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Exploring the functional interaction between POSH and ALIX and the relevance to HIV-1 release

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Background

ALIX (ALG2 interacting protein X) is a multi-functional adaptor protein that plays a central role in the regulation of intracellular protein trafficking and apoptosis. As an ESCRT-associated regulator of protein trafficking, ALIX plays an essential role in retrovirus release, an activity that is dependent on the interaction between the central V-domain and the L-domain consensus sequence YPX_nL in Gag [1,2]. The *trans*-Golgi network RING finger protein POSH (Plenty of SH3) is a scaffold protein that acts as an E3 ligase and augments HIV-1 egress by facilitating the transport of Gag to the cell membrane [3]. Recently, it was reported, that POSH interacts with ALIX and thereby enhances ALIX mediated phenotypes in *Drosophila* [4].

Results

In this study we identified ALIX as a POSH ubiquitination substrate in human cells: POSH induces polyubiquitination of ALIX that is modified on several lysine residues *in vivo* and *in vitro*. This ubiquitination does not destabilize ALIX, which suggests a regulatory function. Consistent with the well known activity of ALIX in virus release that rescues budding of L-domain mutant HIV-1 [2,5], we demonstrated that wild type POSH, but not an ubiquitination inactive RING finger mutant ($POSH^{V14A}$), enhances ALIX mediated release of HIV-$1_{\Delta PTAP}$ variants. In further agreement with the idea of a cooperative function of POSH and ALIX, mutating the YPX_nL-ALIX binding site in Gag completely abrogated augmentation of virus release by overexpression of POSH. However, the effect of the POSH-mediated ubiquitination appears to be auxiliary, but not necessary, as silencing of POSH by RNAi does not disturb ALIX mediated augmentation of virus release.

Conclusion

Thus, the cumulative results identified ALIX as an ubiquitination substrate of POSH and indicate that POSH and ALIX cooperate to facilitate efficient virus release. However, while ALIX is obligatory for the release of YPX_nL-dependent HIV-1, POSH, albeit rate-limiting, may be functionally interchangeable.

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