LAYER-BY-LAYER PARTICLES DELIVER EPIGENETIC SILENCING SIRNA TO HIV-1 LATENT RESERVOIR CELL TYPES

Authors:
Klemm V[1], Czuba-Wojnilowicz E[2], Cortez-Jugo C[2], Turville SG[1], Agarwal A[1], Caruso F[2], Kelleher AD[1], Ahlenstiel CL[1]
[1]Kirby Institute, UNSW Medicine, Sydney, New South Wales 2052, Australia,
[2]ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, and the Department of Chemical and Biomolecular Engineering, The University of Melbourne, Parkville, Victoria 3010, Australia

Background:
Nanomaterials have been employed to facilitate intracellular delivery of small interfering (si)RNA to induce gene silencing via mRNA degradation using the post-transcriptional gene silencing (PTGS) RNA interference pathway. Besides PTGS, siRNAs are also capable of transcriptional gene silencing (TSG) or epigenetic silencing, which targets the gene promoter in the nucleus and prevents transcription via epigenetic modifications, however silencing efficiency is hampered by poor intracellular and nuclear delivery. Here, we describe the use of polyarginine-terminated multilayered particles for delivery of TGS siRNA. The HIV latent reservoir has potential to reactivate and is the major barrier to a HIV cure. It is hypothesized that particles can mediate delivery of TGS-inducing siRNA to the nucleus to induce a block in virus transcription and lock the virus in a “super latent” state.
Methods:
Fluorescently labeled siRNA (promoter-targeted siPromA or control siScrambled) were complexed with multilayered particles formed by the layer-by-layer assembly of poly(styrene sulfonate) and poly(arginine) incubated with HIV-infected cell types (HeLa T4+, HUT78, primary activated/resting CD4+ T cells and monocyte-derived macrophages (MDMs). Nuclear delivery of siRNA was assessed at 48h using deconvolution microscopy to measure i) co-localisation of siRNA with NucBlue stained nuclei, ii) arbitrary line profile and iii) 3D cell profile. Functional HIV-1 gene silencing by particle delivered siRNA was determined using Reverse Transcriptase assay and RT-qPCR of cell associated gag vRNA.
Results:
Image analysis reported successful delivery of siPromA into the nucleus of all infected cell types assessed. Significantly decreased levels of Reverse Transcriptase and gag vRNA levels demonstrated functional silencing of particle delivered siPromA in HeLa T4+ and HUT78 cell lines, and in primary activated CD4+ T cells and MDMs (from three donors), compared to controls (Mock, NP alone, Buffer and siScrambled).
Conclusion:
This works extends conventional nanotechnology-enabled PTGS siRNA delivery to the TGS pathway and paves the way for future studies on particle-delivered siRNA for efficient TGS of various diseases and infections.

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