HIV-1 Nef Inhibits Autophagy in Macrophages through Transcription Factor EB Sequestration

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Abstract

Background: Transcription factor EB (TFEB) is a master gene expression regulator of the classical autophagy-lysosome pathway. Under basal conditions, TFEB is phosphorlyated by MTORC1, resulting in the cytoplasmic retention of TFEB through interactions with 14-3-3 proteins. When MTORC1 is inhibited, TFEB is dephosphorylated, prompting its translocation to the nucleus where it increases the expression of genes encoding lysosomal proteins. HIV-1 Nef acts as an anti-autophagic maturation factor through interaction with beclin-1, an essential autophagy and tumor suppressor protein. In this study, we investigated the role of Nef and TFEB in the modulation of autophagy during HIV-1-infection of human macrophages.

Methods: Monocyte-derived macrophages (MDM) were challenged with 10^5 $TCID_{50}$ HIV-$1_{Ba\text{-}L}$ or HIV-$1_{\Delta Nef}$ per 5 x 10^5 cells. Autophagy and the role of TFEB was assessed using the expression, localization and phosphorylation of autophagy proteins combined with the lipidation of microtubule-associated protein 1 light chain 3B (LC3B) by immunoblotting, qRT-PCR, and fluorescence microscopy or by transducing MDM with shRNA for *TFEB*. Data were analyzed using the Mann-Whitney *U* test.

Results: Following exposure of macrophages to HIV-$1_{Ba\text{-}L}$ or heat-inactivated HIV-1, TFEB is dephosphorylated and translocated to the nucleus. This correlated with an increase in autophagy as evidenced by increased lipidation of LC3B, degradation of sequestosome and increased autophagosome formation ($P < 0.01$) and was attenuated by TFEB silencing ($P < 0.05$). In the presence of productive infection, TFEB phosphorylation and cytoplasmic sequestration started to increase and autophagy markers decrease by 5 days post-infection and by 7 days, levels were similar to the uninfected controls ($P > 0.05$). HIV-$1_{\Delta Nef}$ similarly induced the dephosphorylation and nuclear localization of TFEB that corresponded to an increase in autophagy during initial infection. Conversely, at 7 days post-infection nuclear accumulation of dephosphorylated TFEB and autophagy markers remained elevated ($P < 0.02$).

Conclusions: These results support a model whereby, during initial infection, the interaction between virion surface protein(s) and host receptors serve as a signal for autophagy initiation that is dependent upon the dephosphorylation and nuclear translocation of TFEB mediated by the inhibition of MTORC1. Once HIV-1 establishes a productive infection, Nef down regulates autophagy through the phosphorylation and cytosolic sequestration of TFEB. These findings help to explain how HIV-1 modulates autophagy to promote its own replication and cell survival, and further suggests that disrupting the autophagic balance within the infected cell can inhibit HIV-1 and potentially eliminate the infected cell.

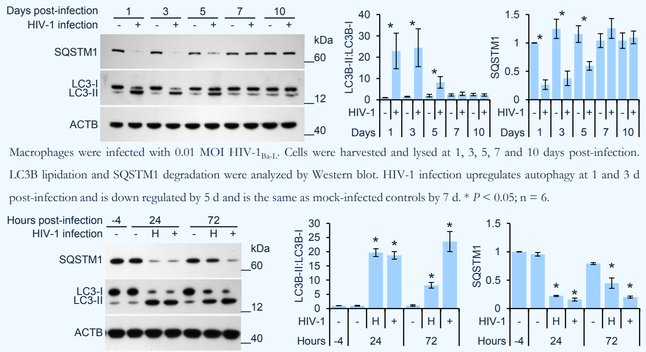
Background

- TFEB is a master gene expression regulator of the classical autophagy-lysosome pathway.
- Autophagy is a major self-degradative process in mammalian cells with roles in cellular homeostasis.
- Under basal conditions, TFEB is phosphorlyated by MTORC1, resulting in the cytoplasmic retention of TFEB by 14-3-3 proteins.
- When MTORC1 is inhibited, TFEB is dephosphorylated, prompting its translocation to the nucleus where it increases the expression of genes encoding lysosomal proteins and the induction of autophagy.
- The conversion of LC3B-I to LC3B-II and its turnover is an indicator of autophagy induction and flux
- Sequestosome 1 (SQSTM1) binds LC3B-II and is degraded when autophagosomes fuse with lysosomes.

Results

Figure 1

HIV-1 infection induces autophagy

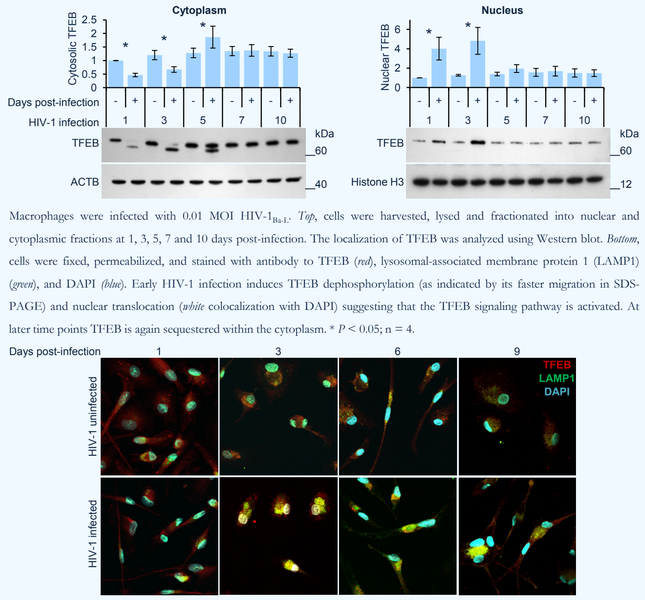


Macrophages were infected with 0.01 MOI HIV-$1_{Ba\text{-}L}$. Cells were harvested and lysed at 1, 3, 5, 7 and 10 days post-infection. LC3B lipidation and SQSTM1 degradation were analyzed by Western blot. HIV-1 infection upregulates autophagy at 1 and 3 d post-infection and is down regulated by 5 d and is the same as mock-infected controls by 7 d. * $P < 0.05$; n = 6.

Macrophages were infected with HIV-$1_{Ba\text{-}L}$ (+), mock infected (-) or with heat-inactivated HIV-$1_{Ba\text{-}L}$ (H) and harvested at 24 and 72 h post-treatment. Heat inactivated HIV-$1_{Ba\text{-}L}$ increased LC3B lipidation and SQSTM1 degradation at 24 h indicating that active HIV-1 infection is not required for the initial induction of autophagy. * $P < 0.05$; n = 4.

Figure 2

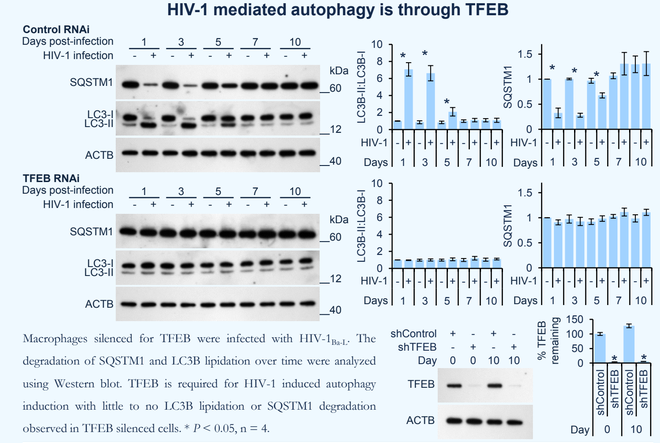
HIV-1 infection induces TFEB dephosphorylation and nuclear localization



Macrophages were infected with 0.01 MOI HIV-$1_{Ba\text{-}L}$. *Top*, cells were harvested, lysed and fractionated into nuclear and cytoplasmic fractions at 1, 3, 5, 7 and 10 days post-infection. The localization of TFEB was analyzed using Western blot. *Bottom*, cells were fixed, permeabilized, and stained with antibody to TFEB (*red*), lysosomal-associated membrane protein 1 (LAMP1) (*green*), and DAPI *(blue*). Early HIV-1 infection induces TFEB dephosphorylation (as indicated by its faster migration in SDS-PAGE) and nuclear translocation (*white* colocalization with DAPI) suggesting that the TFEB signaling pathway is activated. At later time points TFEB is again sequestered within the cytoplasm. * $P < 0.05$; n = 4.

Figure 3

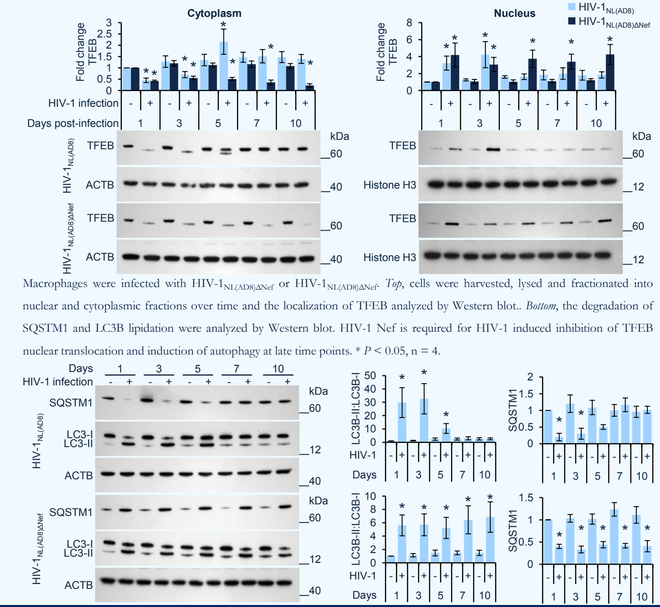
HIV-1 mediated autophagy is through TFEB



Macrophages silenced for TFEB were infected with HIV-$1_{Ba\text{-}L}$. The degradation of SQSTM1 and LC3B lipidation over time were analyzed using Western blot. TFEB is required for HIV-1 induced autophagy induction with little to no LC3B lipidation or SQSTM1 degradation observed in TFEB silenced cells. * $P < 0.05$, n = 4.

Figure 4

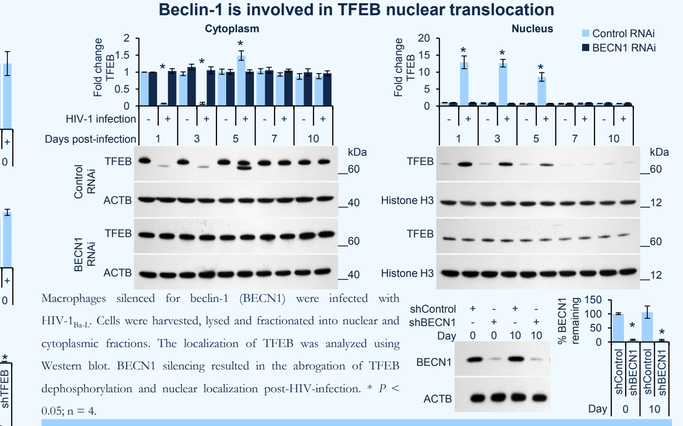
HIV-1 Nef inhibits TFEB nuclear translocation and autophagy



Macrophages were infected with HIV-$1_{NL(AD8)\Delta Nef}$ or HIV-$1_{NL(AD8)\Delta Nef}$. *Top*, cells were harvested, lysed and fractionated into nuclear and cytoplasmic fractions over time and the localization of TFEB analyzed by Western blot.. *Bottom*, the degradation of SQSTM1 and LC3B lipidation were analyzed by Western blot. HIV-1 Nef is required for HIV-1 induced inhibition of TFEB nuclear translocation and induction of autophagy at late time points. * $P < 0.05$, n = 4.

Figure 5

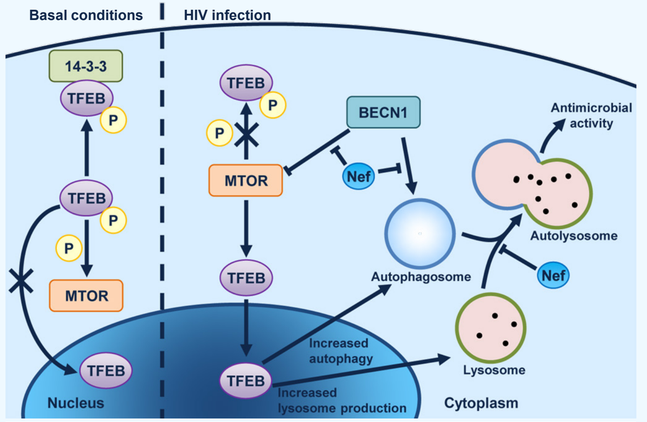
Beclin-1 is involved in TFEB nuclear translocation



Macrophages silenced for beclin-1 (BECN1) were infected with HIV-$1_{Ba\text{-}L}$. Cells were harvested, lysed and fractionated into nuclear and cytoplasmic fractions. The localization of TFEB was analyzed using Western blot. BECN1 silencing resulted in the abrogation of TFEB dephosphorylation and nuclear localization post-HIV-infection. * $P < 0.05$; n = 4.

Conclusions

- HIV-1 infection induces autophagy in macrophages that is dependent upon the dephosphorylation and nuclear localization of TFEB
- HIV-1 Nef inhibits autophagy by binding BECN1, allowing TFEB to be phosphorylated by MTOR



Acknowledgements

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