Identifying the human RNA-induced transcriptional silencing machinery facilitating HIV-1 latency

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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 viral suppression and may provide a HIV gene therapy. However, the mechanisms underlying human epigenetic silencing are poorly understood. Paralleling yeast, Argonaute 1 (Ago1) and siRNA are essential, co-localising components of human RNA-induced transcriptional silencing (RITS) machinery. To date, no other component has been identified. Elucidating a human RITS complex would not only define fundamental mechanisms, but may also offer avenues to enhance RNA-based gene therapy.

Methods:
To promote RITS formation, HeLa $T4^+$ cultures inoculated with HIV-1_{SF162} were transfected with either siPromA or Scrambled control. Following transfection, the cultures were harvested into cytoplasmic, nuclear soluble and nuclear insoluble fractions. Controls included mock infected and mock uninfected cultures. RITS machinery were extracted by immunoprecipitation of Ago1 and candidate proteins identified via mass spectrometry. Following bioinformatic analyses, short-listed candidates were confirmed by western blot and reverse immunoprecipitation. Confirmed candidates will undergo further biomolecular studies.

Results:
Mass spectrometry of siPromA transfected samples identified a total of 1482 proteins in the nuclear soluble fraction and 2035 proteins in the nuclear insoluble fraction. Analysis of these subsets revealed 10 functionally interesting candidates, including proteins with similarities to known yeast RITS complex components. Of those, 8 were confirmed by western blot. Reverse immunoprecipitation pulled down 3 of the 8 candidates. These 3 potential RITS complex protein components are currently undergoing additional protein biochemistry studies to identify the essential Ago1 binding domains required for the protein interaction.

Conclusion:
This study aimed to identify protein components of the human RITS machinery. We have successfully identified key candidates that possess similarities to known RITS proteins in yeast. Further studies will reveal essential Ago1-candidate interactions and may offer new avenues to harness RNA therapeutics for HIV-1.

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