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HIV-1 Vpr Protein Inhibits Telomerase Activity via the EDD-DDB1-VPRBP E3 Ligase Complex*

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Background: Telomerase is an essential enzyme for chromosome stability.
Results: The HIV-1 accessory protein Vpr targets TERT, a catalytic subunit of telomerase, via ubiquitin-mediated degradation.
Conclusion: Vpr inhibits telomerase activity by TERT down-regulation.
Significance: Learning how telomerase is deregulated by HIV-1 Vpr is crucial for understanding HIV-1-associated pathogenesis.

Viral pathogens utilize host cell machinery for their benefits. Herein, we identify that HIV-1 Vpr (viral protein R) negatively modulates telomerase activity. Telomerase enables stem and cancer cells to evade cell senescence by adding telomeric sequences to the ends of chromosomes. We found that Vpr inhibited telomerase activity by down-regulating TERT protein, a catalytic subunit of telomerase. As a molecular adaptor, Vpr enhanced the interaction between TERT and the VPRBP substrate receptor of the DYRK2-associated EDD-DDB1-VPRBP E3 ligase complex, resulting in increased ubiquitination of TERT. In contrast, the Vpr mutant identified in HIV-1-infected long-term nonprogressors failed to promote TERT destabilization. Our results suggest that Vpr inhibits telomerase activity by hijacking the host E3 ligase complex, and we propose the novel molecular mechanism of telomerase deregulation in possibly HIV-1 pathogenesis.

Viral pathogens use host cell machinery for their benefit. For example, of the HIV-1 auxiliary proteins (Vif, Vpu, Vpr (viral protein R), and Nef), Vpu induces degradation of the CD4 receptor by recruiting BTRC/β-TrCP (1). In addition, Vif targets APOBEC3G, a deaminase that destroys viral transcripts, using CUL5-associated E3 ligase and the transcriptional cofactor core-binding factor β (2–6). Similarly, Vpx from HIV-2 targets SAMHD1 (SAM and HD domain-containing protein 1), a viral restriction factor, via E3 ligase (7, 8). Vpr also controls various cellular events, including reverse transcription, nuclear transport of the viral preintegration complex, LTR-mediated transcription, cell cycle arrest at G_2 phase, and apoptosis (9). Moreover, interaction between Vpr and VPRBP (Vpr-binding protein), also known as DCAF1 (DDB1- and CUL4-associated factor 1) (10), implies a crucial role for Vpr in ubiquitination of various cellular proteins.

Maintenance of genomic stability is essential for cell self-renewal and the subsequent transmission of accurate genetic information during cell division (11). In eukaryotes, telomerase overcomes the end replication problem by adding the telomeric repeat sequence TTAGGG to the ends of chromosomes (12). Telomerase is composed of two subunits: TERC, an RNA template, and TERT, a reverse transcriptase (13). TERT expression is limited to stem, germ, and regenerating cells (14). In somatic cells, the absence of telomerase results in the gradual loss of telomeric repeats with every cell division (15). These cells typically undergo growth arrest and cell senescence (16).

Previously, it was shown that HIV-1 down-regulates telomerase activity in peripheral blood mononuclear cells (PBMCs)[2] *in vivo* and *in vitro* (17, 18), $CD4^+$ lymphocytes (19), and lymphoblastic cells (20). Moreover, telomerase reactivation enhances the antiviral activity of T lymphocytes against HIV-1 (21), implying important roles of telomerase regulation in HIV-1-associated disease progression. Herein, we sought to understand the biological function of HIV-1-induced telomerase deregulation. We performed biochemical studies of telomerase and identified that HIV-1 Vpr down-regulates telomerase activity via the host E3 ligase complex.

EXPERIMENTAL PROCEDURES

Mammalian Cell Culture—HeLa, 293T, Jurkat, and SupT1 cells were maintained in Dulbecco's modified Eagle's medium or RPMI 1640 medium containing 10% FBS. HeLa cells stably expressing FLAG-tagged TERT were established by transduction of pMGIB-3FLAG-TERT retrovirus. Chronically HIV-in-

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[2] The abbreviations used are: PBMC, peripheral blood mononuclear cell; TRAP, telomeric repeat amplification protocol; qPCR, quantitative PCR; EDVP, EDD-DDB1-VPRBP complex; LTNP, long-term nonprogressor.

fected H9 cells (H9/HIV-1_{IIIB}) were maintained in RPMI 1640 medium supplemented with 10% FBS.

Flow Sorting and Infection of $CD4^+$ T Cells—Human blood samples were purchased from the Gulf Coast Blood Center (Houston, TX), and PBMCs were purified by the standard Ficoll-Histopaque density gradient centrifugation method. To purify CD4 T lymphocytes from the total PBMCs, cells were first stained with allophycocyanin-conjugated anti-CD3 antibody (clone SP34.2; BD Biosciences) and phycoerythrin-conjugated anti-CD4 antibody (clone L200; BD Biosciences) at 4 °C for 30 min, and then the $CD4^+CD3^+$ cells were collected using a FACSAria cell sorter (BD Biosciences). Purified $CD4^+CD3^+$ T cells were cultured in complete RPMI 1640 medium (10% heat-inactivated FBS) containing 5 μg/ml phytohemagglutinin and 10 units/ml human IL-2 (Sigma-Aldrich) at 37 °C and 5% CO_2 for 48 h. Following the stimulation, cells were washed twice to remove phytohemagglutinin and then resuspended at a concentration of 2×10^6 cells/ml before infection with HIV-1_{IIIB} (4×10^3 $TCID_{50}$/ml in HeLa CD4-LTR/β-gal determined by multinuclear activation of a galactosidase indicator (MAGI) assay) for 2 h. After infection, cells were washed three times with complete RPMI 1640 medium and then cultured in complete RPMI 1640 medium supplemented with 10 units/ml human IL-2 for different durations. Cells were collected for telomeric repeat amplification protocol (TRAP) assay on days 1, 3, 5, 7, and 9. Uninfected cells were used as controls at all time points tested.

Constructs—HIV-1 accessory gene constructs were obtained from the National Institutes of Health AIDS Research and Reference Reagent Program (NIAID). Full-length TERT, Vpr, Vpr mutants, UBC, and DYRK2 (dual-specificity tyrosine phosphorylation-regulated kinase 2) were cloned into HA-tagged pcDNA3.1, FLAG-tagged pcDNA3.1, HA-tagged pWZL, FLAG-tagged pMIGB, and pGEX-6T vectors. The Myc-tagged VPRBP construct was provided by J. Chen (The University of Texas MD Anderson Cancer Center).

Immunoblotting and Immunoprecipitation—Whole cell lysates were prepared in Nonidet P-40 lysis buffer (0.5% Nonidet P-40, 1.5 mM $MgCl_2$, 25 mM HEPES, 150 mM KCl, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 12.7 mM benzamidine HCl, 0.2 mM aprotinin, 0.5 mM leupeptin, and 0.1 mM pepstatin A) for 20 min at 4 °C, followed by centrifugation (14,000 rpm for 10 min). Supernatants were denatured with 5× SDS sample buffer (200 mM Tris-Cl (pH 6.8), 40% glycerol, 8% SDS, 200 mM dithiothreitol, and 0.08% bromphenol blue) at 95 °C for 5 min and resolved by SDS-PAGE. For immunoblot blocking and antibody incubation, 0.1% nonfat dry milk in Tris-buffered saline/Tween 20 (25 mM Tris-HCl (pH 8.0), 125 mM NaCl, and 0.5% Tween 20) was used. SuperSignal West Pico and Femto reagents (Pierce) were used to detect horseradish peroxidase-conjugated secondary antibodies. For immunoprecipitation, cells on 10-cm plates were lysed with 0.3 ml of Nonidet P-40 lysis buffer for 20 min at 4 °C and then spun at 14,000 rpm for 10 min. The cell lysates were incubated overnight at 4 °C with 20 μl of either HA-agarose or FLAG-magnetic beads. Immunoprecipitates were washed four times with Nonidet P-40 lysis buffer, eluted using SDS sample buffer, and analyzed by immunoblotting. The following antibodies were used for immunoprecipitation and immunoblotting: anti-EDD, anti-VPRBP, and anti-DDB1 antibodies (Bethyl Laboratories) and anti-FLAG (M2), anti-c-Myc (9E10), anti-HA (12CA5, HA-7, and 3F10), and anti-TUBA4A (tubulin) antibodies (Sigma).

TRAP Assay—Cells (293T, HeLa, Jurkat, SupT1, and H9) were lysed with 100 μl of Nonidet P-40 lysis buffer containing 400 units of an RNase inhibitor. The cell lysates were analyzed using the TRAP reaction assay with the TRAPeze telomerase detection kit (Millipore) in accordance with the manufacturer's protocol. In addition, a quantitative telomerase detection kit (US Biomax) was used for quantitative PCR (qPCR)-based telomerase activity measurement. RNase-treated samples were included as negative controls.

Transfection—HeLa and 293T cells were transfected with plasmids and siRNAs using Lipofectamine 2000 (Invitrogen) in accordance with the manufacturer's protocol. Control, VPRBP, and CUL4A siRNAs were purchased from Dharmacon.

Semiquantitative PCR—One microgram of RNA extracted from each cell was used to generate cDNA samples (SuperScript II, Invitrogen). Next, cDNA was analyzed using standard PCR.[3]

Ubiquitination Assay—For *in vivo* ubiquitination assay, HeLa cells were transiently cotransfected with pCMV-HA-tagged UBC and pcDNA3.1–3FLAG-tagged TERT and subsequently immunoprecipitated with HA-agarose (HA-7). The precipitates were then immunoblotted with anti-FLAG antibody. *In vitro* ubiquitination reactions were performed at 30 °C for 8 h in 30 μl of ubiquitination reaction buffer (40 mM Tris-HCl (pH 7.6), 2 mM dithiothreitol, 5 mM $MgCl_2$, 0.1 M NaCl, and 2 mM ATP) containing 100 μM ubiquitin, 20 nM UBE1, 100 nM UBE2D2/UBCH5B (all from Boston Biochem), and EDD-DDB1-VPRBP (EDVP) E3 ligase components (50 ng each of EDD, DDB1, VPRBP, and DYRK2). A FLAG-TERT substrate was generated using a TnT-coupled reticulocyte lysate system (Promega). After the ubiquitination reaction, samples were boiled in SDS-PAGE loading buffer. Ubiquitination of TERT was monitored by immunoblotting with anti-ubiquitin and anti-FLAG antibodies.

RESULTS

Vpr Inhibits Telomerase Activity via TERT Protein Down-regulation—We hypothesized that HIV-1 deregulates telomerase activity, which might give rise to HIV symptoms. To test this, we first examined the effect of HIV-1 accessory proteins (Vif, Vpu, Vpr, and Nef) on TERT, a catalytic subunit of telomerase. We ectopically expressed each HIV-1 accessory protein in HeLa cells stably expressing FLAG-TERT (HeLa-TERT cells) and examined the level of TERT protein by immunoblotting. Of note, we confirmed that FLAG-TERT is functionally similar to endogenous TERT protein for telomerase activity (data not shown). Interestingly, the among HIV-1 accessory proteins, Vpr specifically down-regulated TERT protein levels in HeLa-TERT cells (Fig. 1*A*). Additionally, we examined the levels of *TERT* and *TERC* mRNAs by semiquantitative RT-PCR and observed that *TERT* and *TERC* mRNA expression was not down-regulated by Vpr expression (Fig. 1*B*). These data suggest

[3] The sequence information for each primer is available upon request.

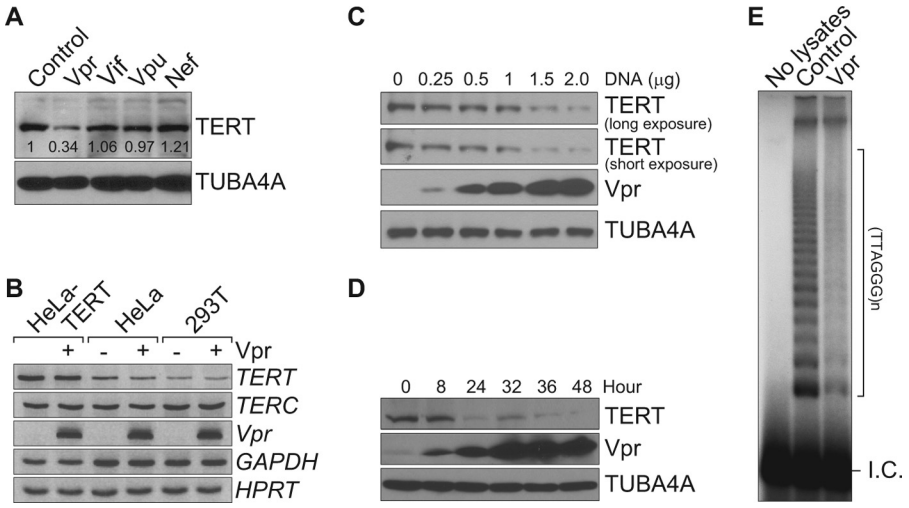

FIGURE 1. **Vpr inhibits telomerase activity by TERT down-regulation.** *A*, down-regulation of TERT protein expression by Vpr. HeLa cells stably expressing FLAG-TERT (HeLa-TERT cells) were transiently transfected with each construct (Vpr, Vif, Vpu, and Nef). Twenty-four hours later, cells were harvested for immunoblot analysis of FLAG-TERT. The level of TERT protein was normalized to TUBA4A (tubulin) and quantified using ImageJ. *B*, no effects of Vpr on *TERT* transcription. HeLa, HeLa-TERT, and 293T cells were transiently transfected with a FLAG-tagged Vpr construct and harvested for gene expression analysis using semiquantitative RT-PCR assays. *GAPDH* and *HPRT* were used as internal controls. *C*, down-regulation of TERT expression by Vpr in a dose-dependent manner. A Vpr construct was transfected into HeLa-TERT cells at different doses. Twenty-four hours later, cells were harvested for immunoblot analysis. *D*, time-dependent effect of Vpr on down-regulation of TERT protein expression. HeLa-TERT cells were transfected with 2 μg of Vpr and collected for immunoblot analysis at different times. Vpr expression in the cells was quantified by immunoblotting (FLAG). TUBA4A was used as an internal control. *E*, suppression of telomerase activity by Vpr expression. HeLa cells were transiently transfected with a Vpr expression plasmid. Twenty-four hours later, cells were collected to quantify telomerase activity using TRAP assays. The telomeric repeat sequence (TTAGGG) increment indicates telomerase activity. *I.C.*, internal control.

that Vpr inhibits TERT levels at the post-transcriptional level. Moreover, we found that Vpr reduced TERT expression in a dose- and time-dependent manner (Fig. 1, *C* and *D*). Next, we examined the effects of Vpr-induced TERT protein down-regulation on telomerase activity using TRAP assays. Consistent with TERT protein down-regulation by Vpr, transient overexpression of Vpr reduced the endogenous telomerase activity in HeLa cells (Fig. 1*E*). These data suggest that Vpr negatively regulates telomerase activity via TERT protein down-regulation.

Vpr Increases TERT Ubiquitination—It was previously shown that Vpr physically binds to the VPRBP E3 ligase substrate receptor (10). VPRBP is a substrate recognition module of E3 ligase complexes, including CUL4A-DDB1 and EDD-DDB1 (22, 23). Importantly, we recently found that TERT protein is phosphorylated and ubiquitinated by the DYRK2-associated EDVP E3 ligase complex (24). Hence, we hypothesized that Vpr down-regulates TERT protein via VPRBP E3 ligase-mediated TERT protein ubiquitination. To address this, we performed *in vivo* ubiquitination assays using HeLa cells. We observed that ectopic expression of Vpr moderately increased TERT protein ubiquitination (Fig. 2*A*). Additionally, *in vitro* ubiquitination assays using recombinant proteins also showed that Vpr enhanced TERT protein ubiquitination (Fig. 2*B*). Of note, TERT protein was constitutively ubiquitinated (Fig. 2, *lane 2*), implying the short half-life of TERT protein. In pulse-chase experiment using [^{35}S]methionine, we found that the half-life of TERT proteins is 2.1 h (24). In addition, treatment with MG132, a proteasome inhibitor, blocked Vpr-induced TERT down-regulation (data not shown). The well demonstrated function of Vpr is to arrest the cell cycle at G_2 phase by activating the ATR (ataxia telangiectasia- and Rad3-related) DNA damage checkpoint (25) via the Vpr-associated CUL4A-DDB1-VPRBP E3 ligase complex (26–28). In our experimental setting, to exclude the possibility that Vpr-induced G_2 arrest may affect TERT protein, we titrated Vpr expression at the subphenotypic level, which did not induce cell cycle arrest, and then examined the effects of Vpr on TERT and telomerase activity. Also, we observed that CUL4A knockdown using siRNAs did not affect Vpr-induced telomerase inhibition (Fig. 2, *C* and *D*). Therefore, our experimental results suggest the cell cycle regulation-independent function of Vpr in targeting TERT. These data suggest that Vpr inhibits telomerase activity by promoting TERT protein ubiquitination and down-regulation.

VPRBP Mediates Vpr-induced TERT Down-regulation—Given the Vpr-VPRBP association (10), we reasoned that Vpr might modulate the E3 ligase activity of VPRBP in target protein ubiquitination. To address this, we took advantage of Vpr mutants. Several studies have identified Vpr mutations (*e.g.* R77Q, Q65R, and R85P) that are pathologically associated with long-term nonprogressors (LTNPs) who have survived without having any HIV-related symptoms (29–31). Hence, the use of Vpr LTNP mutants may provide insight into how Vpr-induced TERT down-regulation is related to HIV pathogenesis. First, we examined whether Vpr LTNP mutants induce TERT protein degradation. We ectopically expressed wild-type Vpr and LTNP mutants in HeLa-TERT cells and examined the level of FLAG-TERT protein by immunoblotting. Intriguingly, Vpr LTNP mutants Q65R, F72L, and R77Q failed to down-regulate TERT protein (Fig. 3*A*). Similarly, Vpr LTNP mutants did not inhibit telomerase activity, as demonstrated by qPCR-based TRAP assays (Fig. 3*B*). Importantly, it has been shown that the Q65R point mutation in Vpr disrupts the leucine-rich motif that is necessary for Vpr-VPRBP interaction (10). Thus, we

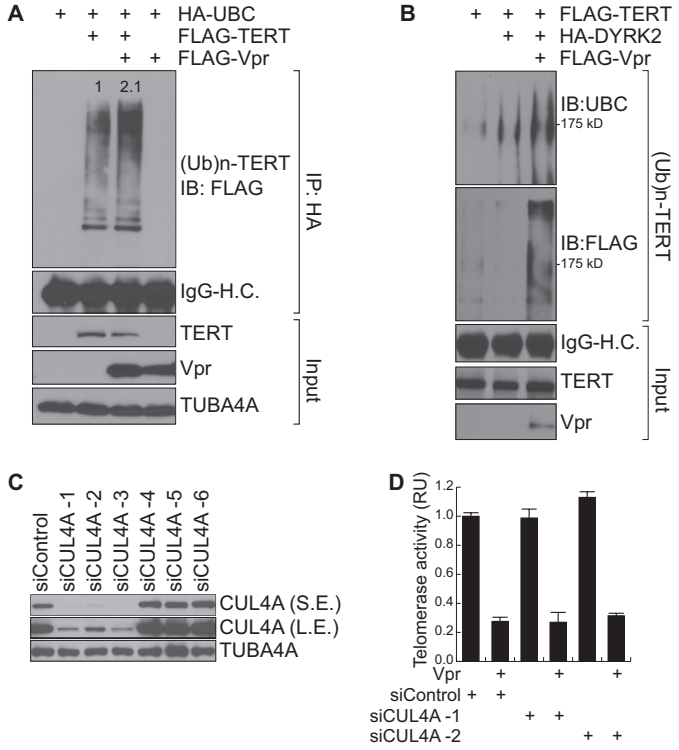

FIGURE 2. **Vpr induces TERT ubiquitination.** *A*, increased TERT ubiquitination by Vpr *in vivo*. HeLa cells were transiently transfected with three plasmids (HA-UBC, FLAG-TERT, and FLAG-Vpr) for *in vivo* ubiquitination assays. Thirty-six hours later, cell lysates were immunoprecipitated (*IP*) with anti-HA antibody (UBC), and TERT ubiquitination (*(Ub)n-TERT*) in the lysates was analyzed by immunoblotting (*IB*; FLAG). Although TERT expression was down-regulated by Vpr (*Input panels*, *lanes 2* and *3*), TERT ubiquitination was increased (*IP: HA panels*, *lanes 2* and *3*). Blots were quantified using ImageJ. *H.C.*, heavy chain. *B*, increased TERT ubiquitination by Vpr *in vitro*. Recombinant TERT and DYRK2 proteins (in the absence and presence of Vpr protein) were incubated with EDVP E3 ligase components, UBE1, UBE2D2, and ATP and analyzed by immunoblotting. *C* and *D*, CUL4A-independent Vpr-induced telomerase inhibition. *C*, deletion of CUL4A using CUL4A siRNA transfection. HeLa cells were transiently transfected with control siRNA (*siControl*) and six different CUL4A siRNAs (*siCUL4A-1–6*) and analyzed by immunoblotting. *S.E.*, short exposure; *L.E.*, long exposure. *D*, Vpr-induced telomerase inhibition in a CUL4A-independent manner. HeLa cells were transiently transfected with siRNAs (control *versus* CUL4A) and Vpr expression plasmids. Twenty-four hours later, cells were analyzed for endogenous telomerase activity using qPCR-based TRAP assays. *RU*, relative units. *Error bars* indicate S.D. ($n = 3$).

hypothesized that Vpr-VPRBP binding is required for TERT protein down-regulation. We examined the interaction between Vpr (wild-type or Q65R mutant) and TERT in HeLa cells using co-immunoprecipitation assays. We observed that Vpr Q65R did not interact with TERT, whereas wild-type Vpr bound to TERT (Fig. 3*C*, *lanes 4* and *5*). Of note, VPRBP in the EDVP E3 ligase complex is responsible for substrate recognition. Thus, Vpr Q65R may be incapable of binding to TERT. As reported previously (26), we observed that Vpr Q65R did not bind to VPRBP in co-immunoprecipitation assays with HeLa cells (Fig. 3*D*), suggesting that Vpr is dependent on VPRBP for TERT interaction. To confirm the requirement of VPRBP for Vpr-induced TERT ubiquitination, we depleted VPRBP in HeLa-TERT cells using VPRBP siRNA and examined the effects of Vpr ectopic expression on TERT protein levels. The depletion of VPRBP indeed inhibited Vpr-induced TERT degradation (Fig. 3*E*, *lanes 3* and *4*). These results suggest that Vpr-VPRBP interaction mediates Vpr-induced TERT ubiquitination.

Vpr Promotes TERT-VPRBP Association—We next examined the impact of Vpr on the EDVP E3 ligase in TERT ubiquitination. Vpr is a small protein (96 amino acids) and consists of three α-helices, which are folded around a hydrophobic core and allow Vpr to bind to other proteins (32). Thus, we hypothesized that Vpr functions as a molecular adaptor to increase the binding affinity between the substrate (TERT) and the E3 ligase substrate receptor (VPRBP). To address this, we examined whether Vpr modulates TERT-VPRBP protein interaction using protein pulldown assays. *In vitro* pulldown assays showed that Vpr enhanced TERT-VPRBP binding (Fig. 4*A*, *lane 4*). However, Vpr Q65R failed to increase TERT-VPRBP binding (Fig. 4*A*, *lane 5*). Furthermore, using co-immunoprecipitation and immunoblot analyses, we observed similar effects of wild-type Vpr on elevation of TERT-VPRBP binding in HeLa-TERT cells (Fig. 4*B*, *lane 2*), whereas Vpr Q65R had no impact on TERT-VPRBP binding (*lane 3*). Next, to determine the physiological interaction of Vpr with the endogenous EDVP complex, we performed a co-immunoprecipitation assay for Vpr and analyzed Vpr-interacting EDVP complex components by immunoblotting in HeLa cells. Indeed, endogenous EDD, DDB1, and VPRBP interacted with wild-type Vpr, but not with Vpr Q65R (Fig. 4*C*). These results suggest that Vpr down-regulates TERT protein via promoting TERT-VPRBP interaction.

Vpr Inhibits Endogenous Telomerase Activity—Although we observed that Vpr and the EDVP E3 ligase complex target TERT protein, we utilized cancer cells that aberrantly or ectopically express TERT. Thus, we addressed whether Vpr suppresses endogenous telomerase activity in immunocytes. First, we examined whether Vpr suppresses the endogenous telomerase activity of Jurkat cells and SupT1-immortalized T cells. We transduced Jurkat and SupT1 cells with retroviruses encoding Vpr and performed TRAP assays. We observed that Vpr expression down-regulated telomerase activity in Jurkat and SupT1 cells. However, Vpr Q65R failed to inhibit telomerase activity in Jurkat and SupT1 cells (Fig. 5*A*). Additionally, HIV-1 infection in HIV-susceptible human $CD4^+$ lymphoblastoid cells (H9) also decreased telomerase activity (Fig. 5*B*). Moreover, we examined the effects of HIV infection on telomerase activity in human $CD4^+$ T cells isolated from PBMCs. Consistently, we observed that HIV infection suppressed telomerase activity in $CD4^+$ T cells (Fig. 5*C*). These results suggest that Vpr inhibits telomerase activity in immunocytes.

DISCUSSION

Herein, we observed that Vpr represses telomerase activity by using the host E3 ligase machinery (Fig. 6). Our results suggest that this process is initiated by Vpr-induced facilitation of TERT loading onto a VPRBP substrate receptor module in the E3 ligase complex, as demonstrated by the increased interaction between TERT and VPRBP by Vpr introduction (Fig. 4, *A* and *B*). Vpr forms a ternary complex with DDB1-VPRBP via a WD*X*R motif in VPRBP (33). Given that the hydrophobic core in Vpr provides the structural platform for strong protein interaction via three α-helices, it is probable that enhancement of TERT-VPRBP binding by Vpr may be due to Vpr-induced conformational change in VPRBP.

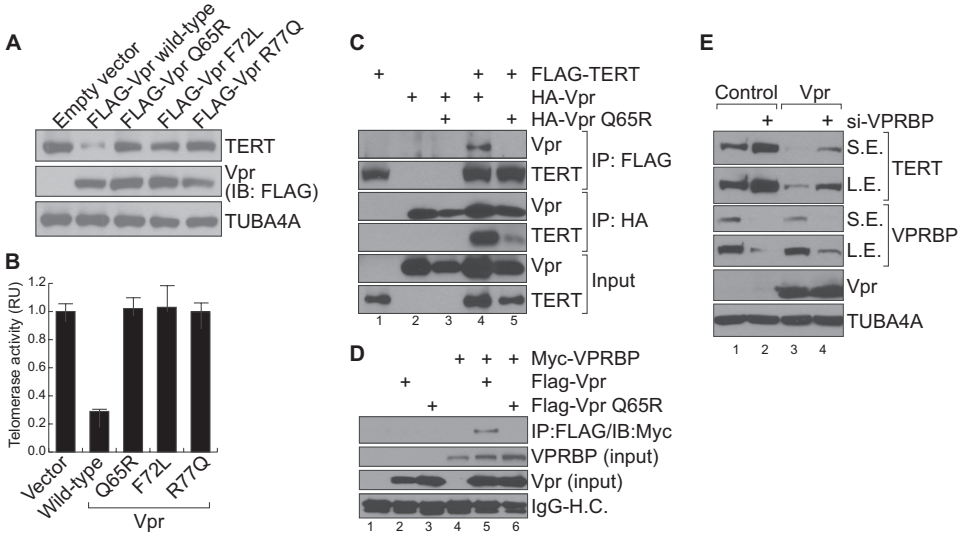

FIGURE 3. **VPRBP mediates Vpr-induced TERT degradation.** *A* and *B*, Vpr-induced TERT down-regulation via VPRBP. HeLa-TERT cells were transiently transfected with plasmids encoding wild-type Vpr or LTNP mutants (Q65R, F72L, and R77Q). Thirty-six hours after transfection, cells were harvested for immunoblotting (*IB*; *A*) and qPCR-based TRAP assays (*B*). *RU*, relative units. *C*, decreased binding of the Vpr LTNP mutant Q65R to TERT. HeLa cells were transiently transfected with the FLAG-TERT, HA-Vpr, or HA-Vpr Q65R plasmid. Cell lysates were then analyzed for interaction between TERT and Vpr (wild-type or Q65R) using reciprocal immunoprecipitation (*IP*) and immunoblotting. *D*, lack of binding of Vpr Q65R to VPRBP. HeLa cells were transfected with VPRBP and Vpr (wild-type or Q65R mutant). Each sample was then subjected to immunoprecipitation and immunoblot analysis. *H.C.*, heavy chain. *E*, VPRBP mediation of Vpr-induced TERT destabilization. HeLa-TERT cells were cotransfected with control or VPRBP siRNA (*si-VPRBP*) with Vpr expression plasmid. Cells were analyzed by immunoblotting for TERT protein levels. *S.E.*, short exposure; *L.E.*, long exposure.

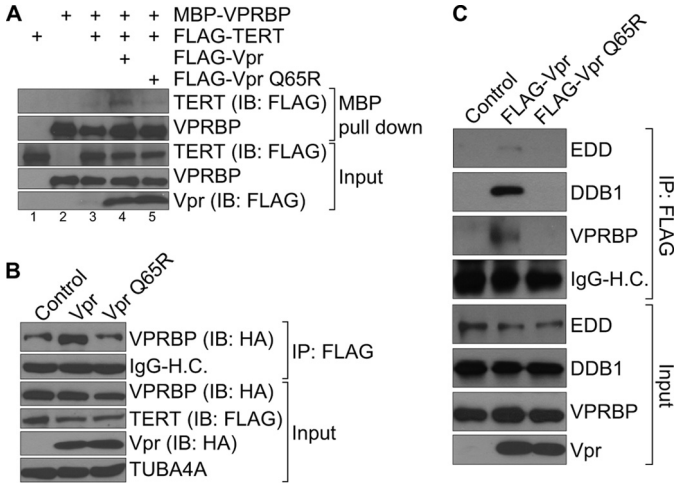

FIGURE 4. **Vpr promotes TERT-VPRBP association.** *A*, Vpr increases TERT-VPRBP interaction *in vitro*. TERT-VPRBP interaction was quantified using *in vitro* binding assays. *In vitro* translated TERT and Vpr (wild-type or Q65R mutant) were incubated with maltose-binding protein (*MBP*)-purified VPRBP protein and analyzed using maltose-binding protein pulldown assays. *IB*, immunoblot. *B*, Vpr promotion of TERT-VPRBP interaction *in vivo*. HeLa-TERT cells were transfected with Vpr (wild-type or Q65R mutant) and analyzed for TERT-VPRBP interaction by immunoprecipitation (*IP*; FLAG-TERT) and immunoblotting (HA-VPRBP). *H.C.*, heavy chain. *C*, association of Vpr with the endogenous EDVP E3 ligase complex. HeLa cells transfected with EDVP components were analyzed for Vpr-EDVP interaction by immunoprecipitation and immunoblotting.

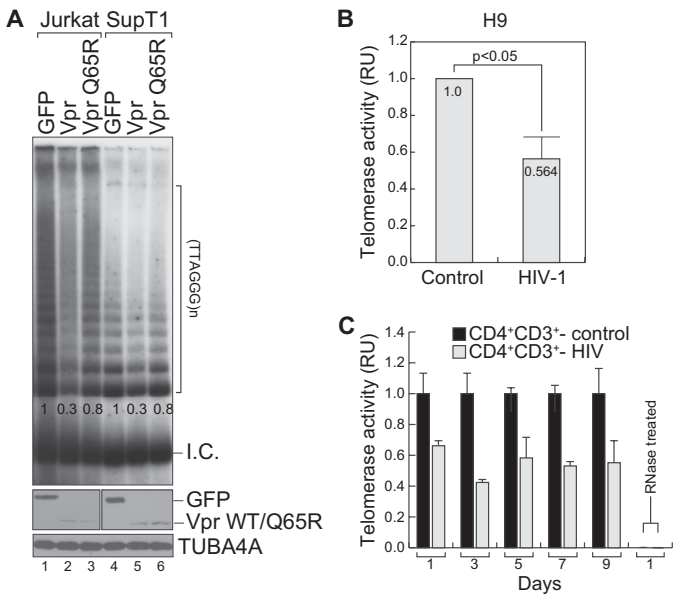

FIGURE 5. **Vpr inhibits endogenous telomerase activity.** *A*, Vpr inhibition of endogenous telomerase activity in immortalized T lymphocytes. Jurkat and SupT1 cells were stably transduced with HA-tagged Vpr (wild-type or Q65R mutant) retrovirus or control HA-tagged EGFP retrovirus. Four days after selection with hygromycin, cells were harvested for TRAP assays. Autoradiography was quantified using ImageJ. *I.C.*, internal control. *B* and *C*, inhibition of telomerase activity by HIV infection. H9 cells (parental) and chronically HIV-infected H9 cells were analyzed for telomerase activity (qPCR-based TRAP assay) (*B*). *RU*, relative units. Human $CD4^+CD3^+$ T cells were isolated from PBMCs and infected with HIV. At each time point, $CD4^+CD3^+$ T cells were collected and analyzed for telomerase activity using qPCR-based TRAP assays (*C*). RNase-treated samples were included as negative controls.

In addition to AIDS-related symptoms, HIV-infected patients also display non-AIDS-related symptoms that are associated with cellular and tissue aging (34). Given the canonical role of telomerase in cellular aging, it is conceivable that HIV-1 Vpr-induced telomerase deregulation might lead to telomere shortening and accelerated aging in non-AIDS-related HIV pathogenesis. Additionally, it is also possible that Vpr may affect non-telomeric roles of telomerase in cell proliferation, stem cell regulation, and gene expression (35–41). Because our scope is limited to the biochemical analyses of Vpr, it is necessary to examine the effects of HIV-1 carrying Vpr (wild-type or mutant) on both telomeric and non-telomeric

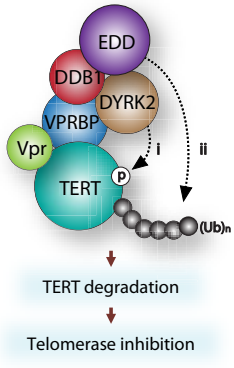

FIGURE 6. **Illustration of the mechanism of Vpr-induced telomerase deregulation.** DYRK2-induced phosphorylation of TERT induces TERT-EDVP association (*arrow i*) in the presence of Vpr. Vpr promotes TERT-VPRBP binding, which facilitates DYRK2-associated EDVP E3 ligase-mediated ubiquitination of TERT (*arrow ii*). Polyubiquitinated (*(Ub)n*) TERT is then targeted by proteasome-mediated degradation, which results in loss of telomerase activity. The decreased telomerase activity elicits telomere crisis during successive cell division and subsequent cell senescence (canonical function of telomerase). Alternatively, telomerase inhibition deregulates signaling pathways, stem cell activity, and gene expression (non-canonical function of telomerase).

functions of telomerase in more physiological conditions. Taken together, our results suggest that Vpr inhibits telomerase activity by hijacking the host E3 ligase.

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