DIAPHANOUS FORMIN 1 KNOCKDOWN DISRUPTS NUCLEAR LOCALISATION 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 and may provide an HIV gene therapy. However, the mechanisms underlying epigenetic silencing are poorly understood. Argonaute 1 (Ago1) and siRNA are essential, co-localising components of RNA-induced transcriptional silencing (RITS) machinery. F-actin is functionally important in the nucleus and interacts biochemically with Ago1. We have previously shown that certain F-actin inhibitors impair siRNA nuclear trafficking. Here we explore the role of Diaphanous Formin 1 (Diaph1) to define fundamental mechanisms that may 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 a Diaph1 shRNA-mediated knockdown (KD) to disrupt the actin cytoskeleton. The Diaph1-KD cultures were compared to HeLa $T4^+$ cells stably transduced with LifeAct and Ago1-GFP. Cells were infected with HIV-1_{SF162} and transfected with fluorescent-labelled siPromA or siScrambled control. Nuclei were stained with NucBlue and time-lapse imaging was performed every 90 seconds over 12 hours. Images were analysed using softWoRx.

Results:
As expected, HeLa $T4^+$ cells expressing LifeAct and Ago1-GFP showed frequent nuclear co-localisation between Ago1-GFP and siPromA (PCC=0.59±0.050, n=14, p=0.004), compared to siScrambled controls, in which no nuclear co-localisation was observed. In contrast, we observed a 78.6% reduction in nuclear co-localisation of Ago1-GFP and siPromA in the Diaph1-KD cultures (n=3). Unexpectedly, nuclear entry of siScrambled controls was observed (n=8) in the Diaph1-KD cultures.

Conclusion:
Diaph1 knockdown does not abrogate nuclear siRNA entry but dysregulates this process making it less specific. Therefore, Diaph1 may be part of a gatekeeping mechanism for siRNA nuclear entry. This study demonstrates the complex role of the actin cytoskeleton in intracellular RITS trafficking during RNA-directed silencing of HIV-1.

Disclosure of Interest Statement:
The authors report no conflict of interest.