Dissecting the Nuclear RNAi machinery Dynamics of Epigenetic Silencing in an HIV Model

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Background:
RNAi is a biologically conserved process of post-transcriptional gene silencing (PTGS) or transcriptional gene silencing (TGS). In TGS, Argonaute 1 (Ago1) protein and promoter-targeted siRNA are essential components mediating epigenetic silencing. We reported nuclear Ago1-siRNA in human cells, however, their trafficking dynamics and essential regions of Ago1 are still unknown.

Methods:
Leica 3D thunder live-cell imaging was performed in HIV-$1_{NL4.3}$-infected HeLa T4+ cells to assess trafficking of AlexaFlour647 tagged promoter-targeted siRNAs and Ago1-GFP. Analysis of intracellular trafficking, vesicle tracking and cell-pose analysis was performed using AIVIA 13.0. Ago1-siPromA-mediated HIV TGS and heterochromatinisation of proviral DNA was measured via Gag RT-qPCR and ChIP assay. Ten Ago1 proteins with truncated functional domains were generated and transcript profiling was performed to assess their role in TGS.

Results:
Live cell image analysis revealed dynamic trafficking of co-localised Ago1-siRNA to subcellular nuclear compartments, with mean vesicle counts in the perinuclear (528.9, $p<0.0001$), inner nuclear membrane (265, $p<0.0001$) and nuclear cavity (264, $p<0.0001$), compared to Ago1 and siScrambled transfected cultures (193, 107 and 86, respectively). Pearson correlation coefficients indicated colocalisation of Ago1-GFP/siPromA-AF647 events with PCC 0.1-1.0 (mean=0.2285, $p<0.0001$) compared to Ago1-GFP/siScrambled-AF647 events with PCC -0.5-+0.5 (mean=0.1625). HIV-1 gag transcripts were 70% reduced in Ago1-siPromA transfected cultures compared to scrambled ($p<0.0001$). ChIP analysis confirmed repressive epigenetic modifications of increased H3K9me3 (5-fold, $p<0.001$), H3K27me3 (1.5-fold, $p<0.01$) and Ago1 (3-fold, $p<0.001$) in siPromA-transfected cultures compared to mock-transfected cultures. Ago1 L1, PIWI, PAZ and N terminal mutants showed increased Gag mRNA expression ($p<0.0001$) compared to Ago1-WT transfected with siPromA, suggesting these regions are important in epigenetic silencing.

Conclusion:
This study demonstrated siRNA-Ago1 trafficking into the nucleus is highly dynamic, with Ago1 L1, PIWI, N and PAZ regions being essential for HIV silencing. Our data supports the role of promoter-targeted siRNA-Ago1 in the Block and Lock HIV functional cure approach.

Disclosure of Interest Statement:
The authors have no conflicts of interest.