Biological Sciences, Microbiology

HIV-1 Vif interacts with TP53 to induce G2 cell cycle arrest and positively regulate viral replication

Taisuke Izumi[a,b], Katsuhiro Io[a], Masashi Matsui[a], Kotaro Shirakawa[a,b], Masanobu Shinohara[a], Yuya Nagai[a], Masahiro Kawahara[a], Masayuki Kobayashi[a], Hiroshi Kondoh[c], Naoko Misawa[d], Yoshio Koyanagi[d], Takashi Uchiyama[e], and Akifumi Takaori-Kondo[a,1]

[a]Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University, 54 Shogoin-Kawaracho, Sakyo-ku, Kyoto 606-8507, Japan

[b]Japanese Foundation for AIDS Prevention, 1-3-12 Misaki-cho, Chiyoda-ku, Tokyo 101-0061, Japan

[c]Department of Geriatric Medicine, Graduate School of Medicine, Kyoto University, 54 Shogoin-Kawaracho, Sakyo-ku, Kyoto 606-8507, Japan

[d]Laboratory of Viral Pathogenesis, Institute for Virus Research, Kyoto University, 53 Shogoin-kawaracho, Sakyo-ku, Kyoto 606-8507, Japan

[e]Kitano Hospital, The Tazuke Kofukai Medical Research Institute, Ougi-machi 2-4-20, Kita-ku, Osaka 530-8480, Japan

[1]To whom correspondence should be addressed. E-mail: atakaori@kuhp.kyoto-u.ac.jp

Running title: Functional interaction between HIV-1 Vif and TP53

Abstract

HIV-1 Vif induces G2 cell cycle arrest; however, the biological significance and mechanism(s) remain totally unclear. Here we demonstrate that the TP53 pathway is involved in Vif-mediated G2 cell cycle arrest. Vif enhances the stability and transcriptional activity of TP53 by blocking the MDM2-mediated ubiquitination and nuclear export of TP53. Furthermore, Vif causes G2 cell cycle arrest in a TP53-dependent manner. HXB2 Vif lacks these activities toward TP53 and cannot induce G2 cell cycle arrest. Using mutagenesis, we demonstrate that the critical residues for this function are located in the N-terminal region of Vif. Finally, we construct a mutant NL4-3 virus with an NL4-3/HXB2 chimeric Vif defective for the ability to induce cell cycle arrest and show that the mutant virus replicates less effectively than the wild type NL4-3 virus in T cells expressing TP53. These data imply that Vif induces G2 cell cycle arrest through functional interaction with the TP53/MDM2 axis and that the G2 cell cycle arrest induced by Vif has a positive effect on HIV-1 replication. This is the first report demonstrating the molecular mechanisms and the biological significance of Vif-mediated G2 cell cycle arrest for HIV-1 infection.

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text

Introduction

HIV-1 Vif plays a crucial role in the viral life cycle by antagonizing host restriction factors APOBEC3G (A3G) and APOBEC3F (A3F) (1, 2). Vif forms a ubiquitin ligase (E3) complex with Cullin5 and Elongin B/C (Vif-BC-Cul5) and functions as a substrate recognition subunit of this complex to induce the ubiquitination and subsequent degradation of A3G/F (3, 4). Recent studies implicate Vif not only as a novel viral protein involved in G2 arrest but suggest that the induction of G2 arrest is a novel Vif function (5, 6). However, neither study addresses the mechanism by which Vif induces G2 arrest or the question of why HIV-1 uses two different viral proteins to arrest cells in the G2 phase of the cell cycle.

Multiple overlapping TP53-dependent and TP53-independent pathways regulate the G2/M transition in response to genotoxic stress (7-9). In the TP53-dependent pathway, TP53 inhibits Cdc2 activity through its transcriptional targets including p21 (10), GADD45 (11), and 14-3-3σ (12). In the TP53-independent pathways, activation of the protein kinases Chk1 and Chk2 by Atm and Atr inhibits Cdc2 by inactivating Cdc25, the phosphatase that normally activates

Cdc2. Vpr has been shown to affect several proteins involved in the G2/M transition including Cdc2, Wee1, Cdc25, Atr, and Chk1 (13-16). These Vpr functions have been interpreted as the molecular mechanisms of Vpr-induced G2 arrest; however, the mechanisms of Vif-induced G2 arrest are totally unknown.

We previously reported that MDM2 is a novel E3 ligase for Vif and induces the ubiquitination and degradation of Vif to regulate HIV-1 replication (17). We showed that Vif binds to the central domain of MDM2, which functions in the MDM2 regulation of TP53 stability and activity. As several proteins have been reported to bind this domain and regulate the effect of MDM2 on TP53, we have herein tested whether Vif regulates TP53 activity, resulting in G2 arrest. We show that Vif stabilizes and activates TP53 to induce G2 arrest in infected cells and that the Vif-induced G2 arrest positively supports HIV-1 replication. Our study reveals that Vif positively regulates viral replication in a TP53-dependent manner. This is the first report demonstrating the biological significance of HIV-1 Vif-induced G2 arrest.

Results

Vif enhances TP53 stability and activity by blocking MDM2.

We previously demonstrated that Vif binds to the central domain of MDM2 (17), which is critical for regulating TP53 stability and transcriptional activity. To test whether Vif affects TP53 transcriptional activity, we first performed luciferase reporter assays using TP53-null H1299 cells (Fig. 1A). Vif blocks the inhibitory activity of MDM2 on TP53-mediated transcription (lane 4 compared with lane 1) for both *p21* and *mdm2* promoters. These data confirmed the functional relationship between Vif and TP53 and we therefore next examined the physical interaction between these two proteins. Co-immunoprecipitation assays using the MDM2-binding-defective TP53 mutant TP53(Gln22,Ser23) showed that Vif was able to bind this mutant (Fig. S1). Using a series of Vif deletion mutants, we determined that the N-terminal region of Vif, consisting of amino acids 4-22, was necessary for TP53 binding (Fig. S2). GST pull-down assays also showed that GST-Vif bound to *in vitro* translated TP53 and the mutant TP53(Gln22,Ser23), but GST-Vif△22 did not (Fig. S3). These data suggest that Vif binds to TP53 directly and independently of MDM2 and that the N-terminal region of Vif is necessary for interaction with TP53. MDM2 specifically targets TP53 for degradation (18, 19), and we

therefore examined the effect of Vif on TP53 protein levels (Fig. 1B). TP53 protein levels clearly correlated with luciferase activities as shown in Fig. 1A and B. Co-expression of MDM2 reduced TP53 levels, while Vif overcame this MDM2 inhibitory effect and restored TP53 protein levels (lane 4 compared with lane 1 in Fig. 1B). Vif also upregulated TP53 levels even in the absence of ectopically expressed MDM2 as seen in luciferase reporter assays (lane 5 compared with lane 2 in Fig. 1B). We assume that this results from the effect of Vif on the function of endogenous MDM2. Vif levels were also downregulated when MDM2 was overexpressed (lane 4 compared with lane 5) as we previously reported (17).

Since MDM2 downregulates TP53 levels by ubiquitination, we examined the effect of Vif on MDM2-induced TP53 ubiquitination (Fig. 1C). The expression of MDM2 induced the ubiquitination of TP53 (lane 4), which was clearly blocked by co-expression of Vif (lanes 5 & 6). This suggests that Vif stabilizes TP53 by blocking MDM2-induced ubiquitination.

Because MDM2 exports TP53 from the nucleus (20), we also examined the possibility that Vif blocked MDM2-mediated nuclear export of TP53 using immunofluorescence studies (Fig. 1D). HA-TP53 was primarily located in the nucleus

when expressed alone (upper panels), but was translocated into the cytoplasm when co-expressed with MDM2 (middle panels). HA-TP53 was redistributed to the nucleus when GFP-Vif was expressed with MDM2 (lower panels), indicating that Vif blocked MDM2-mediated nuclear export of TP53.

Based on the above results, we conclude that Vif enhances TP53 stability and transcriptional activity by blocking MDM2-mediated degradation and nuclear export of TP53.

To further elucidate the mechanisms by which Vif blocks MDM2 function, we examined the physical interactions among these three proteins. Immunoprecipitation assays showed that co-expression of Vif partially blocked the binding of TP53 to MDM2 (Fig. S4), suggesting that the binding of vif to TP53 or MDM2 inhibits the interaction between TP53 and MDM2.

Vif induces G2 cell cycle arrest via the TP53 pathway.

It has recently been shown that Vif induces G2 arrest in HIV-1-infected cells independently of Vpr (5, 6). To test the possibility that Vif induced G2 arrest by stabilizing and activating TP53, we examined the effect of Vif on cell cycle in various cell lines. In TP53-null H1299 cells, Vif alone did not induce G2 arrest, while G2 arrest was induced upon co-expression of ectopic TP53 with Vif (Fig. 2A,

middle panels). Vpr induced G2 arrest even in the absence of TP53 (Fig. 2A, lower panels). In contrast, in 293T cells, which expressed TP53, Vif induced G2 arrest (Fig. 2C, upper panels), and co-expression of *p53* siRNA inhibited Vif-induced G2 arrest (Fig. 2C, lower panels & Fig. 2D). In addition, in HCT116 and its derivative cell lines, Vif induced G2 arrest only in wild-type parental cells (Fig. 2E, upper panels), but not in *p53*$^{-/-}$ or *p21*$^{-/-}$ cells (middle panels and lower panels, respectively). Immunoblot studies of these cells clearly showed that cellular levels of TP53 and P21 were upregulated and those of Cdc2 and CyclinB1 were downregulated in wild-type HCT116 cells when Vif was expressed (Fig. 2F left panels). In contrast, in HCT116 *p53*$^{-/-}$ cells, such changes were not detected (middle panels), whereas in HCT116 *p21*$^{-/-}$ cells, only TP53 levels were upregulated (right panels). These data suggest that Vif induces the upregulation of TP53 and activation of the TP53-induced cascade including upregulation of P21 and downregulation of Cdc2 and CyclinB1, leading to G2 arrest (21), whereas Vpr causes G2 arrest in a TP53-independent manner.

We further examined the effect of Vif on cell cycle in T cells in the context of viral infection. Vif-expressing NL4-3 virus caused G2 arrest in CEM-SS cells and cell cycle

arrest was relieved by treatment with a chemical inhibitor of TP53, Pifithirin-α (Fig. 2G). This finding suggests that viral infection with Vif expression also induces G2 arrest in T cells in a TP53-dependent manner.

The N-terminal region of Vif is critical for the induction of G2 arrest.

We next examined whether other Vif strains could induce G2 arrest and found that Vif derived from the HXB2 strain was not able to induce G2 arrest (Fig. 3A, upper panels). We thus examined the effect of HXB2 Vif on TP53 protein levels and transcriptional activity. NL4-3 Vif overcame the inhibitory effect of MDM2 on TP53 (Fig. 3B & C, lane 4 compared with lane 1), but HXB2 Vif not (lane 7 compared with lane 1). These data suggest that HXB2 Vif lacks the ability to induce G2 arrest via the TP53 pathway. To determine the residues required for this function, we compared the NL4-3, HXB2, and SG3 Vif amino acid sequences as SG3 Vif has also been reported to cause G2 arrest (6)(Fig. 3D). Since the N-terminal region of Vif is important for binding to both TP53 and MDM2 (Fig. S2 and S3), we focused on this region and identified potential residues at position 31, 33, 36, 47, and 50, which were conserved in NL4-3 and SG3 but not in HXB2. We mutated NL4-

3 in these residues and tested the effects of these mutants on cell cycle. We found that both mutants of NL4-3 Vif carrying either the triple NL4-3 to HXB2 mutations at position 31, 33 and 36 or the double mutations at position 47 and 50 lost the ability to induce G2 arrest, whereas the reverse mutations in HXB2 Vif rendered it capable of inducing G2 arrest (Fig. 3A, lower panels). We further examined whether these mutants affect TP53 transcriptional activity. The mutants of NL4-3 Vif to HXB2 defective for the ability to induce G2 arrest lost the ability to overcome the MDM2 activity, whereas the mutant of HXB2 to NL4-3 capable of inducing G2 arrest recuperated the ability to overcome the MDM2 activity (Fig. S5). In addition, we performed co-immunoprecipitation assays to test the binding of these mutants to TP53 (Fig. S6). HXB2 Vif and the mutants of NL4-3 to HXB2 retained the ability to bind TP53 to the similar extent to that of NL4-3 Vif. These data indicate that these amino acids are important for Vif function to induce G2 arrest but dispensable for Vif binding to TP53.

G2 arrest caused by Vif positively supports viral replication.

We also examined cell cycle profiles in T cells upon

infection with VSV-G-pseudotyped NL4-3/ΔEnvΔVpr-Luc viruses using TP53-deficient Jurkat cells (22), permissive CEM-SS and non-permissive CEM cells, which have a functional TP53, and primary $CD4^+$ T cells. We used NL4-3/ΔEnvΔVpr-Luc virus to remove the effect of Vpr on cell cycle and also constructed a chimeric NL4-3/ΔEnvΔVpr-Luc virus replacing the N-terminal aa1-85 with aa1-85 of HXB2 Vif (Fig. 4A). The wild-type NL4-3/ΔEnvΔVpr-luc (Fig. 4B, left panels) induced G2 arrest in CEM-SS, CEM, and primary $CD4^+$ T cells (second, third, and lower panels, respectively), but not in TP53-deficient Jurkat cells (upper panel), whereas the mutant virus expressing a chimeric Vif did not induce G2 arrest in any of these cells (right panels). These data suggest that the N-terminal domain of Vif is critical for inducing G2 arrest and that functional TP53 is necessary for Vif-induced G2 arrest following HIV-1 infection in T cells.

Finally, we examined the effect of Vif-mediated G2 arrest on HIV-1 replication using the NL4-3-Luc virus expressing the NL4-3-Luc/HXB2 chimeric Vif (Fig. 4C). The chimeric virus showed less efficient replication than the parental NL4-3-Luc virus in TP53-active CEM-SS, CEM and $CD4^+$ T cells. Although a ΔVif virus cannot replicate in non-permissive CEM and $CD4^+$ T cells, it can replicate in permissive CEM-SS

cells albeit less efficiently than wild-type virus. In Jurkat cells, NL4-3-Luc virus couldn't efficiently replicate, presumably because of the replacement of *nef* gene with *liciferase*. We therefore constructed the NL4-3/HXB2 chimeric Vif virus in the intact NL4-3 as a backbone and monitored the replication kinetics in TP53-deficient Jurkat cells. There is no differences in replication profiles between wild-type and chimeric Vif viruses (Fig. S7). These results indicate that the G2 arrest induced through the Vif-TP53 interaction has a positive effect on HIV-1 replication.

Discussion

In this study, we demonstrate that Vif enhances TP53 stability and transcriptional activity by blocking MDM2-mediated degradation and nuclear export of TP53, which results in G2 arrest in infected cells. More importantly, we further demonstrate that the Vif-induced G2 arrest positively supports HIV-1 replication.

We first demonstrate that Vif can bind TP53 as well as MDM2 directly. We previously mapped the interaction domain of MDM2 with Vif to amino acids 168-320, which are located in its central acidic and Zn finger domains (17). This central domain is different from the primary TP53-binding site

located in the N-terminal region, but was recently reported as a second TP53-binding site important for regulation of TP53 stability (23-26). Interestingly, several proteins, including P300, P14ARF, and pRB, bind to the central domain of MDM2 and regulate the stability and function of TP53 via MDM2 (24, 27). P300 can bind to TP53 and MDM2 independently and facilitate the MDM2-mediated inhibition of TP53 function (28, 29). In contrast, P14ARF binds to MDM2 and enhances the stability and function of TP53 by blocking MDM2-mediated ubiquitination and nucleo-cytoplasmic shuttling of TP53 (30-32). pRB binds to MDM2 and enhances the stability and apoptotic function of TP53 via MDM2, but not its transcriptional activity (33-35). Vif enhances TP53 stability and transcriptional activity by blocking MDM2-mediated degradation and nuclear export of TP53. According to our data, Vif can bind independently to TP53 as well as MDM2, similar to P300, but its effect on TP53 function is exactly the opposite. On the other hand, Vif is a substrate of an E3 ligase like MDM2 as well as an effecter of MDM2 function, similar to pRB, but its effect on TP53 function is somewhat different. The important finding is that Vif is a protein regulating the TP53/MDM2 axis. This novel interaction also suggests that a possible negative autoregulatory circuit exists among Vif, TP53, and MDM2

(36). Stabilization and activation of TP53 by Vif induces the upregulation of MDM2 (Fig. 1A, upper panel), which in turn downregulates Vif. This circuit keeps cellular Vif levels relatively low, which might be an advantageous level for HIV-1; not too much to affect viral replication, but enough to antagonize A3G/F. One possible reason is that overexpression of Vif affects Gag processing, which impairs viral replication (37).

More interestingly, several groups recently reported that Vif as well as Vpr induced G2 arrest in HIV-1-infected cells (5, 6). Our data clearly demonstrate that the activation of TP53 is the molecular mechanism involved in Vif-induced G2 arrest. Stabilization and activation of TP53 by Vif induces the upregulation of P21, leading to the downregulation of Cdc2 and CyclinB1 levels, resulting in G2 arrest. This is in contrast to Vpr-induced G2 arrest, because Vpr induces G2 arrest via a TP53-independent pathway (Fig. 2A). Several laboratories have recently reported the involvement of the DDB-1-Cul4 E3 complex in Vpr-induced G2 arrest (38, 39), although its target substrates and molecular mechanisms remain unclear. Why does HIV-1 have two different proteins regulating G2/M transition? DNA damage activates checkpoint mechanisms and sustains cells at the G2 phase for DNA repair (7, 8). TP53

is involved in the maintenance rather than the initiation of G2 arrest; for example, wild-type HCT116 cells sustain G2 arrest following irradiation, whereas the isogenic TP53-null derivative initially arrests in G2 but then escapes and enters mitosis (10). It seems quite reasonable for HIV-1 to utilize Vpr and Vif, because each regulates TP53-independent and -dependent pathways to initiate and sustain G2 arrest, respectively.

Furthermore, many reports have described a viral connection between TP53 and cell cycle arrest. Herpesviruses, such as herpes simplex virus (40), human herpesvirus 6 (41), and human cytomegalovirus (42-44) cause cell cycle arrest via the TP53 pathway; however, the viral proteins responsible for TP53 stabilization and the precise mechanisms and significance of cell cycle arrest remain unknown. The data in this study might provide a clue to the mechanism and the significance of cell cycle arrest induced by these viruses. Finally, we demonstrate that the G2 arrest induced by Vif positively supports viral replication. The mutant virus expressing an NL4-3/HBX2 chimeric Vif is defective in its ability to induce G2 arrest, while it can antagonize A3G to the same extent as the NL4-3 parental virus (Fig. S8). Replication assays reveal that this chimeric virus replicates more slowly than wild-type virus in CEM-SS, CEM,

and $CD4^+$ T cells, which express functional TP53 (Fig. 4C) but not in TP53-deficient Jurkat cells (Fig. S7). The disparity noted between wild-type and NL4-3/HBX2 chimeric viruses is attributable to the ability to induce G2 arrest, because they show similar anti-A3G activities (Fig. S8). This finding appears to be consistent with previous observations showing that cells sustained in the G2 phase can produce more virus because of increased LTR and RT activity (45). On the other hand, ΔVif viruses showed a more effective replication profile than an NL4-3/HBX2 chimeric Vif virus in CEM-SS cells, although they cannot replicate in non-permissive CEM and $CD4^+$ T cells because of the presence of A3G. It is conceivable that a chimeric Vif is expressed at higher levels which impedes viral replication by affecting Gag processing (37) because it cannot induce an autoregulatory circuit via the TP53 pathway.

Taken together, we here advocate the novel concept that the interaction between Vif and the TP53/MDM2 axis positively regulates HIV-1 replication. Vif hijacks not only a Cullin5 E3 ligase complex but also the TP53/MDM2 axis to achieve more efficient viral replication. Evolutionarily, HIV-1 seems to have obtained the *vif* gene to antagonize APOBEC3 proteins. Since Vif protein itself impairs viral

replication when overexpressed, we assume that it further interacts with the TP53/MDM2 axis to achieve more efficient viral replication.

Vallunti et al. recently reported that F12-Vif from a non-producer HIV-1 provirus clone F12 has anti-HIV-1 activity despite degrading A3G as efficiently as NL4-3 Vif does (46). The F12-Vif derivative Chim3-Vif is a chimera Vif generated by replacing the domain (aa126-170) of wild-type Vif with that of F12-Vif. Lentiviral transduction of Chim3-Vif protected $CD4^+$ T cells from HIV-1 infection by preventing HIV-1 DNA integration and HIV-1-induced G2 cell cycle delay (47, 48). These data also indicate that modification of cell cycle is important for HIV-1 replication and might be a target for a new therapeutic strategy.

This is the first report to show the molecular mechanisms and the biological significance of G2 arrest induced by Vif and the modification or intervention of this interplay might lead to new therapeutic strategies for HIV-1 infection.

Materials and Methods

Cell culture and virus propagation

HEK293T cells and the TP53-null lung carcinoma cell line H1299 were maintained in Dulbecco's modified Eagle's medium

(Invitrogen Corporation, Carlsbad, CA) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin and glutamine (PSG)(Invitrogen Corporation). The human colorectal carcinoma cell line HCT116 and the isogenic derivative lines HCT116 *p53*$^{-/-}$ and HCT116 *p21*$^{-/-}$ were kindly provided by Dr. B. Vogelstein and cultured in McCoy's 5A medium (Invitrogen Corporation) supplemented with 10% FBS and 1% PSG. The leukemic T cell lines (Jurkat, CEM-SS, and CEM) were maintained in RPMI 1640 medium (Nacalai tesque) containing 10% FBS and 1% PSG. Primary $CD4^+$ T cells were purified by CD4 MultiSort MicroBeads (Miltenyi Biotec) and stimulated with 200 U/ml IL-2 and 1 μg/ml PHA at 37°C for 1 week. All cells were maintained at 37°C in a humidified atmosphere of 5% CO_2. Virus stocks were prepared by transfection of HEK293T cells with HIV-1 NL4-3 derivatives using the calcium phosphate co-precipitation method.
Additional experimental methods are in *SI Materials and Methods.*

Acknowledgments

We thank Dr. K. Strebel for the pNL-A1 plasmid and its derivative mutants, Dr. C. deNoronha for pEGFP-Vpr, Dr. B. Vogelstein for HCT116 and the isogenic derivative lines HCT116 *p53*$^{-/-}$ and HCT116 *p21*$^{-/-}$, Dr. X. Lu for the *mdm2*-Luc

plasmid, Dr. D. Beach for *p21*-Luc plasmid, and Dr. M. Malim for the anti-Vif monoclonal antibody (#319) through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH. This study was partly supported by grants-in-aid from the Ministry of Education, Culture, Sports, Science, and Technology, and from the Ministry of Health, Labour and Welfare, Japan. This study was also partly supported by grants-in-aid from Sankyo Foundation of Life Science.

Competing Financial Interests

The authors declare that they have no competing financial interests.

Figure Legends

Figure 1 Vif enhances TP53 stability and transcriptional activity by blocking MDM2-mediated degradation and nuclear export of TP53.

(A) Vif overcomes the inhibitory activity of MDM2 on TP53-mediated transcription. H1299 TP53-null lung carcinoma cells were co-transfected with 1μg reporter plasmids together with expression vectors for TP53, MDM2, and Vif as indicated. Histograms of TP53 transactivation on *mdm2*- and *p21*-promoters are shown. Values are presented as an average

of three independent experiments. (B) Vif blocks MDM2-mediated degradation of TP53. H1299 cells were transfected with combinations of expression vectors as indicated. Note that the expression vector additions below panel A also refer to panel B. Cell lysates were subjected to immunoblotting with the indicated Abs. The amounts of TP53 were quantified by densitometry and shown as the fold ratio relative to that in lane 2. (C) Vif blocks MDM2-mediated ubiquitination of TP53. HEK293T cells were cotransfected with expression vectors for MDM2 and TP53 together with expression vectors for Vif and FLAG-Ubiquitin (FLAG-Ub) as indicated. Cells were treated with MG132 for 6hr and cell lysates were precipitated with anti-TP53 Ab followed by immunoblotting with the indicated Abs. (D) Vif blocks MDM2-mediated nuclear export of TP53. H1299 cells were transfected with expression vectors for HA-TP53, FLAG-MDM2, EGFP, and EGFP-Vif as indicated. Cells were fixed and probed with anti-HA mAb, and then stained with anti-mouse IgG Ab conjugated with Alexa Fluor 594 dye. Cell nuclei were stained with DAPI dilactate. Samples were examined using a fluorescence microscope, Biozero BZ-8100. Quantification of TP53 localization in the cytoplasm or nucleus is shown on the right side.

Figure 2 HIV-1 Vif causes G2 cell cycle arrest via the TP53 pathway.

(A) H1299 TP53-null cells were transfected with expression vectors for EGFP, EGFP-Vif, or EGFP-Vpr with or without a TP53 expression vector. Two days after transfection, cells were fixed and stained with propidium iodide and analyzed by FACS by gating on GFP(+) cells. The y axis denotes cell counts and the x axis represents DNA content. Cell cycle profiles were analyzed by ModFit LT 3.0 and the percent of cells in G1(Red), G2(Blue) or S(Green) and the G2:G1 ratio are given in the upper right hand corner of each histogram. (B) Cell lysates from the experiment shown in Fig. 2A were subjected to immunoblotting with the indicated Abs. GFP-Vif was clearly detected with anti-Vif mAb, although it was weakly detected with anti-GFP Ab. (C) Knockdown of TP53 relieves Vif-induced G2 cell cycle arrest. HEK293T cells were transfected with expression vectors for EGFP or EGFP-Vif together with *p53* siRNA or control siRNA. Two days after transfection, cell cycle analysis was performed as described above. (D) Cell lysates from the experiment shown in Fig. 2C were subjected to immunoblotting with the indicated Abs. (E) TP53 and P21 are required for G2 cell cycle arrest induced by Vif. HCT116, HCT116 $p53^{-/-}$, and HCT116 $p21^{-/-}$ cells were transfected with expression vectors

for EGFP or EGFP-Vif and analyzed as described above. (F) TP53 and P21 are required for the reduction in Cdc2 and Cyclin B1 levels. Cell lysates from the experiment shown in Fig. 2E were subjected to immunoblotting with the indicated Abs. (G) Representative cell cycle profiles of NL4-3-infected CEM-SS are shown. CEM-SS cells were infected with NL4-3 wild-type or ΔVif virus. Two days after infection, cells were stained with anti-p24 mAb and PI and analyzed by FACS by gating on $p24^+$ cells. Cells were pre-treated with PFT-α (90μM) for 24hr prior to cell cycle analysis.

Figure 3 The N-terminal region is critical for the Vif function of inducing G2 arrest.

(A) Cell cycle profiles of H1299 cells transfected with various mutants of NL4-3 or HXB2 Vif. (B) Luciferase reporter assays and (C) Immunoblotting were performed as shown in Fig. 1. (D) The sequences of the N-terminal domains of NL4-3, SG3, and HXB2 Vif proteins were aligned. Residues that differ only in HXB2 Vif are indicated by asterisks (*).

Figure 4 Replication of HIV-1 in T cells.

(A) Diagram of the chimeric NL4-3/ΔVpr-Luc virus with the N-terminal region (aa 1-85) of HXB2 Vif and no Vpr

expression due to mutation. A luciferase reporter gene replaced the *nef* gene coding sequence. (B) The wild-type NL4-3/ΔEnvΔVpr-Luc (left panels) induces G2 arrest in infected CEM-SS cells, CEM cells and primary $CD4^+$ T cells, but not in TP53-deficient Jurkat cells, while the mutant virus expressing a chimeric Vif (right panels) cannot induce G2 arrest in any of these cells. Two days after infection, cells were stained with anti-p24 mAb and PI and analyzed as the same method in Fig. 2G. (C) CEM-SS, CEM, and primary $CD4^+$ T cells (upper, middle, lower panel, respectively) were challenged with normalized stocks of wild-type, ΔVif, and chimeric Vif NL4-3-Luc viruses and viral replication was monitored as the supernatant accumulation of p24. $CD4^+$ T cells were isolated from PBMC and cells were stimulated with 200U/ml IL-2 and 1μg/ml PHA for 1 week. p24 antigen levels were measured by ELISA.

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