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Single amino acid substitution in the HIV-2 gp36 ectodomain part interacting with BST-2 impairs viral release

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Introduction & Objectives: Tetherin (BST-2) is a host antiviral factor that retains viral particles at the cell surface and causes their endocytosis and destruction. The HIV-2 Env protein is known to counteract tetherin during the viral replication cycle. A study demonstrated that the endocytosis motif GYRPV in the HIV-2 Env cytoplasmic tail (CT) was necessary to antagonize tetherin, but another domain in the gp36 ectodomain was involved in this antagonistic role. As this domain was not clearly defined, we tried to identify it.

Methods: Since the HIV-1 Env protein does not antagonize tetherin, we selected 42 potential amino acids by an *in silico* comparison of the HIV-1 and HIV-2 gp 41 amino acid sequences. We used site directed mutagenesis to introduce mutations at the sites of interest in an infectious clone. HEK293 cells were transfected with the produced clones and the generated viral particles were used to infect H9 cells (MOI=2). A RT-qPCR was performed to quantify the quantity of viral RNA released in the cell culture medium at two, three and six days post-infection. Furthermore, infected cells were treated with subtilisin (a bacterial exotoxic protease) to quantify the number of viral particles tethered at the cell surface in order to assess the antagonistic role.

Results: Among the 42 HIV-2 Env mutants tested, we characterized a mutant (Env N659D) that shows a significant lack of antagonism to tetherin and of release from infected cells. This mutant is ten times less released than wild type virus after six days post-infection. Subtilisin treatment confirmed that this virus is more tethered at the cell surface (seven times more retained at the cell surface).

Conclusion: A single mutation in the HIV-2 gp36 ectodomain hinders the antagonistic role toward human tetherin. We demonstrated precisely that in addition to the endocytosis motif in the CT, an amino acid in the ectodomain is involved in this function. Furthermore, we are performing a CRISPR Cas9 based knockout of *Bst-2* gene expression. These *H9 Bst-2* $^{-/-}$ cells will be infected with HIV-2 wild type virus and Env N659D mutant so as to confirm that this amino acid is involved in the antagonism to tetherin. This finding could refine our understanding of the control of HIV-2, especially the role of tetherin and may open the way to new strategies to control the replication of HIV-1 infection.

Biography

Dufrasne François obtained his master's degree in molecular and cellular biology at the Université Libre de Bruxelles (ULB) in Belgium. He is currently performing his Ph.D. research in the AIDS Reference Laboratory of Prof. Goubau Patrick and Dr. Ruelle Jean at the Université catholique de Louvain (UCL). He also works as assistant and dispenses microbiology practicum to students from Medicine and Biology faculties (UCL). For his thesis, he investigates topological and functional properties of the cytoplasmic domain of the HIV-2 envelope glycoprotein. Dufrasne's research interests are centered around host-pathogen interactions at a molecular level, with a particular focus on how pathogens manipulate signal transduction events to evade or usurp the immune system.

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