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Review article

Functional roles of HIV-1 Vpu and CD74: Details and implications of the Vpu–CD74 interaction

Denise A. Le Noury [a,b], Salerwe Mosebi [a], Maria A. Papathanasopoulos [b], Raymond Hewer [a,*,1]

[a] Centre for Metal-based Drug Discovery, Mintek, Private Bag X3015, Randburg 2125, South Africa
[b] Department of Molecular Medicine and Haematology, Faculty of Health Sciences, University of the Witwatersrand Medical School, Private Bag 3, WITS, 2050, South Africa

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ABSTRACT

HIV-1 Vpu has a variety of functions, including CD4 degradation and the downregulation of MHCII. Downregulation of the MHCII occurs through Vpu binding to the cytoplasmic domain of CD74, the chaperone for antigen presentation. The CD74 cytoplasmic domain also plays a vital role in cell signaling through the activation of an NF-κB signal cascade for the maturation, proliferation and survival of B cells as well as by binding the macrophage inhibitory factor. In view of these functions, it follows that the Vpu–CD74 interaction has multiple downstream consequences for the immune system as it not only impairs foreign antigen presentation but may also have an effect on signal transduction cascades. It is thought that Vpu specifically targets intracellular CD74 while other HIV-1 proteins cannot. Therefore, this protein–protein interaction would be a potential drug target in order to reduce viral persistence. We review the functional importance and specific binding site of Vpu and CD74.

1. Introduction

HIV-1 has several accessory genes, namely *vif*, *vpr*, *nef* and *vpu*. The accessory proteins encoded by these genes play important roles in viral replication [1]. Vif targets the host restriction factor APOBEC3G for proteasomal degradation to prevent the inhibition of viral DNA synthesis [2–4]. Vpr has many functions including activation of cell death and proviral transcription [4,5]. Nef is a functionally diverse protein that is involved in the alteration of cell signaling pathways, disruption of antigen presentation by MHCI and MHCII and alteration of gene and receptor surface expression [4,6]. Lastly, Vpu also has multiple functions within the host cell, some of which are similar to Nef, with the most well-described functions being the degradation of the HIV-1 receptor CD4 and virion release [4,7,8].

Of particular interest, Vpu is only encoded by the genome of HIV-1 and a few simian immunodeficiency virus (SIV) isolates such as SIVcpz (chimpanzee), SIVmon (mona monkey), SIVgsn (greater spot-nosed monkey), SIVmus (mustached monkey) and SIVden (Dent's mona monkey), but is not found within HIV-2 [9]. Related isolates that do not express a functional Vpu protein have far less severity in terms of disease outcome [10] indicating the importance of this viral protein. HIV-1 infections tend to result in chronic immune activation, while HIV-2 infections yield lower levels of immune activation [11] and SIVs of sooty mangabeys and African green monkeys, which do not encode *vpu*, generally also do not cause high levels of immune activation [12]. Furthermore, the functionality of the Vpu protein in the HIV-1 strains of M, N and O has been suggested to be necessary for the spread of the pandemic M strain as this strain expresses a Vpu protein that is not only able to target CD4 for degradation, but also effectively antagonizes tetherin [4,9]. The non-pandemic N and O strains express a Vpu protein that is lacking in one of the primary functions. Although HIV-2 targets tetherin using the Env protein, this has been found to be less effective than that of HIV-1 Vpu [9]. HIV-1 Vpu appears to target the immune system in many different but related ways that are highly effective at sabotaging the immune

Abbreviations: AGTR1, angiotensin II type 1 receptor; β-TrCP, β-transducin repeat-containing protein; CD74-ICD, CD74 intracellular domain; CLIP, class-II-associated Ii-chain peptide; ER, endoplasmic reticulum; ERK-1/2, p44/p42 extracellular-signal-regulated kinase family; HLA-DR/DM, human leukocyte antigen-DR/DM; I-CLiPs, intramembrane cleaving proteases; MCP-1, monocyte chemoattractant protein-1; MIF, macrophage inhibitory factor; NMR, nuclear magnetic resonance; RIP, regulated intramembrane proteolysis; SIV, simian immunodeficiency virus; $TAF_{II}105$, TBP associated factor $_{II}105$; TBP, TATA box binding protein; TFIID, transcription factor IID.

* Corresponding author.
E-mail addresses: denisel@mintek.co.za (D.A. Le Noury), salerwem@mintek.co.za (S. Mosebi), Maria.Papathanasopoulos@wits.ac.za (M.A. Papathanasopoulos), hewerr@ukzn.ac.za (R. Hewer).
[1] Present address: School of Biochemistry, Genetics and Microbiology, University of Kwa-Zulu Natal, Private Bag X01, Scottsville 3209, South Africa.

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response and that work in conjunction with other HIV-1 proteins to more effectively enhance viral replication. As each component of the immune system that is targeted by Vpu is important, the impairment of Vpu function would likely increase the ability of the immune system to respond to HIV-1 infection.

One of the more recently described functions of Vpu is the downregulation of the MHCII, specifically through the interaction with the MHCII-associated invariant chain, or CD74 [13]. As CD74 plays a role in many important cellular functions, including the immune response, it is feasible that the binding of Vpu to this host protein has multiple downstream consequences for the immune response apart from the downregulation of the MHCII. Therefore downregulation of this host protein is beneficial for viral persistence. In this review, we take a look at the multiple functions of both Vpu and CD74 within the infected cell and examine the specific interaction and binding site between these two proteins and consider the possible ramifications of this interaction. Finally, we contemplate on the suitability of this protein–protein interaction as a possible novel therapeutic target for drug intervention.

2. HIV-1 Vpu structure

Vpu is an 81-mer type I integral intracellular membrane protein that is expressed in the later stages of viral infection. Vpu has an N-terminal transmembrane hydrophobic helix (residues 1–27) and two amphipathic helices (residues 35–50 and 58–70) that form part of the cytoplasmic domain and are separated by a linker region (residues 47–58) [14–16]. The linker region has two highly conserved serine residues in the cytoplasmic domain, namely Ser52 and Ser56 [16] that are required for the binding of β-transducin repeat-containing protein (β-TrCP) – a necessity for some functions of Vpu. The transmembrane domain tilts at an angle of approximately 6–15 degrees [17,18] and the linker region between the two cytoplasmic helices is largely flexible, allowing for the second cytoplasmic helix to move and possibly lie parallel to the first cytoplasmic helix [16]. The first cytoplasmic helix of Vpu is considered to lie parallel to and be partly buried in the membrane of the endoplasmic reticulum (ER) to shield the hydrophobic residues, leaving the charged residues exposed. However, the exact conformation of Vpu may be dependent on oligomerization or the interaction with other host proteins [17,19,20]. Using molecular simulation studies, it was shown that there are slight structural differences in the cytoplasmic domain between the phosphorylated and unphosphorylated forms of Vpu, with the phosphorylated protein adopting a more compact structure in comparison to the unphosphorylated protein [19]. Nuclear overhauser effect spectra have suggested that the two cytoplasmic helices lie in an antiparallel formation and that there is likely to be spatial proximity between the C-terminal region and the linker region containing the phosphorylated serine residues [20]. The side chains of the serine residues are thought to be exposed to the buffer or solvent, making these residues accessible to casein kinase II for phosphorylation [20].

Vpu is mostly found to be in the ER and Golgi apparatus but may also be found to some extent at the plasma membrane [14,21]; however Vpu is not found in culture fluid or supernatant, suggesting that the protein is not associated with virions [22]. It is thought that Vpu contains two trafficking signals in the hinge region that lies between the transmembrane domain and the cytoplasmic domain as well as within the second or C-terminal α-helix [9]. It has been suggested that there are deviations in the primary sequences of these trafficking signals between HIV-1 subtypes which would explain why Vpu expressed from different subtypes is not localized within the same cellular compartments. This difference is demonstrated between subtype B and subtype C, where subtype B Vpu localizes mainly within intracellular membranes such as the ER, Golgi and endosomes, while subtype C Vpu is reportedly found mostly at the plasma membrane [9].

3. HIV-1 Vpu functions

The two primary functions of Vpu are the degradation of CD4, the primary receptor protein for HIV-1 [23–25] and the release of new virus particles from infected cells either by inhibition of the host restriction factor, tetherin [26–29] or through the viroporin activity of Vpu in which the transmembrane domain is able to form an ion channel in a separate mechanism to that of the antagonism of tetherin [30–32]. Vpu also seems to undermine or impair the immune system through a variety of methods including the induction of apoptosis in infected cells [33] and the downregulation of MHCII by binding CD74 [13] to name a few.

Possibly the best known function of HIV-1 Vpu is the targeting of CD4 for degradation [15,23–25]. CD4 is integral for the functioning of the immune system as it is involved in multiple functions including the production of cytokines, the enlistment of neutrophils and basophils, assisting with the production of antibodies along with B cells and protection against intracellular and extracellular pathogens, bacteria and parasites [34–38]. After infection with HIV-1, CD4–Env complexes are formed in the ER, trapping CD4. Vpu and CD4 interact *via* their cytoplasmic domains and β-TrCP is then recruited to the Vpu–CD4 complex. The ternary complex consisting of Vpu–CD4–β-TrCP recruits Skp1p and ultimately the E3 ligase complex, leading to the ubiquitination and degradation of CD4. CD4 is unable to bind to β-TrCP in the absence of Vpu, indicating that Vpu is a linker protein between CD4 and β-TrCP [39]. By targeting this protein for degradation, Vpu has a deleterious effect on the immune system.

Yet another protein that is inhibited or antagonized by Vpu is the host restriction factor tetherin. Tetherin is a membrane protein that is induced by interferon-α, is expressed on the cell surface of differentiated B cells and bone marrow stromal cells and functions to harness newly assembled virions to each other as well as to the cell membrane [26,27]. Vpu has been found to co-localize with tetherin [26] and these two proteins interact specifically through the binding of their transmembrane domains [28,29]. Not all cell types require Vpu for virion release. However, those cell types that are dependent on Vpu for this function, but that are deficient in Vpu, show the accumulation of mature and assembled virions at the surface which later are internalized to the endosomes [26]. The ability of Vpu to inhibit tetherin at the surface of the cell relies on both the transmembrane domain of Vpu as well as the conserved serine residues in the cytoplasmic linker region. Previous studies reported the possibility that Vpu downregulates tetherin by trapping it within the Golgi network [40] and degrades tetherin by ER-associated protein degradation [41]. Additionally, it was shown that Vpu rather targeted cell surface tetherin and removed it directly from the plasma membrane [28] thus greatly enhancing virion release. It has further been hypothesized that the antagonism and downregulation of tetherin by Vpu functions to disable anti-HIV antibodies from recognizing infected cells which ultimately abrogates antibody-mediated clearance of the infected cells [42].

Vpu is also able to mediate virus particle release through its transmembrane domain ion channel activity and this mode of virus particle release is distinct from tetherin antagonism [30–32]. The Vpu ion channel, or viroporin, is selective for cations such as sodium and potassium [30] and has been predicted to exist in a pentameric state [43,44]. Predictive molecular simulation studies have generated a model of the Vpu ion channel and suggested this channel would be able to exist in either an open or a closed

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