CD4-TARGETED MRNA DELIVERY OF TAT REVERSES HIV-1 LATENCY

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Background

Interventions that reverse viral latency are needed for an HIV cure. The viral protein Tat drives HIV expression but is toxic and difficult to deliver in a targeted manner. Here, we tested whether CD4-targeted delivery of Tat encoding mRNA could safely reverse latency.

Methods

mRNA encoding wildtype and noncytotoxic Subtype B and D Tat variants were made and transfected into TZM-bls to assess transactivation. Five J-Lat clones were treated with Tat mRNA-LNPs or small molecule agents (romidepsin, prostratin, pabinostat, or AZD5582) and evaluated using flow cytometry, live cell imaging, or single cell multiomic (RNA+ATAC) profiling. Ibalizumab (anti-CD4) was conjugated to mRNA-LNPs to generate CD4-targeted mRNA-LNPs. Primary CD4 T cells were treated with Ibalizumab-targeted Tat or GFP mRNA-LNPs to assess mRNA expression, toxicity, and activation. PBMCs from people living with HIV (PLWH) on ART were treated with CD4-targeted Tat mRNA-LNPs and assayed for p24 expression.

Results

A noncytotoxic Tat variant demonstrated similar transactivation activity in TZM-bls when compared to wildtype variants and drove HIV expression in neighboring, nontransfected cells, indicating secretion of active Tat. Tat mRNA transfection of J-Lat clone 10.6 resulted in dose-dependent viral reactivation. Multiomic analysis of treated J-Lat 10.6 revealed significant changes in expression of four genes ($|\log2\text{-fold change}| > 0.25$, $p < 0.05$): HIV-1 (0.37 log2-fold increase), MTRNR2L12 (0.33 log2-fold decrease), RPS10 (0.31 log2-fold decrease), and SNHG25 (0.26 log2-fold decrease). Minimal changes to ATAC accessibility of the proviral genome 2-3 days post-treatment were seen despite GFP signal in ~60% of cells. All J-Lat clones reactivated, albeit heterogeneously, upon treatment with Tat mRNA-LNPs or Romidepsin, but less so with other agents. Ibalizumab-targeting of Tat or GFP mRNA-LNPs resulted in efficient mRNA delivery and expression in rhesus and human CD4 T cells, with no observed cytotoxicity (Live/Dead, Annexin V), change in activation markers (CD69, CD25, CD38, HLA-DR), or widespread changes to gene expression in bulk RNAseq. CD4-targeted Tat mRNA-LNP treatment of PBMCs from three PLWH with inducible reservoirs resulted in p24 expression in both cellular pellet (0.05-0.5 pg/ml) and supernatant (0.15-0.2 pg/ml).

Conclusions

CD4-targeted Tat mRNA-LNPs selectively drive viral gene expression without evidence of cell toxicity. These findings show that viral transcriptional programs, such as latency, can be modified using CD4 cell-targeted mRNA gene therapy.