Published in final edited form as:

Trends Microbiol. 2014 December ; 22(12): 662–664. doi:10.1016/j.tim.2014.10.008.

The end of Nef's tether

Ruth Serra-Moreno

Department of Biological Sciences, College of Arts and Sciences, Texas Tech University, 8th and Canton Avenue, Lubbock, TX 79409-3131, USA

Abstract

Tetherin represents an important barrier for successful cross-species transmissions of primate lentiviruses. HIV-1 overcame this obstacle by using Vpu as a countermeasure. However, Kluge and collaborators now show that HIV-1 group O uses Nef to antagonize tetherin, and that this activity may have contributed to its spread in West-Central Africa.

Keywords

HIV; Nef; tetherin; restriction

Viruses and their hosts are under a constant arms race for survival. These host–pathogen interactions result in genetic conflicts in which hosts evolve barriers (restriction factors) to block virus infections, and viruses are forced to evolve mechanisms to circumvent them. One such a barrier is tetherin (BST-2): a membrane protein that anchors progeny virions to the cell surface, preventing virus spread [1]. The majority of simian immunodeficiency viruses (SIVs) have evolved the Nef accessory protein to counteract tetherin [1]. Nef interacts with the cytoplasmic domain of non-human primate tetherin to promote its internalization and accumulation in lysosomal compartments. Thus, Nef efficiently removes tetherin from sites of virus assembly and release [1]. Remarkably, Nef targets a five amino acid region in the N-terminus of tetherin (G/D₁₄IxK₁₈), which is missing in the human orthologue. Therefore, the lack of those residues renders human tetherin resistant to SIV Nef [1].

Due to the absence of sequences in human tetherin that would confer susceptibility to Nef, human immunodeficiency viruses (HIV-1 and HIV-2) switched from Nef to other viral proteins to effectively overcome this hurdle. HIV-2 uses its envelope glycoprotein (Env) to counteract human tetherin [1]. Env interacts with the ectodomain of tetherin to promote its internalization and sequestration within the *trans*-Golgi network (Figure 1, blue arrows). The four groups of HIV-1 (M, N, O, and P) originated after the transmission of SIVs infecting chimpanzees and gorillas. Again, after those transmissions, the Nef proteins of the

© 2014 Elsevier Ltd. All rights reserved.

Corresponding author: Serra-Moreno, R. (ruth.serra-moreno@ttu.edu).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

resulting viruses were unable to antagonize tetherin in the new host. In the case of HIV-1 group M (responsible for the AIDS pandemic), Vpu adapted to acquire anti-tetherin activity [1]. Vpu interacts with the transmembrane domain of tetherin to promote its intracellular sequestration and/or degradation (Figure 1, orange arrows). HIV-1 group N Vpus counteract human tetherin poorly [1], a factor that may have contributed to the limited spread of this group in the human population. HIV-1 group P has only been found in two individuals from Cameroon, and currently there is no information about its ability to antagonize tetherin. HIV-1 group O viruses have infected around one hundred thousand individuals in West-Central Africa. Despite this relatively high incidence, the mechanism of tetherin antagonism by O viruses is not well understood [1, 2].

In the current issue of *Cell Host & Microbe*, Kluge and collaborators addressed this lingering question by performing functional analyses of contemporary HIV-1 group O Nef proteins (O-Nef) against tetherin. Under conditions of tetherin overexpression, O-Nefs had a marginal effect on enhancing virus release, while they potently downregulated the surface levels of tetherin. Notably, O-Nef proteins were more efficient at downregulating human than gorilla tetherin, suggesting that HIV-1 group O efficiently adapted to humans after its transmission from gorillas. Under a more physiological scenario, not only O-Nefs proved to be effective tetherin antagonists, but also strongly enhanced virus release from primary human CD4⁺ T cells [3]. Therefore, these observations suggest that previous studies likely missed the anti-tetherin potential of HIV-1 group O by testing tetherin antagonism under conditions of protein overexpression. In a sophisticated approach, the authors inferred the Nef sequence of the most recent common ancestor (MRCA) of the currently circulating O-Nef proteins. The sequence of the O-MRCA Nef was cloned and the resulting protein was tested for its ability to counteract tetherin. Remarkably, O-MRCA Nef counteracted both human and gorilla tetherin, and exhibited similar potency at counteracting human tetherin to the currently circulating O-Nef alleles [3], implying that this function was acquired prior to the spread of HIV-1 group O.

SIVs antagonize non-human primate tetherin by using multiple regions in Nef [4]. Similarly, the anti-tetherin activity of HIV-1 group O mapped to several domains in Nef, indicating that the determinants for tetherin antagonism are complex. In particular, residues near the highly conserved di-leucine motif (ExxxLL) affected anti-tetherin activity without altering other Nef-related functions. In contrast to SIV Nef [4–6], O-Nef targets a conserved dual tyrosine motif (Y₆DY₈) in human tetherin [3]. This dual tyrosine motif and the di-leucine motif in Nef correspond to the binding sites for adaptor proteins 1 and 2 (AP-1 and AP-2), suggesting that O-Nef takes advantage of the cellular trafficking pathways to downmodulate tetherin – a fact that has been observed in other primate lentiviruses [1, 4, 6]. Despite some similarities, the mechanism of tetherin antagonism differs from SIV Nef. Whereas SIV Nef promotes the endocytosis of tetherin through a clathrin- and AP-2-dependent pathway [1, 4, 7], O-Nef impairs the anterograde transport of human tetherin to the cell surface by sequestering it within the *trans*-Golgi network [3] (Figure 1, red cross). Analogous cases of convergent evolution are found in HIV-2 and certain SIVs, in which the inability of Nef to counteract tetherin exerted a selective pressure that resulted in Env acquiring anti-tetherin

activity. However, the determinants for tetherin antagonism map to different regions in Env [8, 9].

Besides serving as a potent restriction factor, tetherin functions as a viral sensor by signaling through nuclear factor- κ B (NF- κ B) and inducing pro-inflammatory responses [1]. This activity is successfully counteracted by HIV-1 group M Vpu (M-Vpu) and the O-MRCA Nef. However, because contemporary O-Nefs inherently activate NF- κ B, this activity masked their potential effect on tetherin-induced NF- κ B activation [3]. Human tetherin is expressed in two isoforms, in which the short version (s-tetherin) is not capable of activating NF- κ B signaling, but is more resistant to M-Vpu antagonism [10]. Likewise, O-Nefs failed at counteracting this tetherin variant [3]. S-tetherin is the result of an alternative start codon and lacks 12 amino acids in the N-terminal domain (including the YDY motif) [10], rendering it resistant to O-Nef. We do not have enough information about the contribution of each tetherin isoform in virus restriction *in vivo*, and therefore, we do not know how the lack of countermeasures against s-tetherin affects virus spread and pathogenesis for HIV-1 groups M and O. We could envision a scenario though, in which this arms race might lead to increased levels of s-tetherin to effectively block virus replication.

The results presented by Kluge and collaborators show that unlike other HIVs, adaptation of HIV-1 group O in humans did not require switching the anti-tetherin activity from Nef to other viral proteins, but targeting a different region in human tetherin – a factor that likely contributed to their spread in West-Central Africa. In addition, this study shows that HIV-1 Nef has the potential to acquire anti-tetherin activity under selective pressure, highlighting once again the adaptability of the primate lentiviruses to tetherin: an important barrier for lentiviral transmissions.

Acknowledgements

We want to thank the funding agencies and Texas Tech University for continuous support of our research program. Dr. Serra-Moreno is funded through NIH/NIAID R21AI106400.

References

1. Sauter D. Counteraction of the multifunctional restriction factor tetherin. *Front Microbiol.* 2014; 5:163. [PubMed: 24782851]
2. Sauter D, et al. Tetherin-driven adaptation of Vpu and Nef function and the evolution of pandemic and nonpandemic HIV-1 strains. *Cell Host Microbe.* 2009; 6:409–421. [PubMed: 19917496]
3. Kluge SF, et al. Nef proteins of epidemic HIV-1 group O strains antagonize human tetherin. *Cell Host Microbe.* 2014 <http://dx.doi.org/10.1016/j.chom.2014.10.002>.
4. Serra-Moreno R, et al. Tetherin/BST-2 antagonism by Nef depends on a direct physical interaction between Nef and tetherin, and on clathrin-mediated endocytosis. *PLoS Pathog.* 2013; 9:e1003487. [PubMed: 23853598]
5. Jia B, et al. Species-specific activity of SIV Nef and HIV-1 Vpu in overcoming restriction by tetherin/BST2. *PLoS Pathog.* 2009; 5:e1000429. [PubMed: 19436700]
6. Zhang F, et al. Nef proteins from simian immunodeficiency viruses are tetherin antagonists. *Cell Host Microbe.* 2009; 6:54–67. [PubMed: 19501037]
7. Zhang F, et al. SIV Nef proteins recruit the AP-2 complex to antagonize tetherin and facilitate virion release. *PLoS Pathog.* 2011; 7:e1002039. [PubMed: 21625568]

8. Le Tortorec A, Neil SJ. Antagonism and intracellular sequestration of human tetherin by the HIV-2 envelope glycoprotein. *J Virol.* 2009; 83:11966–11978. [PubMed: 19740980]
9. Serra-Moreno R, et al. Compensatory changes in the cytoplasmic tail of gp41 confer resistance to tetherin/BST-2 in a pathogenic Nef-deleted SIV. *Cell Host Microbe.* 2011; 9:46–57. [PubMed: 21238946]
10. Cocka LJ, Bates P. Identification of alternatively translated tetherin isoforms with differing antiviral and signaling activities. *PLoS Pathog.* 2012; 8:e1002931. [PubMed: 23028328]

Figure 1.

Mechanisms of tetherin antagonism by HIVs. HIV-1 group M uses Vpu to overcome restriction by tetherin. Vpu physically interacts with tetherin through its transmembrane domain and promotes tetherin ubiquitination and degradation, or sequestration at perinuclear compartments (orange arrows). HIV-2 uses the envelope glycoprotein (Env) to antagonize tetherin. Env interacts with the ectodomain of tetherin and, by virtue of harboring an endocytosis motif in its cytoplasmic tail (YxxΦ), promotes the internalization and sequestration of tetherin within the *trans*-Golgi network (blue arrows). HIV-1 group O viruses use Nef to antagonize tetherin. O-Nef targets a conserved dual tyrosine motif (YDY) in the cytoplasmic domain of tetherin to block the anterograde transport of tetherin to the plasma membrane (red cross).