Xenophagy in HIV infection: actual knowledge and future therapies

Sergio Tejedor Félez, Degree in Biochemistry

UAB Universitat Autònoma de Barcelona

Background

HIV (human immunodeficiency virus) is able to escape from the immune response and antiretroviral drugs. Despite this, **HIV depends on its ability to avoid intrinsic cellular defenses**. The presence of virulence factors against autophagy proves the essential role of **xenophagy** in HIV infection. Xenophagy response is also implicated in antigen presentation extending its function to adaptive immunity.

Hypothesis

To restore the xenophagy response disrupted by viral infection could be a therapy to fight against HIV infection. The viral protein which seems to have a central role in this response is *nef*, mainly through *nef*/beclin-1 interaction. Research of a drug to counteract this interaction could be the way to find a therapy against HIV infection and AIDS (acquired immune deficiency syndrome) progression.

Objectives and methods

The objective of the study is to define the xenophagy response during HIV infection and propose a therapy strategy or investigation line. To achieve this, I have focused in recent publications and literature about the subject and bioinformatic analyzing tools. Protein structure analysis and homology search were performed with pBLAST, FATCAT and T-Coffee servers. Template construction was achieved with SwissModel software. Docking studies have been carried out with AutoDock 4.0, ClusPro, SwarmDock, ZDOCK and RossettaDock softwares. The modeling of the best clusters obtained from docking studies was performed with ROSIE server. The images were generated with UCSF Chimera software and Photoshop CS6.

Xenophagy response

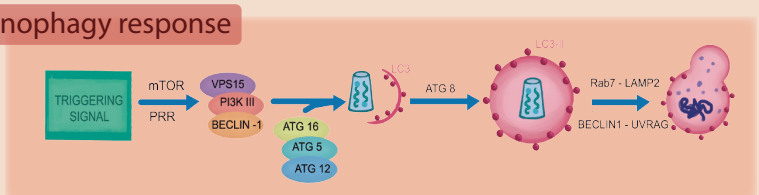

Figure.1: Most xenophagy triggering signals converge in the **inhibition of mTOR** (mammalian target of rapamycin) **or PRR** (Pattern Recognition Receptor) **activation**. This leads to the activation of **PI3K complex** (class III phosphatidylinositol-3-OH kinase) which triggers the **autophagophore formation**. The **elongation** begins **guided by LC3**. Finally it **fuses** with the lysosome **through LAMP-2/Rab7 and beclin-1/UVRAG interaction**. Beclin-1 is involved in vesicle nucleation and autophagosomal maturation besides lysosomal fusion. Image designed with Photoshop CS6 software.

HIV disturbs autophagy

Bystander cells
- *env* ↑autophagy and promotes cell death unless the cell become infected
- secreted *tat* downregulates autophagy to avoid premature degradation

Early infection stages
- Viral genome transcription begins
- *gag*/LC3 and *nef*/beclin-1 co-localization trigger phagophore formation

Latency
- *Tat* protein translates viral and cell proteins. ↑ER stress = ↑autophagy
- ↑autophagy = ↓*tat* = ↓*gag* and *nef* = recovering normal autophagy

Late infection stages
beclin-1/*nef* interaction avoids autophagosome-lysosomal fusion. This avoids viral particles degradation and enhances *gag* processing and viral reproduction

Proteins involved in autophagy disruption

env
Autophagy induction will occur wether there is an infection or not

gag
gag/LC3-II interaction occurs in a *nef* dependent manner

tat
Necessary for transcription, also there are drugs to counteract its effects

nef
Prevents the last steps of xenophagy and disrupts antigen presentation

Mutated or knockout *nef* strain decreases viral replication and AIDS progression

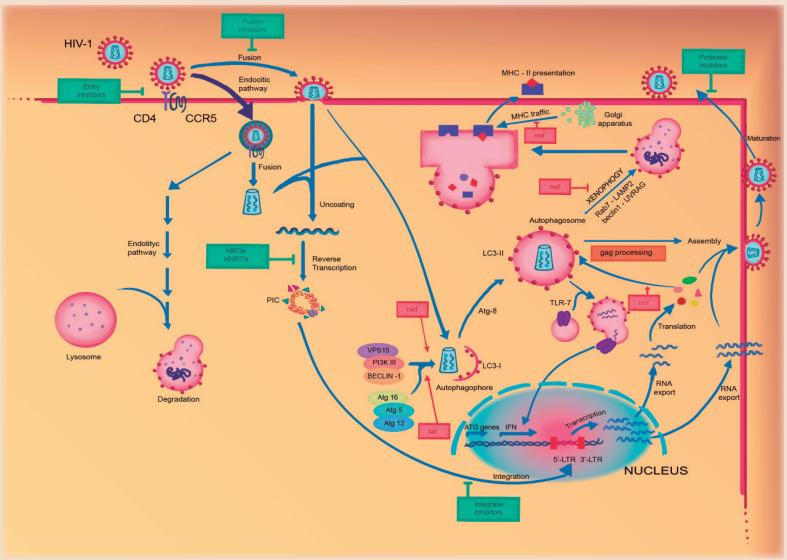

Figure 2: Global integrated scheme of the molecular cell cycle of HIV and xenophagy response against the infection. Viral entrance triggers autophagy response due to *env* mediated effects. Occurs mostly in an endocytic pathway. This avoids fusion inhibitors but exposes the virus to lysosomal degradation. Viral genome keeps integrated and transcriptionaly active until cell death. Infection can enter in a latency phase. In green frames, current targets in HIV antiretroviral therapy are highlighted.

Xenophagy response and its principal involved proteins in the different steps of the process are picted. Firstly xenophagy is initiated by cargo receptors that recognize foreign material. Then, autophagosome formation is mediated by LC3 and engulfs the foreign material. Finally, it fuses with the lysosome to degrade the content. *Tat* and *nef* proteins promote autophagy first steps and *nef* also blocks autophagosome-lysosomal fusion through *nef*/beclin-1 interaction. This generates autophagosome accumulation, which is useful for the virus for *gag* processing and for increasing viral protein flux to cytoplasmatic membrane. The downregulation of autophagosome formation also prevents MHC (major histocompatibility complex) presentation and the initiation of adaptive immune response. MHC presentation is also disrupted by *nef* protein through disruption of Golgi's vesicle traffic. TLR-7 (toll-like receptor) has a role in HIV recognition and could start antiviral response through IFN-α (interferon) transcription among others.

Image designed with Photoshop CS6 software.

Proposed therapy

To counteract nef could be the best way to fight against HIV infection. The use of **beclin-1 mimetic peptide** would retain *nef* and block its mediated effects. The peptide will be based on the 267-299 region of beclin-1(previously described by Shoji-Kawata, S. et al., Nature 2013) and will cover from 55 to 121 *nef* residues, this represents **over 30% of total viral protein**.

This peptide, **combined with common ART** (antiretroviral) cocktails would be able to stop HIV progression. Other autophagic enhancers could be added, but there is not enough description to ensure their efficacy.

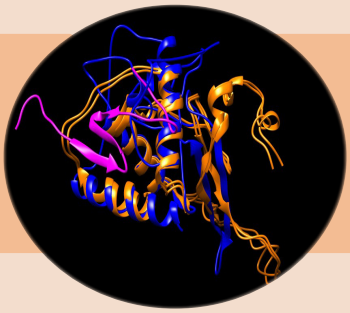

Figure 3: Beclin-1 peptide (magenta) interaction with HIV *nef* protein (orange). GAPR1 (Golgi-Associated Plant Pathogenesis-Related protein 1) structure (blue) is aligned with *nef* to point out that the interacting region is homologous in both proteins. The models were obtained from SwissModel, builded from 4ddp (beclin-1 peptide), 3rea (*nef*) and 4aiw (GAPR-1) PDB codes. The interaction was determined by different docking servers and modeled by ROSIE server. The *nef*/GAPR-1 alignment was obtained with FATCAT server. Image designed with UCSF Chimera software.

Expected results

HIV has got mechanisms to avoid xenophagy response. Blocking these mechanisms would diminish replication and also infection efficiency of HIV. Investment on this investigation line would help us to obtain an efficient therapy to treat HIV infection and AIDS symptoms.

Difussion plan

In order to spread this theory and its argues the best way would be the publication in an high impact journal of this area. Also to contact researchers to collaborate with them in further investigation.
This work will be presented to Universitat Autònoma de Barcelona and will remain at their DDD (digital documents deposit).

Conclusions

Beclin-1 mimetic peptide is a great candidate to target *nef*

Autophagy disrutpion, mainly *nef* mediated, is **essential for an efficient HIV infection** and replication

The *nef*/beclin-1 interaction **covers over 30% of nef** protein

The interaction **with the peptide is stronger** than with the full beclin-1

We are targeting a **viral, non a host protein**

The molecular **homology to hCG therapy**, currently approved, would facilitate the new therapy acceptance

Positive reported results from in vitro and in vivo assays

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