DISRUPTION OF ACTIN-RELATED PROTEIN 3 (ARP3) ABROGATES NUCLEAR LOCALIZATION OF HIV-1 LATENCY-INDUCING SIRNA

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Background: Epigenetic silencing is a conserved process that can be mediated by RNA. We have developed an siRNA, siPromA, which targets the HIV-1 5`LTR to induce virus suppression. As a potential HIV-1 gene therapy, the mechanisms underlying epigenetic silencing are poorly understood. Argonaute 1 (Ago1) and siRNA are essential components of RNA-induced transcriptional silencing (RITS) machinery. We have shown Ago1 co-localises with siPromA, and F-actin interacts biochemically with Ago1, as well as co-localising in the nucleus of HIV-1 infected cells. Elucidating the role and regulation of actin in epigenetic silencing could define fundamental mechanisms and provide targets to enhance gene therapy.

Methods: Live imaging (DeltaVision Elite) was performed to elucidate dynamics of RITS transport into the nucleus, using HeLa $T4^+$ cells stably transduced with LifeAct, Ago1-GFP and an actin-related protein 3 (ARP3) shRNA-mediated knockout (KO) to disrupt the actin cytoskeleton. The ARP3-KO cultures were compared to HeLa $T4^+$ cells stably transduced with LifeAct and Ago1-GFP. Cells were infected with $HIV\text{-}1_{SF162}$ and transfected with fluorescent-labelled siPromA-647 or siScrambled control (siScPromA-647). Nuclei were stained with NucBlue and time-lapse imaging was performed every 90seconds over 12hours. Images were analysed using softWoRx.

Results: As expected, HeLa $T4^+$ cells expressing LifeAct and Ago1-GFP showed nuclear co-localisation between Ago1-GFP and siPromA-647 (PCC=0.510±0.0436, n=18, p=0.0005), compared to siScrambled controls. Arbitrary line intensity profiles and 3D models also confirmed RITS nuclear localisation in these cultures. In contrast, we observed signal co-localisation of Ago1-GFP and siPromA-647 in the cytoplasm of ARP3-KO cultures, which was confirmed by significant Pearson correlation coefficient (PCC) values (0.607±0.0226, n=46, p<0.0001), but no signal or colocalisation in the nucleus. Arbitrary line intensity profiles and 3D models also confirmed a lack of nuclear entry.

Conclusion: This study demonstrates the importance of the actin cytoskeleton in intracellular trafficking of RITS machinery during RNA-directed silencing of HIV-1.

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