MULTIPLEXING MIR-SHRNA USING SELF-CLEAVING 2A PEPTIDES FOR AN RNA-BASED HIV GENE THERAPY.

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Background:
The latent reservoir poses a major challenge for achieving an HIV cure. Our RNA-based block and lock HIV cure approach can effectively silence the reservoir using promoter-targeted short-interfering (si)RNA. In silico analysis has revealed three promoter-targeted siRNAs together can silence >99% of global HIV subtypes. Here we aim to develop a combination HIV gene therapy via lentiviral delivery of these siRNA as microRNA short-hairpin RNAs (miRshRNAs), with self-cleaving T2A peptide linkers.

Methods:
Lentiviral constructs were generated with Pol II or III promoters (SFFV or U6 respectively) driving expression of siRNA (CCR5 and/or promoter-targeted PromA) sequences as miRshRNA on a miR-30 backbone, with combination miRshRNAs linked with T2A (thosea asigna virus 2A) self-cleaving peptides. MOLT4 cell lines stably expressing lentiviral expressed miRshRNA/s targeting CCR5/PromA were made and CCR5 silencing was assessed by flow cytometry using CCR5-APC antibody. miRNA-shRNA processing and expression of siRNA was assessed with miRNA extraction using miRNeasy (Qiagen) and RT-qPCR. miRshRNA-induced HIV silencing was assessed using HIV_{BAL} infection in Magic5 cells and measuring Gag expression by flow cytometry.

Results:
Pol II (SFFV) driven constructs reported equivalent CCR5 silencing by single constructs and multiplexed construct (both 45%, p=0.0004) compared to Scram. Pol III (U6) driven constructs did not show comparable silencing between single (30%) and multiplexed constructs (4%). SFFV-driven constructs showed successful processing and expression of single and multiplexed miR-shRNAs comparable to reference standards, SNORD44 and SNORD 48 (C_t values of 25-27). Significant silencing of Gag in HIV-$_{BAL}$ infected Magic5 cells by Pol II multiplexed constructs (83%, p<0.001) was also observed compared to controls.

Conclusion:
This study demonstrates T2A peptide linkers can be used for multiplexing miR-shRNAs when driven by a Pol II promoter. This multiplexing approach is adaptable for other viruses where multiple siRNA are required for broad-spectrum protection.

Disclosure of Interest Statement:
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