Creation and characterization of HIV nanoLuc CHME-5 cell line

Lee Campbell, NIDA Intramural Research Program, 251 Bayview Blvd #200, Baltimore, MD 21224

Microglia play a key role in the pathogenesis of Human Immunodeficiency Virus (HIV)-associated neurocognitive disorders (HAND) due to their productive infection by HIV. This results in the release of neurotoxic viral proteins and pro-inflammatory compounds which impair the functionality of surrounding neurons. Because models of HIV infection within the brain are limited, we aimed to create a novel microglia cell line capable of recreating several hallmarks of HIV infection. We utilized CRISPR/Cas9 gene editing technology to integrate a modified HIV provirus into CHME-5 immortalized microglia to create HIV-nanoLuc CHME-5. In the modified provirus, the Gag-Pol region is replaced with a luciferase; nanoLuc, which allows for rapid assay of HIV long terminal repeat (LTR) activity using a luminescent substrate. The remaining portion of the provirus encoding neurotoxic accessory proteins (e.g. tat, rev, nef, gp120) remains intact. Using molecular assays, we confirmed that CRISPR/Cas9 conferred target integration of the modified HIV provirus at the safe harbor locus ROSA26. Additionally, provirus activity can be differentially modulated by pharmacological, molecular and viral mechanisms. Increased luciferase expression was observed by treatment of lipopolysaccharide (LPS), tumor necrosis factor α (TNF-α) and the HIV viral protein tat. Conversely decreases are observed when treated with the nuclear factor-κB (NF-κB) inhibitor sulfasalazine, or by CRISPR/Cas9 mediated knockout of the TLR-4 receptor. To examine whether this cell line could cause neuronal injury/alterations, we co-cultured HIV-nanoLuc CHME-5 with primary cortical neurons expressing the calcium indicator GCaMP. Using time series measurements, we observe decreases in synchronous neuronal firing rate with the addition of HIV-nanoLuc CHME-5 in comparison to parental CHME-5 microglia. Overall these data suggest that HIV-nanoLuc CHME-5 may be a novel tool to enhance the study of HIV mediated neuropathology in a high-throughput manner.