIV-1 genome that potentially inactivates essential genes required for infectivity in the absence of Vif (11). Although Vif has been shown to inhibit A3G translation and promote A3G degradation through the core binding factor β-mediated proteosomal degradation (12,13), the action of Vif is not absolute, and a few copies of A3G are thought to be encapsidated into virions (14), probably through interaction with HIV-1 gRNA (15,16) and/or a number of cellular RNAs, including 7SL RNA (17–19). A3G-catalyzed G→A hypermutated HIV-1 genomes have been isolated from blood cells of HIV-1–infected patients at different stages of infection (20,21). A3G encapsidation into HIV-1 virions requires interactions with the nucleocapsid (NC) domain of a viral Gag protein, suggesting that incorporation of A3G into HIV virions occurs during viral assembly (18,22–24). In T cells, A3G is present in RNase-sensitive ribonucleoprotein (RNP) complexes localized in the cytoplasm (25–27) and enriched at mRNA processing bodies (26–31). Cellular proteins interacted with the RNP complex might also be important for A3G encapsidation. However, there is a little knowledge about the molecular mechanism and host cellular
proteins responsible for A3G encapsidation into HIV-1 virions (32, 33).

GC-associated nuclear protein (GANP) that was discovered as a protein upregulated in GC B cells during immune responses is physically associated with AID through its C-terminal side region (34). GANP is a component of transcription and export complex 2 interacted with RNP complexes and involving mRNA export in mammals (35, 36). GANP mutant mice studies have shown a strong correlation between the levels of GANP expression and SHM at the rearranged IgV loci. B cell–specific GANP-transgenic mice augment the generation of high-affinity Abs against the immobilized Ags in vivo (37), suggesting that GANP is an important functional AID partner in generation of high-affinity Abs in GC B cells. Recently, we have shown that GANP facilitates AID recruitment from the cytoplasm to the nucleus (38). GANP also augments AID targeting to the rearranged IgV region through interaction with many proteins composed of RNP complex and regulation of chromatin modification for nuclease occupancy at the selective IgV region site (39).

Given the similarity among AID/APOBEC proteins, we have explored a possibility that GANP interacts with A3G to regulate its localization in HIV-1 virions. In this study, we show that GANP is a cellular protein that is upregulated in CD4+ T cells and physically interacts with A3G to facilitate its targeted encapsidation into the HIV-1 virion.

Materials and Methods

Abs
The following Abs were purchased: β-actin (AC-15), hemagglutinin (HA)-7, and FLAG-M2 (from Sigma-Aldrich); HA (ab9110) and RNase A (ab6611) from Abcam; HA (C29F), calnexin (no. 2433), and β-tubulin (9F3) from Cell Signaling Technology; mouse IgG (sc-2025), rabbit IgG (sc-2027), and GANP (sc-83297) from Santa Cruz Biotechnology; and GANP (11054-AP) from Proteintech. Abs for Gag p24 (VAK4) (40) and Gag p17 (LG20-13-15) (41) were used. Anti-A3G (no. 9968) serum was provided from the National Institutes of Health AIDS Research and Reference Reagent Program. Allophycocyanin-conjugated anti-human CD4 (BioLegend), FITC-conjugated anti-CD69 (BD Biosciences), and PE-conjugated anti-CD25 (BD Biosciences) Abs were used for FACS staining.

T cell activation

PBMCs were isolated from healthy volunteers using a protocol approved by the Ethics Committee of Kumamoto University Faculty of Life Sciences. T cells were purified using a Pan T Cell Isolation Kit II and an automatic magnetic cell sorter (autoMACS) (Miltenyi Biotec). CD4+ T cells (1 × 10⁷) purified using CD4 MicroBeads were activated with 40 IU/ml IL-2, then stained with anti-CD4, anti-CD69, and anti-CD25 for purification by a FACSaria II cell sorter (BD Biosciences).

Cell culture

The 293T cells were maintained in DMEM. RPMI 1640 medium supplemented with 10% heat-inactivated FBS (Invitrogen) was used for culturing H9 T cells and Ramos B cells.

RNA purification, cDNA synthesis, and quantitative real-time PCR analysis

Total RNA was isolated using an RNasey Mini Kit (Qiagen) from cell lines. To measure the amount of cellular RNAs in cell-free virions, the viral RNA was isolated using a QIAamp Viral RNA Mini Kit (Qiagen). Gene expression was calculated and normalized to the amount of GAPDH expression in cells and of HIV-1 5’-LTR region of virions. RNA was reverse transcribed using a SuperScript III first-strand synthesis system (Invitrogen), and quantitative real-time PCR (qRT-PCR) was performed using MESA BLUE qPCR MasterMix Plus (Eurogentec) on the Applied Biosystems 7500. Gene-specific primers used in this study are listed in Supplemental Table I.

Plasmids

FLAG-GANP (38), A3G-HA (42), untagged A3G (43), wild-type (WT) and ΔVif pNL4-3 MLacE-R (42), and WT pNL4-3 proviral vectors were used.

Transfection and cell lysis preparation, immunoprecipitation, and Western blotting

Cells were transfected with FuGENE HD transfection reagent (Roche Diagnostics). Forty-eight hours after transfection, cells were harvested and lysed using TNE buffer (10 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40, 0.5% Triton X-100 [pH 7.5]) with a protease inhibitor mixture (Nacalai Tesque). Immunoprecipitation (IP) and Western blotting (WB) were performed as described previously (38). In some experiments, cellular and viral RNAs in cell lysates were removed by a treatment with 25 μg/ml RNase A for 30 min at 37°C before IP.

Viral preparation

Virus-like particles (VLPs) were produced from HIV-1 Gag mutant constructs (44). Vesicular stomatitis virus G protein (VSV-G) pseudotyped HIV-1 and ΔVif HIV-1 virions with a luciferase reporter were produced from WT pNL4-3 and ΔVif pNL4-3 in 293T cells. For the multiple-round infection assay, Vif proficient env-intact pNL4-3 vector was used. VLPs were produced from HIV-1 Gag mutant constructs. Virus particles or VLPs were harvested from the culture supernatant and purified by centrifugation on 20% sucrose cushion at 35,000 × g for 1 h. Relative amounts of HIV-1 virions in viral preparations were calculated based on p24 titer by ELISA (HIV-1 p24 Ag ELISA kit; ZeptoMetrix).

Fractionation of HIV-1 virion cores

HIV-1 virions were fractionated as reported by Soros et al. (45) with some modification. The virus pellet was treated with the hypotonic buffer containing 0.25% Triton X-100 (TX-100 buffer) for 2 min at 25°C and spun at 14,000 rpm for 8 min at 4°C to collect the supernatant (first supernatant). The insoluble pellet was incubated with the TX-100 buffer for 5 min and centrifuged at 14,000 rpm for 8 min at 4°C to separate into the second supernatant and the final pellet that was enriched for virion cores.

Viron immunoprecipitation

Detection of the protein-bound RNA in virion was performed either with anti-HA (for A3G) or anti-FLAG (for GANP) by modification of the RNA chromatin IP assay (38, 46). The virion amount was adjusted with p24 and fixed with 1% formaldehyde and cross-linked with 0.25 M glycine (pH 7.0) to preserve the RNA–protein interaction, then washed and centrifuged at 16,000 rpm. Virions were cross-linked and lysed for the IP of the RNA/ protein complex by the method described previously (46). A3G-bound or GANP-bound RNAs were detected by qRT-PCR using primers designed for various regions of HIV-1 gRNA listed in Supplemental Table I.

Mutation analysis in HIV-1 proviral DNA

Genomic DNA was isolated from cells at 48 h postinfection. A 408-bp region of HIV-1 pol was amplified using the high-fidelity PrimeSTAR HS DNA polymerase (TaKaRa Bio) and subsequently digested with DpnI to remove the plasmid DNA used for transfection. PCR products were purified by agarose gel electrophoresis and ligated to the pCR4 Zero Blunt TOPO sequence vector (Invitrogen) to be sequenced using an ABI3130 genetic analyzer with BigDye Terminator (Applied Biosystems).

Small interfering RNA treatment

293T cells (2.5 × 10⁵ cells/well) in Opti-MEM GlutaMAX medium were transfected with 10 nM small interfering (si)RNA using Lipofectamine RNAiMAX (Invitrogen) transfection reagent. H9 cells (5 × 10⁵ cells) were transfected with 2 μM siRNA using the Amaxa Nucleofection Kit V (program A-30) according to the manufacturer’s protocol. All siRNA against GANP and negative control (siControl) were obtained from Sigma-Aldrich. The duplex sense sequences of siGANP-A and siGANP-B were 5′-CCUGAUCACUGCCGCUUUUTT-3′ and 5′-GCAUGGAAAGCUUAAUAAUUUTT-3′, respectively.

Infection assays

In single-round infectivity assay, 293T cells (1.5 × 10⁵), activated CD4+ primary T cells (5 × 10⁶), and H9 cells (5 × 10⁶) were infected with similar amounts (2 ng p24 equivalent) of ΔVif HIV-1 viruses and cultured for 48 h. Cells were lysed and assayed for luciferase activity as described previously (42). For multiple-round infection assays, H9-targeted cells (5 × 10⁵) were infected with WT HIV-1 viruses (20 ng p24 equivalent) in the presence of polybrene (8 μg/ml). After 3 h, cells were washed and cultured in RPMI 1640 medium. Multiple-round infection of HIV-1 was monitored as p24 production in the culture supernatant at 5, 7, 10, and 12 d postinfection.
**Statistical analysis**

Differences were analyzed statistically by the unpaired two-tailed Student’s t test and p values < 0.05 were considered significant.

**Results**

**A3G interacts with GANP in T cells**

We examined the expression of GANP and A3G in CD4$^+$ T cells from PBMCs after stimulation with PMA, PHA plus IL-2, or anti-CD3/CD28 beads plus IL-2 for 3 d in vitro (Fig. 1A). A3G mRNA expression is increased in stimulated T cells (47). A3G and ganp mRNAs were increased by stimulation with anti-CD3/CD28 beads plus IL-2: a 6.4-fold increase for A3G and a 19.2-fold increase for ganp after stimulation (Fig. 1A). Expression of A3G and GANP was examined by WB in cell lines (293T, Ramos, and H9) (Fig. 1B). A3G and GANP were expressed at a higher level in H9 T cell and Ramos B cell lines (Fig. 1B, 1C) in whole-cell lysates (WCLs).

Next, we examined whether GANP interacts with A3G as a complex for efficient targeting. FLAG-tagged GANP (FLAG-GANP) and A3G tagged-HA (A3G-HA) expression vectors were used to transfect 293T cells. WCLs were further immunoprecipitated with anti-FLAG Ab and analyzed using anti-HA Ab. A3G-HA was present in the IP precipitate with FLAG-GANP (Fig. 1D). A similar IP experiment using anti-GANP and anti-A3G Abs showed co-IP of the endogenous A3G and GANP in H9 cells and CD4$^+$ T cells from PBMCs (Fig. 1E). These results show that A3G interacts with GANP in T cells.

**GANP enhances A3G encapsidation in VLPs**

The Gag protein alone is sufficient for A3G packaging into VLPs, and thus we examined GANP incorporation into VLPs with WT and a series of myristoylated Gag mutants (42) (Fig. 2A). As previously shown, A3G is encapsidated into WT Gag, d10-110, and d10-277, but not into Zw-t-p6 (Fig. 2B, left panel; A3G-HA). We found that GANP alone is encapsidated in WT Gag, mutant Gag d10-110, or Zw-t-p6, although with less efficiency, but not in d10-277 (Fig. 2B, left panel; FLAG-GANP). These data indicate that different regions of Gag are required for A3G and GANP encapsidation in VLPs. Encapsidation of GANP requires the capsid region, whereas A3G encapsidation requires the NC region. No A3G was observed in the Zw-t-p6 VLPs without GANP (Fig. 2B, A3G-HA). Notably, A3G can be encapsidated into the Zw-t-p6 VLPs with
coexpressed GANP (Fig. 2B, right panel). These data suggest that GANP may actively mediate an NC-independent A3G encapsidation into Zwt-p6 VLPs in the absence of HIV-1 gRNA.

Next we examined whether GANP is assembled and encapsidated into virions. Viruses were produced from 293T cells using HIV-1 proviral vector pNL4-3LucE-R (WT pNL4-3) and Vif-deficient variant (ΔVif pNL4-3) pseudotyped with the VSV-G envelope (42). Virions from 293T cells transfected with pNL4-3 and FLAG-GANP, or with mock (empty vector), were subjected to WB analysis to determine the amount of GANP incorporation after normalization to p24 protein content. FLAG-GANP was detected in both WT and ΔVif HIV-1 virions, but the level of incorporated FLAG-GANP in WT virions was slightly less compared with the level of FLAG-GANP in ΔVif HIV-1 virions (Fig. 3A). The absence of cellular organelles in virion preparations was confirmed by WB against calnexin, an endoplasmic reticulum membrane marker protein. The data clearly indicated that GANP is encapsidated into HIV-1 virions and suggest that Vif protein inhibits not only A3G but also GANP encapsidation. The influence of A3G and GANP on their mutual encapsidation into ΔVif HIV-1 virions was examined. Encapsidated A3G and GANP were quantified by WB from purified ΔVif HIV-1 virions produced in 293T cells transfected with ΔVif pNL4-3, FLAG-GANP, and A3G (0, 2, 10, or 50 ng). FLAG-GANP was detected in all virion preparations and was not affected by the expression of A3G (Fig. 3B), indicating that A3G does not affect GANP encapsidation in the absence of Vif.

We next examined whether GANP affects the incorporation of A3G into HIV-1 virion compartments. First, ΔVif HIV-1 virions were produced from the 293T cells transfected with ΔVif pNL4-3, A3G-HA, and FLAG-GANP or a mock (empty vector) control. Virions were subjected to fractionation to determine whether A3G and GANP are located in the same virion compartments. FLAG-GANP was detected solely in the virion pellet fraction, presumably composed of virion cores (Fig. 3C). Thus, GANP is incorporated exclusively into the virion cores, but not in any other virion compartment (Fig. 3C). In contrast, A3G was present in all three virion fractions, which is consistent with earlier studies (16, 45). In the absence of FLAG-GANP, most encapsidated A3G was...
present in supernatant fractions, whereas ~10% of the A3G was present in the pellet. FLAG-GANP expression markedly increased (2.5-fold) the amount of A3G in the pellet core fraction (Fig. 3D). In contrast, A3G levels in non-core matrix fractions (first and second supernatants) decreased 3- and 10-fold, respectively (Fig. 3D). GANP overexpression led to a significant increase in A3G encapsidation into the virion pellet fraction while reducing A3G incorporation in the HIV-1 virion outside core region (Fig. 3D).

GANP binds to HIV-1 gRNA

A modified RNA chromatin IP assay, designated virion-IP, was carried out to examine whether encapsidated GANP and A3G associate with HIV-1 gRNA. GANP and A3G were found to associate with HIV-1 gRNA within virions (Fig. 4A). The virion-IP assay suggested that both A3G and GANP associated with HIV-1 gRNA inside the virion core (Fig. 4B). Using the region-specific primers for qRT-PCR for GANP-bound fragments, we probed GANP binding to different regions of HIV-1 gRNA (Fig. 4C). The data showed that GANP can associate with all regions spanning the entire HIV-1 gRNA (H1 to H6), but it has a significantly higher binding to the H5 region where the Rev-responsive element is located (Fig. 4D). In virions encapsidated with both GANP and A3G, the A3G binding preference was at H1 (stem-loop 1) and H5 regions of HIV-1 gRNA (Fig. 4E).

GANP modulates cellular RNA incorporation with A3G

A3G encapsidation can either increase or decrease the encapsidation of cellular RNAs (15, 17, 48, 49). Thus, we investigated whether GANP, A3G, or GANP plus A3G are able to mediate the packaging of various cellular RNA molecules into virions. A3G alone reduced the encapsidation of U4, U5, U6, and hY5, but it did not cause any change in 7SL RNA encapsidation (Fig. 5A, gray bars). NC-independent GANP packaging in virions was consistent with observed changes in encapsidation of cellular RNAs in FLAG-GANP (Fig. 5B). GANP alone had small but significant effects on the encapsidation of U2, U4, and U6 cellular RNAs, but not on encapsidation of cellular RNAs hY1, hY3, hY4, and hY5 (Fig. 5B). A3G and GANP coexpression significantly reduced U2, U4, U6, and hY4, but still maintained 7SL RNA in virions (Fig. 5B, open bars), implying that A3G and GANP cooperate in the assembly of HIV-1 gRNA together with the preferential cellular RNAs, such as 7SL RNA, for their efficient encapsidation (17, 48).

A cleaved form of 7SL RNA, known as S domain fragment with several cleavage sites, was found in Zwt-p6 VLPs (Fig. 5C) (50).

FIGURE 4. GANP is associated with HIV-1 gRNA. (A) Association of GANP and A3G with viral gRNA. The ΔVif HIV-1 virions produced in the presence of FLAG-GANP or A3G-HA were subjected to virion-IP assay using anti-FLAG or anti-HA. qRT-PCR was used to quantify the fraction (percentage) of HIV-1 gRNA. IgG was used as a control. (B) GANP and A3G association with HIV-1 gRNA in virion cores. The ΔVif HIV-1 virion core pellet prepared in Fig. 3C was subjected to the virion-IP assay. (C) Schematic representation of HIV-1 regions examined for association with GANP. (D) GANP binds to different HIV-1 gRNA regions within the virion. Binding regions were quantified by qRT-PCR and normalized to the GANP binding to the 5' LTR. (E) Comparison of FLAG-GANP and A3G-HA binding regions of HIV-1 gRNA in ΔVif HIV-1 virions produced in the presence of both FLAG-GANP and A3G-HA. Data represent the means ± SD calculated from three experiments. *p < 0.05, **p < 0.01.
Virion-IP analysis showed that A3G and GANP have a significant binding to 7SL RNA in virions (Fig. 5D). In the presence of A3G, GANP reduced the level of cleaved S domain of 7SL RNA in Zwt-p6 VLPs (Fig. 5E), suggesting that GANP-mediated packaging of HIV-1 gRNA and cellular 7SL RNA.

GANP enhances A3G-mediated anti–HIV-1 activity

The ΔVif HIV-1 virions were first generated from 293T cells in the presence of A3G and FLAG-GANP or A3G and mock (empty vector) control and then added for secondary infection against 293T cells. Virions subsequently produced from the secondary infected 293T cells were subjected to mutational analysis of the HIV-1 pol gene. Consistent with previous reports (42), a high frequency of G→A hypermutation (2.08 × 10^{-3}) was observed at the pol gene when viruses were produced with A3G alone (A3G plus mock) (Fig. 6A). In the presence of FLAG-GANP, A3G-catalyzed G→A mutations were significantly increased (89%) in the same region (Fig. 6A). The G→A mutations occurred preferentially at A3G hotspot motifs GG→AG (Fig. 6B, flags). Thus, GANP overexpression that increases A3G encapsidation into virion cores enhances C→U deamination, resulting in accumulation of G→A mutations in the genome of ΔVif HIV-1.

The effect of GANP overexpression on A3G-mediated anti–HIV-1 activity was examined using a single-round infectivity assay (42). To confirm GANP effect on A3G encapsidation and anti–HIV-1 activity, we carried out the single-round infection assay for ΔVif HIV-1 produced from endogenous GANP-knockdown cells. Two different siRNAs targeting distinct regions of the ganp gene severely reduced endogenous GANP expression in 293T cells (Fig. 6D). The ΔVif HIV-1 virions were generated from the 293T siControl and GANP-knockdown cells after cotransfection with ΔVif pNL4-3 and A3G-HA (Fig. 6D, left panel). As expected, downregulation of endogenous GANP markedly suppressed A3G encapsidation in ΔVif HIV-1 virions (Fig. 6D, blot data). The reduction in A3G encapsidation into the virion core coincided with a higher infectivity of ΔVif HIV-1 virions prepared from GANP-knockdown cells compared with siControl. The results showed the similar increase of HIV-1 infectivity against H9 cells and primary CD4+ T cells in a single-round infection assay with the VSV-G envelope (Fig. 6D, right panel).

GANP is important for restriction of WT Vif-proficient HIV virus

The potential role of GANP in restriction of HIV-1 infectivity was further studied using a multiple-round infection assay in H9 cell lines.
line that expresses endogenous GANP and A3G. The level of endogenous GANP from 2 to 4 d after transfection in siGANP-treated H9 cells was ∼2-fold lower compared with the siControl H9 cells (Fig. 7A). WT Vif-proficient HIV-1 viruses were produced in siGANP-treated or siControl-treated 293T cells. Multiple-round infection was performed in GANP-knockdown H9 cells as the

FIGURE 6. GANP enhances A3G-catalyzed G→A hypermutation and promotes A3G anti–HIV-1 activity. (A) Mutation analysis of 408-nt pol region of HIV-1 proviral DNA. Virions produced in 293T cells with a reduced amount of A3G (2 ng) in combination with or without FLAG-GANP were infected to the target cells and subjected to sequence analysis. (B) A3G-catalyzed mutation spectra in the HIV-1 pol region (nucleotide positions, 2137–2544). Flags indicate mutations at an A3G hotspot motif (GG→AG). (C) GANP overexpression in 293T virus-producing cells enhances A3G-mediated inhibition of ΔVif HIV-1 infectivity in a single-round infection assay. The ΔVif HIV-1 virions were produced with a low dose (0, 2, 10, or 50 ng) of A3G with and without FLAG-GANP and a similar amount of virions from each preparation (2 ng p24 equivalent) was used to infect 293T cells and CD4+ primary T cells. Relative viral infectivity was calculated by the luciferase activity in WCLs of infected cells at 48 h postinfection. (D) Single-round infection assay in H9 cells. The ΔVif HIV-1 virions containing A3G were produced in 293T cells treated with either siControl or siGANP. A3G was examined by WB in both WCLs and virion core fractions. Relative viral infectivity was calculated based on the luciferase activity in WCLs of infected H9 cells and CD4+ primary T cells. Data represent the means ± SD calculated from three experiments. *p < 0.05, **p < 0.01.
target cells to examine infectivity of the WT HIV-1 virions with the intact envelope (Fig. 7B). Knockdown of GANP in both virus-producing and target cells showed a dramatic increase (50-fold) in WT HIV-1 production as monitored by the p24 protein levels at day 12 in GANP-knockdown compared with control cells (Fig. 7B). These data suggest that HIV-1 virions produced in cells with a limited amount of GANP exhibited a significantly higher infectivity compared with HIV-1 virions produced in cells expressing a high level of GANP.

Discussion

HIV-1–infected patients are now treated with a highly active antiretroviral therapy. HIV-1 viral load and the disease progression are controlled by the immune responsiveness of the infected individuals and balance with the production of serum Abs and generation of CD8+ CTLs. Innate and adaptive immune responses to HIV-1 infection are complex and involve multiple viral and host factors (51). A3G is a host cellular factor that has been shown to inactivate HIV-1 virus lacking Vif (10). For the WT HIV-1 virus, the antiviral activity of A3G might not be sufficient to shut down the viral infection at the initial infection stage because the Vif protein inhibits expression and causes degradation of A3G in the infected cells. Vif-mediated inhibition of A3G in HIV-1–infected cells, however, is not complete. A3G action on HIV-1 genomes is readily observed in patients at different stages of HIV-1 infection (20, 21). In addition to direct action on HIV-1 replication, A3G has been shown to enhance the ability of the immune system to recognize HIV-1–infected cells and to activate HIV–specific CD8+ CTLs (52). A3G mutator activity produces defective HIV-1 pro-viruses expressing truncated or misfolded viral proteins, which can be served as the pool of MHC class I–restricted HIV-1 Ags (52). Thus, A3G-mediated C→U conversions on HIV-1 cDNA play an important role to evoke the immunogenicity of HIV-infected cells and CTL activation.

A3G encapsidation in HIV-1 virions is required for its antiviral activity. When expressed at a low level, active A3G is packaged primarily in HIV-1 virion cores (45). Only those A3G molecules packaged into the virion cores and that associate with viral gRNA are “biologically active,” that is, have an ability to restrict HIV-1 infectivity (14, 16, 45). GANP is upregulated in activated CD4+ T cells (Fig. 1), and GANP overexpression caused a higher level of encapsidated active A3G in virions (Fig. 3C, 3D), leading to a marked elevation of G→A hypermutation (Fig. 6A, 6B). Consistent with active A3G encapsidation in virions, ΔVif HIV-1 virions produced in 293T cells with overexpressed GANP exhibited significantly lower infectivity compared with the control cells (Fig. 6C). Conversely, HIV-1 virions produced from GANP down-regulated 293T or H9 cells appeared to have considerably higher infectivity, compared with the virions produced in control cells, in either single-round or multiple-round infection assays (Figs. 6D, 7B). A significant increase in infectivity of WT HIV-1 virions propagated in GANP-knockdown H9 T cells in multiple-round infection assays (Fig. 7) suggests that GANP has an effect on the packaging of a very low level of A3G present in T cells infected with Vif-proficient HIV-1. Overall, our data showing that GANP promotes A3G packaging into virion (Fig. 3D) and enhances A3G antiviral activity indicate a key role of GANP in recruiting A3G to its physiological targets, thereby leading to observed G→A hypermutation of HIV-1 genome.

GANP alters the location of encapsidated A3G inside the ΔVif HIV-1 virions. Although A3G is packaged both in the virion core and non-core fractions (45), we have determined that GANP promotes A3G encapsidation into the virion core compartment (Fig. 3C, 3D), presumably through the stable association with HIV-1 gRNA (Fig. 4A, 4B). Coexpression of GANP and A3G decreased encapsidation of cellular RNAs, except 7SL RNA (Fig. 5A), suggesting that cellular RNA-mediated packaging of A3G into virions appears to be nonspecific and is inhibited by GANP GANP-mediated localization of A3G into the virion cores is likely to occur during maturation of the virus particles, when the appearance of Gag- and Gag-Pol–cleaved proteins leads to structural re-arrangements and formation of the virion particles. Because GANP itself is incorporated into the virion, it may recruit A3G into the virion through interaction and with the RNP complex containing HIV-1 gRNA. Thus, GANP–facilitated A3G encapsidation into virion represents an additional pathway of packaging A3G into the virion, which was supported by a previous report showing that A3G incorporation into the virion core can be mediated through its interaction with HIV-1 gRNA or 7SL RNA (50).

GANP interacts with A3G (Fig. 1) and with AID, another member of the APOBEC family of cytidine deaminases (38). In B cells, AID localizes primarily in the cytoplasm but acts on genomic DNA in the nucleus. GANP–facilitated recruitment of AID into the nucleus and targeting AID to the IgV region initiate
SHM (38, 39). A3G, however, appears exclusively in the cytoplasm and is recruited to HIV-1 virions during the assembly and maturation of the viral particles. In this study, we reveal an important regulatory role for GANP as a key cellular factor that regulates A3G packaging into the HIV-1 virion. Thus, GANP interacts with both cytidine deaminases, AID and A3G, and helps to facilitate their targeting the intended physiological targets in vivo.

The effect of GANP on A3G encapsidation into the virion consequently leading to G→A hypermutation of the viral genome might have a potential use in HIV-1 vaccine development. Co-expression of GANP and A3G in HIV-1-infected cells causes an increase in production of defective HIV-1 mutant proviruses expressing truncated, hypermutated, or misfolded viral proteins. These hypermutated proviruses do not produce infectious particles but generate a sustained pool of “naturally occurring” inactive HIV-1 variants. With a good selection strategy, A3G-generated HIV-1 inactive variants can be selected to use as naturally occurring multiple-type HIV-1 vaccines that are effective on a broad spectrum of viral clades in many areas, including North and South America, Eurasian, African, and Asian countries.

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Disclosures

The authors have no financial conflicts of interest.

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