Virology 477 (2015) 10–17

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Determinants for degradation of SAMHD1, Mus81 and induction of G_2 arrest in HIV-1 Vpr and SIVagm Vpr

Ana Beatriz DePaula-Silva [a], Patrick A. Cassiday [a], Jeffrey Chumley [a,1], Alberto Bosque [a], Carlos M.R. Monteiro-Filho [a], Cathal S. Mahon [b], Kelsey R. Cone [c], Nevan Krogan [b], Nels C. Elde [c], Vicente Planelles [a,*]

[a] Division of Microbiology and Immunology, Department of Pathology, University of Utah School of Medicine, 15 North Medical Drive East #2100, Salt Lake City, UT 84112, USA
[b] Department of Cellular and Molecular Pharmacology, University of California San Francisco, San Francisco, CA, USA
[c] Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, UT, USA

ARTICLE INFO

Article history:
Received 24 September 2014
Returned to author for revisions
13 October 2014
Accepted 29 December 2014
Available online 22 January 2015

Keywords:
HIV-1
SIV
Vpr
Mus81
Agm
SAMHD1
DCAF1
G2 arrest
Ubiquitination
MLN4924

ABSTRACT

Vpr and Vpx are a group of highly related accessory proteins from primate lentiviruses. Despite the high degree of amino acid homology within this group, these proteins can be highly divergent in their functions. In this work, we constructed chimeric and mutant proteins between HIV-1 and SIVagm Vpr in order to better understand the structure–function relationships. We tested these constructs for their abilities to induce G_2 arrest in human cells and to degrade agmSAMHD1 and Mus81. We found that the C-terminus of HIV-1 Vpr, when transferred onto SIVagm Vpr, provides the latter with the *de novo* ability to induce G_2 arrest in human cells. We confirmed that HIV-1 Vpr induces degradation of Mus81 although, surprisingly, degradation is independent and genetically separable from Vpr's ability to induce G_2 arrest.

Introduction

The HIV-1 genome encodes structural proteins (Gag, Pol and Env), regulatory proteins (Tat and Rev), and accessory proteins such as Vif, Vpr, Vpu and Nef. HIV-2, SIVmac and SIVsmm encode Vpr, Vpx, Vif and Nef as accessory proteins. Vpr and Vpx from HIV-2, SIVmac and SIVsmm are highly related to each other and are thought to have arisen through gene duplication (Tristem et al., 1992). SIVagm encodes Vif, Vpr and Nef. A Vpu homolog is not found in the HIV-2/SIVmac/SIVsmm or the SIVagm phylogenetic groups.

From the entry step to the time of release from the host cell, lentiviruses encounter several restriction factors that function to inhibit viral infection and are considered innate immune effector mechanisms. TRIM5α, APOBEC3G, sterile alpha motif (SAM) and HD domain-containing protein 1 (SAMHD1) and tetherin are examples of host restriction factors. These restriction factors are typically overcome by the accessory proteins encoded by lentiviruses (reviewed in (Strebel, 2013)).

Three of the four accessory proteins in each HIV-1 (Vif, Vpr and Vpu) and HIV-2 (Vif, Vpr and Vpx) antagonize innate immunity by a common mechanism, the ubiquitin–proteasome system (UPS). These proteins modify the specificity of cullin-RING ubiquitin ligases (CRUL) such that non-cognate proteins are modified and later degraded.

Vpr has been associated with induction of cell cycle arrest in G_2 and apoptosis (He et al., 1995; Jowett et al., 1995; Stewart et al., 1997). Vpr induces these effects via activation of the ATR kinase (Roshal et al., 2003), a result of manipulation of the ubiquitin ligase $CRUL4^{DDB1/DCAF1}$ (Belzile et al., 2007; DeHart et al., 2007; Hrecka et al., 2007; Le Rouzic et al., 2007; Schrofelbauer et al., 2007; Wen et al., 2007). Recently, the ubiquitination target for the Vpr/CRUL4 complex has been identified (Laguette et al., 2014). Vpr induces premature activation of the SLX4 complex (SLX4com) (Laguette et al., 2014). Vpr increases the binding of DCAF1 to the scaffold protein SLX4 and, together with the polo-like kinase-1 (PLK1), promotes SLX4com remodeling, which results in Mus81 degradation (Laguette et al., 2014). As a consequence of the

* Corresponding author.
E-mail address: vicente.planelles@path.utah.edu (V. Planelles).
[1] Present address: ARUP Laboratories, 500 Chipeta Way, Salt Lake City, UT 84108 USA.

http://dx.doi.org/10.1016/j.virol.2014.12.040
0042-6822/

untimely SLX4com activation, replication forks are processed incorrectly causing cell cycle arrest in G_2 (Laguette et al., 2014). The authors showed that by activating SLX4, Vpr prevents the viral DNA from stimulating cellular DNA sensors, which would normally trigger a type-I interferon response (Laguette et al., 2014)

Nuclear magnetic resonance (NMR) studies indicate that HIV-1 Vpr is comprised of three bundled alpha helices connected by short flexible loops and flanked by flexible amino- and carboxy-terminal unstructured regions (Morellet et al., 2003). The region on Vpr that binds to DCAF1 was mapped within the third α-helix, involving a leucine-rich motif (DeHart et al., 2007; Le Rouzic et al., 2007). The Vpr Q65R amino acid substitution within this region disrupts the interaction between Vpr and DCAF1 resulting in inability to induce G_2 arrest (DeHart et al., 2007; Le Rouzic et al., 2007). The C-terminal unstructured region of Vpr is predicted to be required for interaction with the target, since the mutant R80A within that region is capable of interacting with DCAF1 but is unable to cause G_2 arrest (DeHart et al., 2007). Furthermore, Vpr R80A acts as a dominant-negative protein because it binds to DCAF1 and blocks the Vpr-binding site (DeHart et al., 2007; Le Rouzic et al., 2007).

Vpx is encapsidated in HIV-2 and SIVmac virions and antagonizes SAMHD1 (Hrecka et al., 2011; Laguette et al., 2011). SAMHD1 interferes with the ability of the virus to efficiently synthesize viral cDNA during reverse transcription because it reduces the available cellular pools of dNTPs (Goldstone et al., 2011). Consequently, SAMHD1 diminishes the capacity of the virus to infect macrophages, dendritic cells and quiescent T-cells wherein dNTP levels are normally very low (Hrecka et al., 2011; Laguette et al., 2011). Vpx triggers degradation of SAMHD1 by associating with DCAF1 and recruiting SAMHD1 to the $CRUL4^{DDB1/DCAF1}$ E3 ligase. SAMHD1 is subsequently ubiquitinated and degraded by the proteasome. Inhibition of the interaction between Vpx and DCAF1 by the mutant Vpx (Q76A) results in failure to degrade SAMHD1. HIV-1 Vpr and SIVmac Vpx appear to bind to similar or overlapping regions on DCAF1, although the amino acid residues in DCAF1 involved in interaction with either accessory protein are not identical (Cassiday et al., 2014).

African green monkeys (AGM) are endemically infected with several strains of simian immunodeficiency viruses collectively known as SIVagm (Jin et al., 1994). This group of primate lentiviruses encode three accessory genes: Vpr, Vif and Nef. SIVagm Vpr is bifunctional because it can induce G_2 arrest and also antagonize SAMHD1. Unlike HIV-1 Vpr, SIVagm Vpr is highly species-specific, as it induces G_2 arrest in AGM cells but not in human ones (Fletcher et al., 1996; Planelles et al., 1996), and is able to degrade agmSAMHD1 but not human SAMHD1 (hSAMHD1) (Lim et al., 2012).

In order to understand the structure–function relationships for HIV-1 Vpr and SIVagm Vpr we constructed chimeric proteins by exchanging homologous domains of HIV-1_{NL4-3} Vpr and SIVagm.gri Vpr. We then investigated the ability of the different chimeras to cause cell cycle arrest in G_2 and to induce degradation of SAMHD1 and Mus81. We also investigated whether the structural requirements toward degradation of Mus81 are the same as those required for induction of G_2 arrest by HIV-1 Vpr.

Results

To study the structure–function relationships in HIV-1 Vpr and SIVagm.gri (henceforth, SIVagm) Vpr, a set of 4 chimeras and 2 truncations were constructed as shown in Fig. 1A. The exchange points for the chimeras were designed based on the published nuclear magnetic resonance (NMR) structure of HIV-1 Vpr and the high degree of amino acid homology with SIVagm Vpr (Fig. 1B).

Determinants required for induction of G_2 arrest

It was previously suggested that the C-terminal unstructured region of HIV-1 Vpr was required to interact with a putative G_2 arrest-related cellular factor (DeHart et al., 2007; Di Marzio et al., 1995; Le Rouzic et al., 2007). In support of the previous notion, the point mutant Vpr(R80A), although capable of interacting with DCAF1, was unable to induce G_2 arrest, and behaved as a dominant-negative protein by competing with wild-type Vpr for DCAF1 binding (DeHart et al., 2007).

We first constructed two truncations in HIV-1 Vpr (Vpr1–80 and Vpr1–84; Fig. 1A) encoded by lentiviral vectors (Verrier et al., 2011). HeLa cells were then transduced with VSV-G-pseudotyped lentivirus vectors (Supplemental Fig. 1) encoding HIV-1 Vpr, HIV-1 Vpr (R80A) or each of the indicated truncations. As shown in Fig. 2A and B, HIV-1 Vpr, but not HIV-1 Vpr (R80A), HIV-1 Vpr (1–80), or HIV-1 Vpr (1–84) induced cell cycle arrest. The percentages of cells transduced with the corresponding lentivirus vectors are shown in Supplemental Fig. 2A.

While HIV-1 Vpr is able to induce cell cycle arrest in human and non-human primate cells, SIVagm Vpr arrests AGM, but not human cells (Planelles et al., 1996). We asked whether transposition of the C-terminal domain (HIV-1 Vpr residues 78–96) onto SIVagm Vpr (Ch1) would confer upon SIVagm Vpr a *de novo* ability to induce G_2 arrest in human cells. As shown in Fig. 2C and D, Ch1 was able to induce G_2 arrest in human cells. Therefore, the inability of SIVagm Vpr to function in human cells can be overcome by a determinant within the C-terminal domain of HIV-1 Vpr.

Using the inactive truncation HIV-1 Vpr(1–80) as the recipient, we asked whether grafting the C-terminus of SIVagm Vpr (Ch2) would enable induction of G_2 arrest in human cells. HeLa cells transduced with the Ch2 also underwent cell cycle arrest (Fig. 2C and D). The C-terminal domain of SIVagm Vpr is intrinsically capable of recruiting the target leading to G_2 arrest in human cells when in the context of HIV-1 Vpr. One possible explanation for the previous observation is that, while DCAF1 is highly conserved across primate lentiviruses (Berger et al., 2014), the target protein may be variable. In addition, these observations suggest that the interaction of Vpr with the target may be dependent, in part, on determinants that lie upstream of the C-terminal domain of Vpr.

In contrast with the above findings, transposition of the N-terminal unstructured region of HIV-1 Vpr to SIVagm Vpr (Ch3) did not confer upon this chimera the ability to induce G_2 arrest in human cells (Fig. 2C and D). The reciprocal exchange (Ch4), which contained most of HIV-1 Vpr with the N-terminus of SIVagm Vpr, was still capable of inducing arrest in human cells (Fig. 2C and D). The above results indicate that the N-terminal unstructured region of HIV-1 Vpr is not required to induce cell cycle arrest.

In an effort to test whether the chimeras maintained the capacity to fold correctly, we verified their abilities to interact with DCAF1. HIV-1 Vpr, SIVagm Vpr and chimeras Ch1 to Ch4 were able to Co-IP with DCAF1 (Fig. 3, lanes 1 and 4). In contrast, the mutant HIV-1 Vpr (Q65R), did not efficiently co-IP with DCAF1 (Fig. 3)

The amino terminal domain of SIVagm Vpr is required for degradation of agmSAMHD1

SIVagm Vpr is bi-functional in AGM cells, where it can arrest cells in G_2 and induce degradation of agmSAMHD1. However, SIVagm Vpr, when expressed in human cells, is unable to perform either function (Lim et al., 2012). We wished to analyze which domain(s) in SIVagm Vpr may be important for targeting agmSAMHD1 for degradation. To that end, we tested the chimeras between SIVagm Vpr and HIV-1 Vpr. AgmSAMHD1 was degraded by SIVmac Vpx and SIVagm Vpr (Fig. 4A, lanes 5 and 6,