RESOLVING THE INTERSECTION OF HIV AND F-ACTIN IN THE CONTEXT OF CELL-CELL VIRAL SPREAD

Ospina Stella A[1], Aggarwal A[1], Narayan K[2], Turville S[1]

[1]The Kirby Institute, [2]NIH National Cancer Institute

Background: Subversion of the host F-actin cytoskeleton is a recurring mechanism among intracellular pathogens. Both early and late stages of the HIV-1 life cycle have been associated with F-actin-dependent processes. Direct cell-to-cell transmission is orders of magnitude more efficient than infection by cell-free virus, yet it is particularly dependent on the host cell cytoskeleton. Our over-reaching aim is to mechanistically resolve how HIV-1 interacts with cellular F-actin networks during egress and how this influences viral spread.

Methods: Using CRISPR/Cas9 gene edition, we have systematically depleted F-actin regulators in the myeloid cell line U937, and combined high-resolution fluorescence microscopy, live cell imaging, and functional viral-transfer assays to interrogate how this affects actin-rich structures, as well as cellular and viral behaviour during infection.

Results: Using a fluorescence complementation technique, we confirmed a close (<15nm) and specific physical association of HIV Gag and F-actin in living cells. Depletion of F-actin regulators (incl. Formins, Arp2/3 and nucleation factors) revealed their role in driving formation of different cellular structures (i.e. filopodia, veils and dendritic processes), while maintaining total F-actin content, cell size and viability. Assessment of viral transfer in manipulated cells also revealed the ability of particular regulators to modulate cell-cell viral transmission, despite having little impact on free virus release.

Conclusion: Our results confirm a physical association between HIV-Gag and F-actin, and suggest that HIV can functionally interact with an Arp2/3-related regulator to promote cytoskeletal re-organization, leading to F-actin structures which facilitate cell-cell viral spread. Further work is needed to fully resolve the link between HIV and F-actin in order to understand its critical evolutionary importance and how to exploit it.

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