RESOLVING THE MECHANISMS OF CELL TO CELL SPREAD OF HIV

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Background: Despite effective anti retroviral therapy, a cure for HIV remains elusive. Cell to cell spread of HIV is a significant contributor to virus infection and persistence but the mechanisms underlying the process are poorly understood. In recent studies, we have observed newly budding HIV virions tethered to peripheral cytoskeleton through long finger like projections called filopodia. Using this unique and easily resolved phenotype, we endeavoured to decipher the link that tethers HIV to cortical F-actin. We also investigated how this tethering can mediate HIV spread between cells and finally, using virus as a tool, how F-actin is fundamentally regulated in cells of our immune system.

Methods: After screening lineage-restricted expression of F-Actin regulators we systematically depleted F-actin regulators in the myeloid cell line U937 using shRNA knockdowns. The various filopodial phenotypes were then examined using live imaging, high resolution fluorescent imaging and correlative FIB-SEM. Mass spectroscopy was performed determine various actin regulators that get incorporated into budding virions.

Results: Our studies revealed Arp2/3 complex to be the cellular actin regulator that tethers HIV to the cytoskeleton. We resolved this phenotype by generating a cell that primarily has Arp2/3 dependent F-Actin/filopodial activity. Mass spectroscopy analysis of virions released from these cells showed differential enrichment of specific regulators. Volume EM imaging revealed HIV buds localized at F-actin branch points indicating Arp2/3 dependent nucleation. Additionally, we found HIV buds enriched on areas of high positive membrane curvature. Expression of HIV mutants with inability to outwardly bend the membrane acted as dominant negatives by abrogating virus budding and filopodia formation.

Conclusions: Taken together, our studies provide strong evidence for how HIV is tethered to cellular cytoskeleton. We propose that the HIV link to cortical F-actin provides an evolutionary advantage for the virus, as it is key to co-ordinating viral transfer during cell-cell spread.