P-A7

HIV-1 Vpu Antagonizes Rhesus Macaque and Chimpanzee BST-2 Through Cytoplasmic Domain Interactions

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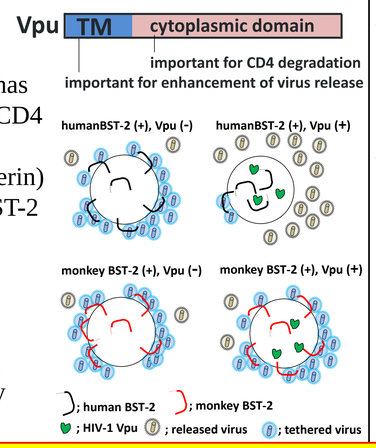
Background

HIV-1 Vpu consists of the cytoplasmic domain and the transmembrane (TM) domain. Vpu has two main functions; degradation of CD4 and enhancement of virus release.

Human host protein, BST-2 (Tetherin) was identified as a target of Vpu. BST-2 inhibits HIV-1 virus release but its inhibitory activity is antagonized by Vpu. Further research showed HIV-1_{NL4-3} Vpu cannot antagonize monkey or murine BST-2. However, mechanism of the species-specificity is still not known.



Aims & Approaches

Aims

To gain further insights into the species-specificity of BST-2 antagonism by Vpu, we focused on the physical interaction between Vpu and BST-2 and asked whether physical interaction is important, necessary, or sufficient for the antagonism of BST-2 by Vpu.

Approaches

- We took advantage of two phenomena (panel I).
  - (i) Vpu of HIV-1_{DH12} is less species restricted than HIV-1_{NL4-3} Vpu and antagonizes both human and rhesus BST-2 (Shingai&Yoshida et al, JVI, 2011).
  - (ii) A single a.a. change in rhesus BST-2 (I48T) renders the protein sensitive to HIV-1_{NL4-3} Vpu (Yoshida et al. Frontiers in Microbiology, 2011).
- To assess the physical interaction between Vpu and BST-2 we used BiFC assay (see Method section).

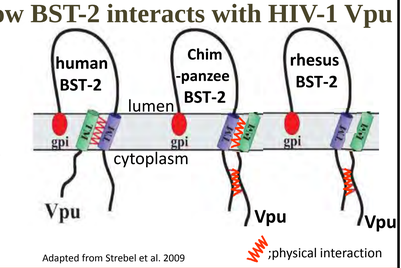
- panel I-

Vpu	BST-2/Tetherin: human	rhesus	rhesus I48T	chimpanzee
HIV-1 NL4-3 Vpu	antagonized	NOT antagonized	(ii) antagonized	antagonized
HIV-1 DH12 Vpu	antagonized	(i) antagonized	antagonized	(not tested)
SIVcpz Vpu (MB897)	NOT antagonized	(not tested)	(not tested)	NOT antagonized

Conclusions

- **Physical interaction between BST-2 and Vpu was observed in live cells by BiFC assay (see Method section).**
- **Human and rhesus BST-2 interact with NL4-3 Vpu via their TM and CYT domains, respectively.**
- **Cytoplasmic interaction occurs between chimpanzee BST-2 and NL4-3 Vpu also.**
- **Physical interaction is necessary but not sufficient for the BST-2 antagonism by HIV-1 Vpu.**

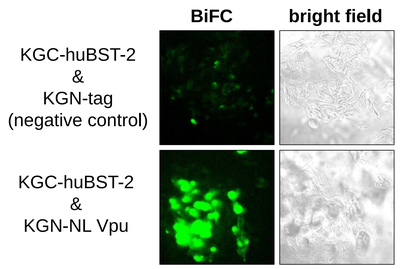
How BST-2 interacts with HIV-1 Vpu



Adapted from Strebel et al. 2009

Experiments & Results

Fig. 1 Human BST-2 physically interacts with HIV-1 Vpu in live cells.

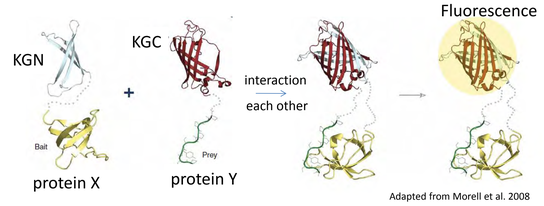


To assess the physical interaction between human BST-2 and HIV-1_{NL4-3} Vpu, we transfected 293T cells with BiFC-tagged plasmids (KGC-huBST-2 and NL4-3 Vpu-KGN) and analyzed cells with microscopy. We observed strong BiFC signal between human BST-2 and NL4-3 Vpu (lower left panel).

Method

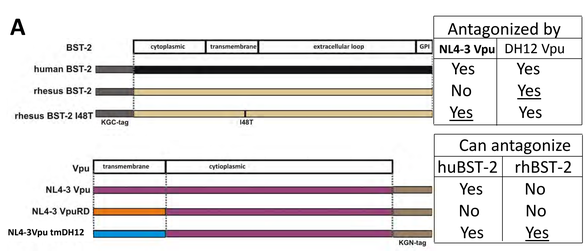
What is BiFC assay?

BiFC (Bimolecular Fluorescence Complementation) assay is based on the association of complementary fragments of protein (KGC&KGN) similar to Yeast-Two-Hybrid assay.
Interaction of the complementary BiFC fragments causes fluorescence (Kusabira-Green like GFP) that can be detected by microscopy and FACS.



Adapted from Morell et al. 2008

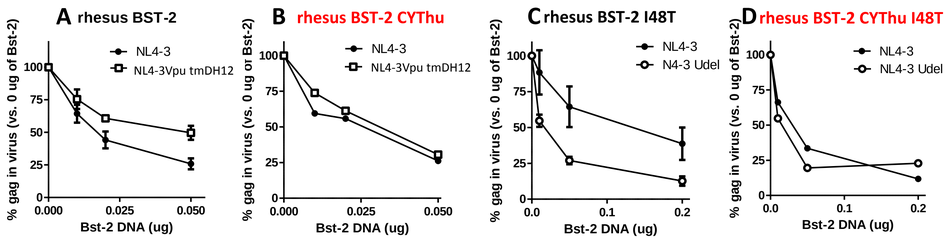
Fig. 2 Physical interaction is not sufficient for the BST-2 antagonism by Vpu.



(A) We generated BiFC-tagged rhesus BST-2 I48T and NL4-3 Vpu_{tmDH12}. (B) After confirming equal amount of protein, we compared MFI of BiFC signal in 293T cells by FACS.

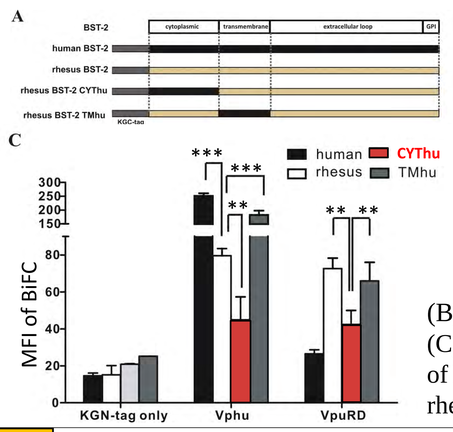
(C) We found that mutation of I48T in rhesus BST-2 is not critical for the interaction with NL4-3 Vpu and that TM of DH12 Vpu is not important for the interaction with rhesus BST-2. ***; P<0.0001, n.s.; not significant

Fig. 4 Physical interaction is necessary for the BST-2 antagonism by Vpu.



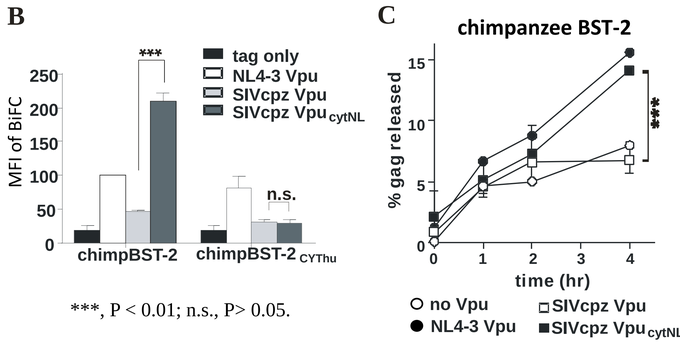
NL4-3 Vpu_{tmDH12} antagonizes rhesus BST-2 (A) but not rhesus BST-2_{CYThu} (B).
NL4-3 Vpu antagonizes rhesus BST-2_{I48T} (C) but not rhesus BST-$2_{CYThu\ I48T}$ (D).
We found that physical interaction is necessary for the BST-2 antagonism by HIV-1 Vpu.

Fig. 3 Human BST-2 interacts with NL4-3 Vpu via its TM domain. Rhesus BST-2 interacts with NL4-3 Vpu via its CYT domain.



We hypothesized that rhesus BST-2 interacts with Vpu via its CYT domain because rhesus BST-2 interaction is not dependent on TM domain of Vpu in Fig. 2C.
(A) We generated rhesus BST-2 mutants carrying human BST-2 CYT or TM domain.
(B) We confirmed similar amount of protein.
(C) We found that Vpu interacts with TM domain of human BST-2 and cytoplasmic domain of rhesus BST-2. ***; P<0.0001, **; P<0.01

Fig. 4 The cytoplasmic domain of HIV-1 Vpu contributes to the physical interaction with, and antagonism of, chimpanzee BST-2.



***, P < 0.01; n.s., P> 0.05.

(A) Amino acid of the cytoplasmic domains of the BST-2 variants used in this experiment are shown. (B) We found that the cytoplasmic domain of HIV-1_{NL4-3} Vpu contributes to the physical interaction with chimpanzee BST-2. (C) We found SIVcpz Vpu_{cytNL} antagonized chimpanzee BST-2 but not chimpBST-2_{CYThu} through the cytoplasmic domain interactions.