

# Comparative proteomics of wild-type and nef-deleted HIV-1 particles

Christelle Brégnard, Olivier Danos, Stéphane Basmacioquallari

► **To cite this version:**

Christelle Brégnard, Olivier Danos, Stéphane Basmacioquallari. Comparative proteomics of wild-type and nef-deleted HIV-1 particles. *Retrovirology*, BioMed Central, 2011, 8 (Suppl 2), pp.P3. inserm-00637226

**HAL Id: inserm-00637226**

**<https://www.hal.inserm.fr/inserm-00637226>**

Submitted on 31 Oct 2011

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



POSTER PRESENTATION

Open Access

# Comparative proteomics of wild-type and nef-deleted HIV-1 particles

Christelle Brégnard<sup>1,2</sup>, Olivier Danos<sup>1,2,3</sup>, Stéphane Basmacioquillari<sup>1,2\*</sup>

From *Frontiers of Retrovirology 2011*

Amsterdam, The Netherlands. 3-5 October 2011

## Background

Human and Simian Immunodeficiency viruses acquired auxiliary genes that allowed a better adaptation of viruses to their hosts. Among these genes, *nef* was shown to play an important role in the onset of immunodeficiency syndromes. Humans infected with viruses harboring major deletions in *nef* resulting in no Nef expression ( $\Delta nef$ ) remain symptom-free for a significantly longer period of time than individuals infected with wild type (WT) viruses. Single round infection assay also reveal that WT viruses are 5-10 fold more infectious than their  $\Delta nef$  counterparts. This phenotype relies on a function of Nef that takes place during viral particles biogenesis. A proteomic analysis of WT and  $\Delta nef$  viruses was conducted to identify differences responsible for the higher infectivity of WT viruses.

## Materials and methods

Virions were harvested from cell culture supernatant of 293T cells transfected either with pNL4-3 or pNL4-3 *Xho* in which the Nef ORF has been disrupted. Supernatants were then ultracentrifuged over two 20 and 60% sucrose cushions. Viral like particles were harvested at the 60-20% interface, diluted in PBS and ultracentrifuged again. Pelleted material was then subjected to Differential Gel Electrophoresis (DIGE) or Isobaric Tagging for Relative and Absolute Quantification (ITRAQ). Proteins enriched either in WT or  $\Delta nef$  virions were selected for further characterization in order to investigate their role in virus infectivity. Candidate proteins were over expressed in WT or  $\Delta nef$  single round infection competent virus-producing cells, alternatively, siRNA was used to silence candidate protein expression.

## Results

DIGE and ITRAQ revealed that Nef regulates the incorporation/ exclusion of cellular proteins into/from virions. Both methods showed that Glucosidase II and ERM proteins are enriched in WT and  $\Delta nef$  virions, respectively. In addition, ITRAQ which is more sensitive than DIGE, pointed out other differences, among which CD81, ALG-2 and EHD4, all enriched in  $\Delta nef$  virions, were selected for further characterization, based on their involvement in mechanisms that potentially affect virus biogenesis. Over expression or silencing CD81 in virion-producing cells decreased or increased virus infectivity, respectively. In addition, CD81 decreased virus release. Both effects were observed in HXBc2 env pseudotyped virions, not on VSV-G pseudotyped virions. Silencing Ezrin also increased HXBc2 pseudotyped virus infectivity. Although Ezrin over expression affected neither virus infectivity nor release, over expression of the FERM domain of Ezrin significantly decreased virus release. Glucosidase II, ALG-2 and EHD4 are presently under investigation.

## Conclusions

The presence of a different set of cellular proteins in WT and  $\Delta nef$  HIV-1 particles might affect virus infectivity. We clearly demonstrated that Ezrin and CD81 are less abundant in WT virions than in  $\Delta nef$  virions, which correlates with a higher infectivity of WT virions. This could be recapitulated by artificially manipulating the expression level of both proteins in virion producing cells. Interestingly, Nef potently increased the infectivity of virions produced in cells depleted from CD81, or, to lower extent, Ezrin. This suggests a partial overlap between the ability of Nef to exclude CD81/Ezrin from virions in the course of their biogenesis, and to increase virus infectivity.

<sup>1</sup>Hôpital Necker-Enfants Malades, Université Paris Descartes, Paris, 75743 Cedex 15, France

Full list of author information is available at the end of the article

**Author details**

<sup>1</sup>Hôpital Necker-Enfants Malades, Université Paris Descartes, Paris, 75743 Cedex 15, France. <sup>2</sup>INSERM U845, Paris, 75730 Cedex 15, France. <sup>3</sup>Cancer Institute, University College London, London, UK.

Published: 3 October 2011

doi:10.1186/1742-4690-8-S2-P3

**Cite this article as:** Brégnard *et al.*: Comparative proteomics of wild-type and nef-deleted HIV-1 particles. *Retrovirology* 2011 **8**(Suppl 2):P3.

**Submit your next manuscript to BioMed Central  
and take full advantage of:**

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at  
[www.biomedcentral.com/submit](http://www.biomedcentral.com/submit)

