DIRECT VISUALIZATION OF NANOPARTICLE DELIVERY OF EPIGENETIC GENE SILENCING RNA TO THE NUCLEUS OF HIV-1 INFECTED ACTIVATED AND RESTING CD4+ T CELLS

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Background: The latent virus reservoir is a major barrier to HIV cure. We are investigating a targeted functional cure approach utilizing epigenetic silencing RNA that potently inhibit virus transcription. Efficient delivery of anti-HIV agents, such as lentiviral vectors or siRNA, for gene therapy in CD4+ Tcells is a substantial challenge. This study visualizes siRNA delivery in CD4+ Tcells using nanoparticle technology.

Methods: Human primary CD4+ Tcells were activated using anti-CD2/CD3/CD28 beads and infected with VSV-G pseudotyped HIV expressing an mOrange-reporter or live HIV-$1_{NL4.3}$ expressing GFP and an envelope with high CD4 affinity. In parallel, resting human primary CD4+ T cells were infected using the same live virus. Epigenetic silencing siRNA, siPromA, or its scrambled control, were delivered 24h post-infection in activated cultures or 5 days post-infection in resting cultures using a novel nanostructured film.To assess viral infection and siRNA location, CD4+ Tcell cultures were imaged using a DeltaVision-microscope. Arbitary line intensity profiles were utilized to determine signal overlap and subcellular location.

Results: 81% and 71% of activated CD4+ Tcells were infected with Pseudotyped virus or live HIV-$1_{NL4.3}$ virus, respectively. Nuclear localization of siPromA was observed only in infected CD4+ Tcells, with 12% of mOrange positive cells and 40% of GFP positive cells showing nuclear siPromA signal. 23% of resting CD4+ Tcells were infected with variant HIV-$1_{NL4.3}$ virus as judged by GFP. 15% of these cells showed a nuclear siPromA signal, confirmed by arbitrary line. In contrast, while siScrambled was detected in all CD4+ Tcells, it was only detected in the cytoplasm. Virus suppression, determined by RT assay, will be presented.

Conclusions: This is the first study using nanoparticle technology to deliver epigenetic silencing siRNA into the nucleus of CD4+ Tcells. These results provide a pathway for targeting latent reservoirs by achieving uptake and release of RNAi vectors into the nucleus of resting CD4+ Tcells.

Disclosure of Interest Statement:
The authors declare that there is no conflict of interest.