EXPLORING THE EFFECT OF RNA-DIRECTED EPIGENETIC SILENCING OF HIV-1 IN ASTROCYTES, A CELL TYPE RELEVANT TO THE CNS RESERVOIR

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Background: Our laboratory has previously reported the efficacy of short-interfering RNA (siRNA), namely siPromA and 143, which target the HIV-1 5' long terminal repeat (LTR) region to induce epigenetic silencing. This phenomenon has been confirmed in a wide range of cell types relevant to the HIV latent reservoir, such as $CD4^+$ T-cells and macrophages. However, to date there have been no investigations involving the epigenetic silencing phenomena in cell types relevant to the HIV latent reservoir in the CNS. This project aims to characterise RNA-induced epigenetic silencing in a human glial cell line, SVG astrocytes, using various anti-HIV promoter-targeted siRNAs.

Methods: The human fetal astrocyte cell line, SVG, was infected with VSV-G pseudotyped HIV-$1_{NL4\text{-}3\ \Delta env}$ carrying a mOrange reporter and subsequently transfected with AlexaFluor 647-labelled siPromA, si143, si143T and corresponding specificity controls siScrambled and siM2 (carrying two mismatches). The Cytell™-Cell-Imaging-System was used to determine the level of infection and the level of suppression following siRNA transfection, as determined by expression of the mOrange reporter over a four-day time course. High-resolution fluorescence microscopy using the DeltaVision-Elite-Imaging was performed at 48hr post-transfection to determine siRNA localization, which is expected to be nuclear in silenced cultures.

Results: Transfection of siPromA, si143 and si143T in HIV-$1_{NL4\text{-}3}$ infected SVG-A cells resulted in a significant decrease of mOrange labeled viral protein expression from 100% to 73.8 $\pm$ 2.9% (p=0.017), 65.5 $\pm$ 3.4%, (p=0.001) and 62.2 $\pm$ 4.5%, (p=0.001) at day 4, respectively. All treatments were normalized to an infected, mock-transfected culture. DeltaVision analysis demonstrated both siPromA, si143 and si143T were observed inside the nucleus, whereas, the negative controls siScrambled and siM2 were localized to the cytoplasm.

Conclusion: This study is the first demonstration of nuclear localisation of epigenetic silencing-inducing siRNA in SVG-astrocytes, which may indicate that the silencing phenomena can occur in this specialised CNS cell type.