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Original article

Comparative study on the structure and cytopathogenic activity of HIV Vpr/Vpx proteins

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Abstract

The three-dimensional (3-D) structure of human immunodeficiency virus type 2 (HIV-2) Vpr/Vpx was predicted by homology modeling based on the NMR structure of human immunodeficiency virus type 1 (HIV-1) Vpr. The three proteins similarly have three major amphipathic α-helices. In contrast to HIV-1 Vpr, Vpr/Vpx of HIV-2 have a long N-terminal loop and clustered prolines in the second half of the C-terminal loop. HIV-2 Vpx uniquely contains a long region between the second and third major helices, and bears several glycines in the first half of the C-terminal loop. Instead of the glycines, there is a group of hydrophilic amino acids and arginines in the corresponding regions of the two Vprs. To compare the cytopathogenic potentials of HIV-1 Vpr and HIV-2 Vpr/Vpx, we examined the production of luciferase as a marker of cell damage. We further analyzed the characteristics of cells transduced with *vpr*/*vpx* genes driven by an inducible promoter. The results obtained clearly show that structurally similar, but distinct, HIV Vpr/Vpx proteins are detrimental to target cells.

Keywords: HIV-1; HIV-2; Vpr; Vpx; Homology modeling; Cytopathogenic activity

1. Introduction

All human and simian immunodeficiency viruses (HIVs and SIVs) isolated so far carry an accessory gene, *vpr*, in their genomes [1]. HIV type 2 (HIV-2) and SIVs isolated from rhesus (SIVmac) and sooty mangabey (SIVsm) monkeys constitute an independent sub-group (HIV-2 group) within primate immunodeficiency virus groups, and carry a *vpx* gene in addition to *vpr* [2]. Recently, SIVs from the mandrill (SIVmnd-2), red-capped mangabey monkey (SIVrcm) and drill (SIVdrl) have been reported to have both *vpr* and *vpx,* like viruses of the HIV-2 group [3–5]. A complete set of SIVmac *vpr* and *vpx* is required to cause AIDS efficiently in rhesus monkeys [6–8]. The *vpr* and *vpx* share considerable sequence similarity [2,5], and encode small proteins of approximately 100 amino acids. The three-dimensional (3-D) structure of HIV-1 Vpr determined by NMR is characterized by three major α-helices surrounded by N and C-terminal loops [9]. Structural analyses of Vpr/Vpx proteins other than HIV-1 Vpr, however, have not yet been carried out.

HIV-1 Vpr has been demonstrated to display cytopathogenic activities such as cell cycle arrest at the G_2 phase (G_2 arrest) [10–15] and apoptosis [15–17]. It was reported that the Vpr of HIV-2 group arrests cells at the G_2 phase [14,18–21], while Vpx does not [13,14,18,19,21]. The role of Vpr/Vpx of the HIV-2 group for apoptosis has not yet been well documented. While the virological significance of G_2 arrest and apoptosis induced by Vpr remains unclear, the ability to induce G_2 arrest is conserved among various primate immunodeficiency viruses [19,20,22]. The cytopathogenic potential of

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Vpr/Vpx, however, has not been compared yet under the same experimental conditions.

In this study, the 3-D structure and cytopathogenic activity of HIV-1 Vpr and HIV-2 Vpr/Vpx were compared. To analyze the framework of HIV-2 Vpr/Vpx, homology modeling based on the HIV-1 Vpr structure was performed. To evaluate their cytopathogenic activity, cells transiently or stably transfected with various expression vectors for HIV Vpr/Vpx were characterized biochemically and biologically. We demonstrate here that HIV-1 Vpr and HIV-2 Vpr/Vpx are structurally quite similar, but have distinct characteristics, and also that these proteins are detrimental to target cells.

2. Materials and methods

2.1. Homology modeling

Sequence alignment of HIV-1 Vpr and HIV-2 Vpr/Vpx was performed by the Clustal W program [23]. On the basis of this alignment, the 3-D structure of HIV-2 Vpr/Vpx was predicted from the NMR structure of HIV-1 Vpr (Protein Data Bank (PDB) code 1ESX) [9] by the MODELLER 6v2 program [24], and a diagram was generated by RASMOL software [25]. The amino acid sequence of HIV-1 Vpr and those of HIV-2 Vpr/Vpx are from HIV-1 P896 (GenBank accession no. U39362) [26] and HIV-2 GH-1 (GenBank accession no. M30895) [27] isolates, respectively. The sequences of *vpr*/*vpx* in HIV-2 GH-1 are identical with those in pGL-AN [28,29] used in this study.

2.2. Expression vectors

Vector pME18Neo-Fvpr was used to express HIV-1 Vpr with a FLAG tag at the N-terminus [30]. Expression vectors for HIV-2 Vpr and Vpx with FLAG tags designated pME18Neo-Fvpr2 and pME18Neo-Fvpx were constructed by replacement of the *vpr* of pME18Neo-Fvpr with *vpr* and *vpx*, respectively. *Vpr*/*vpx* were amplified by polymerase chain reaction (PCR) using pGL-AN [28,29] as a template. Vectors, a pGL3-Control Vector (Promega, Madison, WI, USA), pSG-Vif cFLAG [31], and pSG-Gag (p24) cFLAG [32] were used to express luciferase, HIV-1 Vif and HIV-1 Gag-p24, respectively. These vectors were transiently transfected into 293T cells [33] by the calcium phosphate coprecipitation method as previously described [34]. 293T cells were cultured in Eagle's minimal essential medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) [33].

2.3. Full-length viral clones

For construction of full-length viral clones with tags at the 5′ site of *vpx*/*vpr* designated pGL-xFrH and pGL-xHrF, the *Xba* I-*Eco*R I fragment of pGL-AN [28,29] (nucleotides 5064-5756) was cloned into pUC19 to construct a subcloning vector pUC-GL(Xb-Ec). The FLAG and HA sequences were then introduced right after the ATG codon of *vpx*/*vpr* genes in pUC-GL(Xb-Ec) by a QuikChange Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA), and the resultant DNA fragment was cloned back into pGL-AN [28,29] to construct pGL-xFrH and pGL-xHrF, as shown in the text. These clones were transiently transfected into 293T cells [33] by the calcium phosphate coprecipitation method as above [34].

2.4. Establishment of HeLa Tet-Off cell lines

Expression vectors based on pBI-EGFP under the control of an inducible and bidirectional promoter (Clontech, Palo Alto, CA, USA) were used to express EGFP and HIV Vpr/Vpx simultaneously. Vectors designated pBI-EGFP/Vpr, pBI-EGFP/Vpr2 and pBI-EGFP/Vpx to express HIV-1 Vpr, HIV-2 Vpr, and HIV-2 Vpx with a FLAG tag at N-terminus, respectively, were constructed by insertion of *vpr*/*vpx* and FLAG sequences into pBI-EGFP. HIV-1 *vpr* and HIV-2 *vpr*/*vpx* were amplified by PCR using pNL432 (GenBank accession no. AF324493) [34] and pGL-AN [28,29] as templates, respectively. Transient transfection into HeLa Tet-Off cells, cultured as described in the Tet Systems User Manual, was performed by the calcium phosphate coprecipitation method [34]. For establishment of Tet-Off/control and Tet-Off/Vpx, HeLa Tet-Off cells were transfected with pBI-EGFP or pBI-EGFP/Vpx as described above [34], and cultured in the selection medium described in the manual. These cell lines were maintained in Eagle's minimal essential medium supplemented with 10% heat-inactivated FBS in the presence of G418 (0.1 mg/ml), hygromycin B (0.1 mg/ml) and doxycycline (0.1 μg/ml). Induction of Vpr/Vpx and EGFP from these cells was achieved by removal of doxycycline from the culture medium.

2.5. Luciferase assay

Luciferase assays were performed with a Luciferase Assay System (Promega, Madison, WI, USA).

2.6. Western immunoblotting

Western immunoblotting was performed essentially as previously described [35]. Cell lysates for immunoblotting were prepared from 293T and HeLa Tet-Off cells transfected with various clones by CHAPS (3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate)/DOC (deoxycholate) [35] or Laemmli's sample [32] buffer, and resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were electrophoretically transferred to polyvinylidene fluoride membranes, and the membranes were treated with an ANTI-FLAG M2 Monoclonal Antibody (Ab) (Sigma–Aldrich, St. Louis, MO, USA), anti-EGFP Ab (Living Colors A.v. Peptide Ab, BD Biosciences, Palo Alto, CA, USA) or anti-HA Ab (Monoclonal Ab, HA.11, BAbCO, Berkeley, CA, USA). For visualization, ECL Plus Western Blotting Detection Reagents (Amersham Biosciences, Buckinghamshire, England) were used.