Virology 440 (2013) 190–203

Contents lists available at SciVerse ScienceDirect

Virology

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

HIV-1 Vpu affects the anterograde transport and the glycosylation pattern of NTB-A

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ARTICLE INFO

Article history:
Received 3 December 2012
Returned to author for revisions
22 February 2013
Accepted 25 February 2013
Available online 22 March 2013

Keywords:
Vpu
NTB-A
SLAMF6
HIV-1
SIV
Pulse-chase
N-linked glycosylation
Tetherin

ABSTRACT

HIV-1 Vpu induces downregulation of cell surface NTB-A to evade lysis of HIV-1 infected cells by NK cells. Here we show that Vpu affects the anterograde transport and the glycosylation pattern of NTB-A by a mechanism that is distinct from the Vpu induced downregulation of CD4 and tetherin. In the presence of Vpu, only the high mannose form of NTB-A was detectable, suggesting that Vpu prevented the formation of the mature form of NTB-A. This phenomenon is associated with the ability of Vpu to downregulate cell surface NTB-A by retention of NTB-A within the Golgi-compartment. Furthermore, the Vpu-mediated effect on NTB-A glycosylation is highly conserved among Vpu proteins derived from HIV-1 and SIV and corresponds to the level of downregulation of NTB-A. Together, these results suggest that the reduction of NTB-A from the cell surface is associated with the Vpu-mediated effect on the glycosylation pattern of newly synthesized NTB-A molecules.

Introduction

The human immunodeficiency virus type 1 (HIV-1) accessory protein Vpu is a 15–20 kDa oligomeric type 1 integral membrane phosphoprotein (Cohen et al., 1988; Maldarelli et al., 1993; Strebel et al., 1988), which is encoded exclusively by HIV-1 and related simian immunodeficiency viruses (SIVs), but not by the majority of SIVs and HIV-2. Vpu induces the degradation of newly synthesized CD4, by retaining it in the endoplasmic reticulum (ER) (Magadan et al., 2010) and thereby targeting CD4 to the ER-associated protein degradation (ERAD)-pathway (Binette et al., 2007; Magadan et al., 2010; Schubert et al., 1998; Willey et al., 1992). The cytoplasmic domain of Vpu contains a pair of highly conserved serine residues, which are constitutively phosphorylated by the casein kinase 2 (CK-2) (Schubert et al., 1994). This phosphorylation allows binding of the ß-transducin repeat containing protein (ß-TrCP), a component of the Skp1-Cullin-F-box (SCF) E3 ubiquitin ligase complex (Butticaz et al., 2007; Margottin et al., 1998), which induces polyubiquitination of the cytoplasmic domain of CD4 (Magadan et al., 2010). Consequently, CD4 is retranslocated into the cytosol and degraded by the 26S proteasome (Binette et al., 2007; Magadan et al., 2010; Schubert et al., 1998). Furthermore, it was shown that Vpu supports HIV-1 virion release by counteracting the cellular restriction factor tetherin (also known as CD317, BST-2 or HM1.24) (Neil et al., 2008; Van Damme et al., 2008). The ability of Vpu to enhance virion release directly correlates with its ability to downregulate cell surface tetherin. The transmembrane (TM) domain of Vpu is essential for interaction with tetherin and consequently for the downregulation of tetherin from the cell surface (Banning et al., 2010; Bolduan et al., 2011; Dube et al., 2010; Iwabu et al., 2009; Rong et al., 2009). This physical interaction likely enables Vpu to trap newly synthesized and recycling tetherin molecules at the level of the trans-Golgi network (TGN) in order to reduce cell surface exposure of the restriction factor (Dube et al., 2010; Schmidt et al., 2011).

In addition to its role in tetherin antagonism and CD4 degradation, the TM domain of Vpu was found to form a non-selective, voltage gated ion channel in planar lipid bilayers (Ewart et al., 1996; Schubert et al., 1996b), which was later continued for the

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0042-6822/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.virol.2013.02.021

full length molecule (Marassi et al., 1999). Randomization of the TM domain prevents Vpu's ion channel formation and impairs its ability to regulate virion release (Schubert et al., 1996a), indicating a correlation between the ion channel activity of Vpu and its augmentation of virus release. However, it was shown that ion channel activity of Vpu is dispensable for counteraction of tetherin (Bolduan et al., 2011; Kuhl et al., 2011; Skasko et al., 2011).

Recently, it has been described that Vpu induces downregulation of the coactivating NK cell receptor NK, T-cell, B-cell antigen (NTB-A) (also termed CD352 or SLAMF6), as well as the activating NK cell receptor PVR/CD155, from the cell surface to evade lysis of HIV-1 infected cells by NK cells (Matusali et al., 2012; Shah et al., 2010). However, the molecular mechanism of these Vpu induced downregulations, particularly of NTB-A, has not been elucidated so far.

NK cell-mediated degranulation requires two signals from activating and coactivating receptors (Sowrirajan and Barker, 2011). The coactivating NK cell receptor NTB-A, which facilitates NK cell-mediated cytotoxicity (Bottino et al., 2001; Flaig, Stark, and Watzl, 2004), is a type I transmembrane glycoprotein and a member of the signaling lymphocytic activation molecule (SLAM) receptor family (Bottino et al., 2001). It has been suggested that the Vpu induced downregulation of cell surface NTB-A is mechanistically distinct from the downmodulation of CD4 and tetherin (Shah et al., 2010). Furthermore, Vpu does neither increase internalization rates nor alters the steady state protein level of NTB-A (Shah et al., 2010).

Here we demonstrate that in the presence of Vpu only the high mannose form of NTB-A is detectable, suggesting that Vpu prevents the formation of complex/hybrid-type glycosylated NTB-A. Furthermore, this phenomenon is associated with the transport of NTB-A to the cell surface.

The second cytoplasmic alpha helix of Vpu and/or its localization in the Golgi-compartment appears to be critical to prevent the formation of the mature form of NTB-A. The importance of

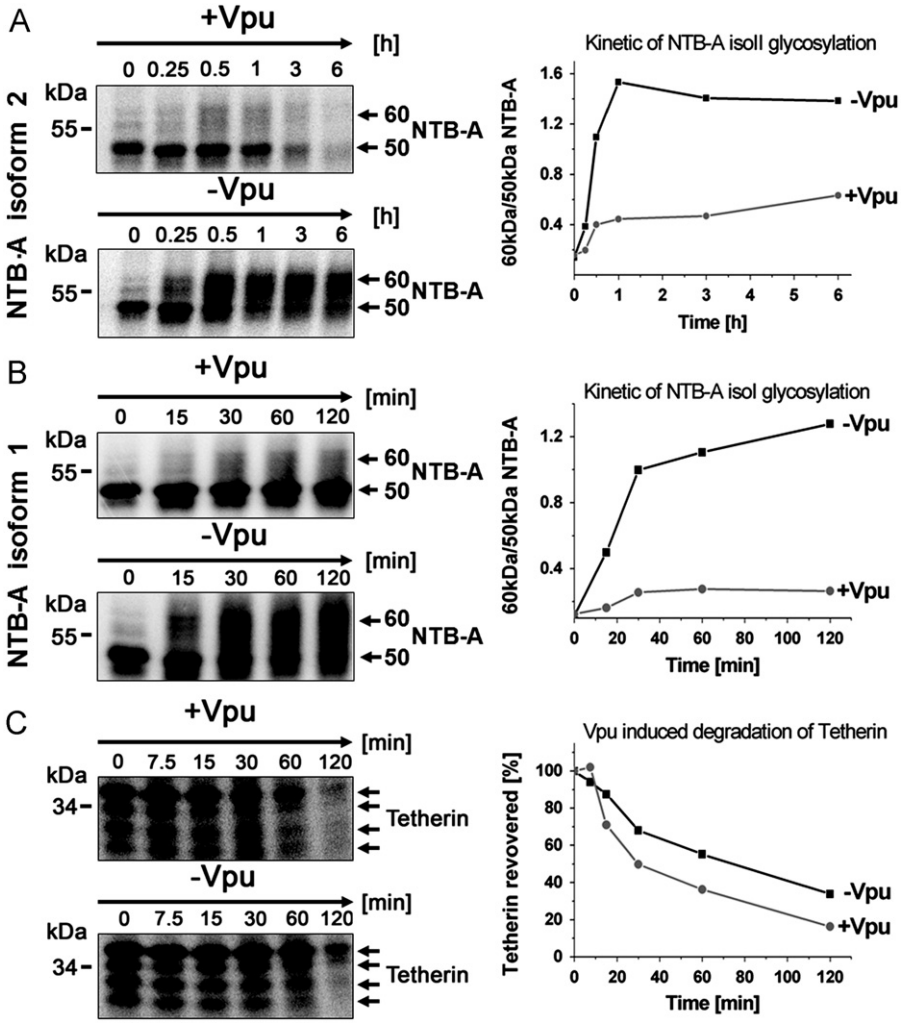

Fig. 1. The effect of *wt* Vpu on the glycosylation pattern of NTB-A and tetherin analyzed via *pulse-chase* kinetic analysis. (A) Parallel cultures of 293T cells were cotransfected with pNTB-A-2 and either AU1-tagged *wt* Vpu or an empty vector control. 24 h post transfection cells were incubated in methionine free medium for 30 min and were then pulse labeled for 7 min with [^{35}S]-methionine. Cells were chased for up to 6 h and at each time point indicated cells were collected. NTB-A was immunoprecipitated with an anti-NTB-A antibody, analyzed by SDS-PAGE and autoradiography. Quantification of the kinetics of NTB-A isoform 2 glycosylation is given on the right. (B) Parallel cultures of 293 T cells were cotransfected with NTB-A isoform 1 and either AU1-tagged *wt* Vpu or an empty vector control. 24 h post transfection cells were incubated in methionine free medium for 30 min and were then pulse labeled for 7 min with [^{35}S]-methionine. Cells were chased for up to 2 h, and at each time point indicated cells were collected. NTB-A was immunoprecipitated with an anti-NTB-A antibody, analyzed by SDS-PAGE and autoradiography. Quantification of the kinetics of NTB-A isoform 1 glycosylation is given on the right. (C) Parallel cultures of 293T cells were cotransfected with Flag-tagged tetherin and either AU1-tagged *wt* Vpu or an empty vector control. 24 h post transfection cells were incubated in methionine free medium for 30 min and were then pulse labeled for 7 min with [^{35}S]-methionine. Cells were chased for up to 2 h and at each time point indicated cells were collected. Flag-tagged tetherin was immunoprecipitated with anti-Flag M2 affinity matrix, analyzed by SDS-PAGE and autoradiography. Quantification of the Vpu induced degradation of tetherin is given on the right.