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

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Xin Wang†, Shailbala Singh§, Hae-Yun Jung¶, Guojun Yang†, Sohee Jun‡, K. Jagannadha Sastry§, and Jae-Il Park†‡¶
From the Departments of †Experimental Radiation Oncology and §Immunology and the ¶Program in Cancer Biology, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030 and the §Graduate School of Biomedical Sciences at Houston, The University of Texas Health Science Center and MD Anderson Cancer Center, Houston, Texas 77030

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 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 substate 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 G2 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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**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. To ectopically express each HIV-1 accessory protein (Vif, Vpu, Vpr, and Nef) on TERT, a catalytic subunit of telomerase activity was 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 MgCl₂, 0.1 mM NaCl, and 2 mM ATP) containing 100 μM ubiquitin, 20 μM UBE1, 100 μM UBE2D2/UBCH5B (all from Boston Biochem), and EDD-DDB1–VPRBP (EDV) 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.

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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. 1E). 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. 2A). Additionally, in vitro ubiquitination assays using recombinant proteins also showed that Vpr enhanced TERT protein ubiquitination (Fig. 2B). Of note, TERT protein was constitutively ubiquitinated (Fig. 2, lane 2), implying the short half-life of TERT protein. In pulse-chase experiment using [35S]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 G2 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 G2 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 TERT protein by immunoblotting. Intriguingly, Vpr LTNP mutants Q65R, F72L, and R77Q failed to down-regulate TERT protein (Fig. 3A). Similarly, Vpr LTNP mutants did not inhibit telomerase activity, as demonstrated by qPCR-based TRAP assays (Fig. 3B). 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
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. 4A, lane 4). However, Vpr Q65R failed to increase TERT-VPRBP binding (Fig. 4A, 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. 4B, 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. 4C). 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. 5A). Additionally, HIV-1 infection in HIV-susceptible human CD4+ lymphoblastoid cells (H9) also decreased telomerase activity (Fig. 5B). 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. 5C). 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 WD40 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.
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
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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REFERENCES

1. Margottin, F., Bour, S. P., Durand, H., Selig, L., Benichou, S., Richard, V., Thomas, D., Strebel, K., and Benarous, R. (1998) A novel human WD protein, h-βTrCP, that interacts with HIV-1 Vpu connects CD4 to the ER degradation pathway through an F-box motif. Mol. Cell 1, 565–574
2. Yu, X., Yu, Y., Liu, B., Luo, K., Kong, W., Mao, P., and Yu, X. F. (2003) Induction of APOBEC3G ubiquitination and degradation by an HIV-1 Vif-Cul5-SCF complex. Science 302, 1056–1060
3. Marin, M., Rose, K. M., Kozak, S. L., and Kabat, D. (2003) HIV-1 Vif protein binds the editing enzyme APOBEC3G and induces its degradation. Nat. Med. 9, 1398–1403
4. Sheehy, A. M., Gaddis, N. C., and Malim, M. H. (2003) The antiretroviral enzyme APOBEC3G is degraded by the proteasome in response to HIV-1 Vif. Nat. Med. 9, 1404–1407
5. Mariani, R., Chen, D., Schröfelbauer, B., Navarro, F., König, R., Bollman, B., Münk, C., Nymark-McMahon, H., and Landau, N. R. (2003) Species-specific exclusion of APOBEC3G from HIV-1 virions by Vif. Cell 114, 21–31
6. Jäger, S., Kim, D. Y., Hultquist, J. F., Shinoh, K., LaRue, R. S., Kwon, E., Li, M., Anderson, B. D., Yen, L., Stanley, D., Mahon, C., Kane, J., Frank-Skiba, K., Cimermancic, P., Burlingame, A., Sali, A., Craik, C. S., Harris, R. S., Gross, J. D., and Krogan, N. J. (2012) Vif hijacks CBF-β to degrade APOBEC3G and promote HIV-1 infection. Nature 481, 371–375
7. Hreeca, K., Hao, C., Gierszewska, M., Swanson, S. K., Kesik-Brodacka, M., Srivastava, S., Flores, L., Washburn, M. P., and Skowronski, J. (2011) Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein. Nature 474, 658–661
8. Laguette, N., Sobhian, B., Casartelli, N., Ringead, M., Chable-Bessia, C., Ségéral, E., Yatim, A., Emiliani, S., Schwartz, O., and Benkirane, M. (2011)

SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. Nature 474, 654–657
9. Kogan, M., and Rappaport, J. (2011) HIV-1 accessory protein Vpr: relevance in the pathogenesis of HIV and potential for therapeutic intervention. Retrovirology 8, 25
10. Zhao, L., I., Mukherjee, S., and Narayan, O. (1994) Biochemical mechanism of HIV-1 Vpr function. Specific interaction with a cellular protein. J. Biol. Chem. 269, 15577–15582
11. Cech, T. R. (2004) Beginning to understand the end of the chromosome. Cell 116, 273–279
12. Shippen-Lentz, D., and Blackburn, E. H. (1990) Functional evidence for an RNA template in telomerase. Science 247, 546–552
13. Anteixier, C., and Luc, N. F. (2006) The structure and function of telomerase reverse transcriptase. Annu. Rev. Biochem. 75, 493–517
14. Meyerson, M., Counter, C. M., Eaton, E. N., Ellisen, L. W., Steiner, P., Caddel, S. D., Ziaugra, L., Beijersbergen, R. L., Davidoff, M. J., Liu, Q., Bacchetti, S., Haber, D. A., and Weinberg, R. A. (1997) hTERT: the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization. Cell 90, 785–795
15. Bodnar, A. G., Ouellette, M., Frolikis, M., Holt, S. E., Chiu, C. P., Morin, G. B., Harly, C. B., Shy, J. W., Lichtsteiner, S., and Wright, W. E. (1998) Extension of life-span by introduction of telomerase into normal human cells. Science 279, 349–352
16. Harly, C. B., Futcher, A. B., and Greider, C. W. (1997) Telomeres shorten during ageing of human fibroblasts. Nature 345, 458–460
17. Ballon, G., Ometto, L., Righetti, E., Cattelan, A. M., Masiero, S., Zanchetta, M., Chieco-Bianchi, L., and De Rossi, A. (2001) Human immunodeficiency virus type 1 modulates telomerase activity in peripheral blood lymphocytes. Oncogene 20, 417–424
18. Bostik, P., Dodd, G. L., Patel, S. S., Kadivar, H., and Ansari, A. A. (2002) Effect of productive in vitro human immunodeficiency virus or simian immunodeficiency virus infection on lymphoid and nonlymphoid cells. J. Infect. Dis. 185, 999–1001; author reply 1001–1002
19. Franze, O., Adama, R., Pollicita, M., Comandini, A., Laidusi, A., Perno, C. F., Aquaro, S., and Bonmassar, E. (2007) Telomerase activity, hTERT expression, and phosphorylation are downregulated in CD4 + T lymphocytes infected with human immunodeficiency virus type 1 (HIV-1). J. Med. Virol. 79, 639–646
20. Reynoso, R., Minces, L., Salomon, H., and Quarello, J. (2006) HIV-1 infection downregulates nuclear telomerase activity on lymphoblastoid cells without affecting the enzymatic components at the transcriptional level. AIDS Res. Hum. Retroviruses 22, 425–429
21. Fauce, S. R., Jamieson, B. D., Chin, A. C., Mitsuysasu, R. T., Parish, S. T., Ng, H. L., Kitchen, C. M., Yang, O. O., Harly, C. B., and Efrros, R. B. (2008) Telomerase-based pharmacologic enhancement of antiviral function of human CD8 + T lymphocytes. J. Immunol. 181, 7400–7406
22. Maddika, S., and Chen, J. (2009) Protein kinase DYRK2 is a scaffold that facilitates assembly of an E3 ligase. Nat. Cell Biol. 11, 409–419
23. Huang, J., and Chen, J. (2008) VprBP targets Merlin to the Roc1-Cul4A-DDB1 E3 ligase complex for degradation. Oncogene 27, 4056–4064
24. Jung, H.-Y., Wang, X., Jun, S., and Park, J.-I. (2013) Dyrk2-associated EDD-DDB1-VprBP E3 ligase inhibits telomerase by TERT degradation. J. Biol. Chem. 288, 7522–7526
25. Roshal, M., Kim, B., Zhu, Y., Nghiem, P., and Planelles, V. (2003) Activation of the ATR-mediated DNA damage response by the HIV-1 viral protein R. J. Biol. Chem. 278, 25879–25886
26. Belizne, J.-P., Duisit, G., Rougeau, N., Mercier, J., Finzi, A., and Cohen, E. A. (2007) HIV-1 Vpr-mediated G2 arrest involves the DDB1-CUL4AVPRBP E3 ubiquitin ligase. PLoS Pathog. 3, e85
27. Tan, L., Ehrlich, E., and Yu, F. X. (2007) DDB1 and Cul4A are required for human immunodeficiency virus type 1 Vpr-induced G2 arrest. J. Virol. 81, 10822–10830
28. Le Rouzic, E., Morel, M., Ayinde, D., Belaïdouni, N., Letienne, J., Transy, S., and Bacchetti, S. (2007) HIV-1 Vpr-mediated G2 arrest involves the DDB1-CUL4AVPRBP E3 ubiquitin ligase. Proc Natl Acad Sci USA 104, 18154–18159
29. Zhang, L., Huang, Y., Yuan, H., Tuttleton, S., and Ho, D. D. (1997) Genetic characterization of vif, vpr, and vpu sequences from long-term survivors of

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FIGURE 6. Illustration of the mechanism of Vpr-induced telomerase deregulation. DYRK2-induced phosphorylation of TERT induces TERT-EDV P binding, which facilitates DYRK2-associated EDVP E3 ligase-mediated ubiquitination of TERT (arrow i). 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 (canonal function of telomerase). Alternatively, telomerase inhibition deregulates signaling pathways, stem cell activity, and gene expression (non-canonical function of telomerase).
human immunodeficiency virus type 1 infection. Virology 228, 340–349
30. Wang, B., Ge, Y. C., Palasanthiran, P., Xiang, S. H., Ziegler, J., Dwyer, D. E., Randle, C., Dowton, D., Cunningham, A., and Saksena, N. K. (1996) Gene defects clustered at the C-terminus of the vpr gene of HIV-1 in long-term nonprogressing mother and child pair: in vivo evolution of vpr quasispecies in blood and plasma. Virology 223, 224–232
31. Lum, J. J., Cohen, O. J., Nie, Z., Weaver, J. G., Gomez, T. S., Yao, X. J., Lynch, D., Pilon, A. A., Hawley, N., Kim, J. E., Chen, Z., Montpetit, M., Sanchez-Dardon, J., Cohen, E. A., and Badley, A. D. (2003) Vpr R77Q is associated with long-term nonprogressive HIV infection and impaired induction of apoptosis. J. Clin. Invest. 111, 1547–1554
32. Morellet, N., Bouaziz, S., Petitjean, P., and Roques, B. P. (2003) NMR structure of the HIV-1 regulatory protein VPR. J. Mol. Biol. 327, 215–227
33. Le Rouzic, E., Belaidouni, N., Estrabaud, E., Morel, M., Rain, J. C., Transy, C., and Margottin-Goguet, F. (2007) HIV1 Vpr arrests the cell cycle by recruiting DCAF1/VprBP, a receptor of the Cul4-DDB1 ubiquitin ligase. Cell Cycle 6, 182–188
34. Deeks, S. G. (2011) HIV infection, inflammation, immunosenescence, and aging. Annu. Rev. Med. 62, 141–155
35. Park, J.-I., Saretzki, G., Peters, H., Wappler, I., Evans, J., Hole, N., von Zglinicki, T., and Lako, M. (2005) Overexpression of telomerase confers growth advantage, stress resistance, and enhanced differentiation of ESCs toward the hematopoietic lineage. Stem Cells 23, 516–529
36. Yang, C., Przyborski, S., Cooke, M. J., Zhang, X., Stewart, R., Anyfantis, G., Atkinson, S. P., Saretzki, G., Armstrong, L., and Lako, M. (2008) A key role for telomerase reverse transcriptase unit in modulating human embryonic stem cell proliferation, cell cycle dynamics, and in vitro differentiation. Stem Cells 26, 850–863