Transcriptomic Assays of Immunometabolic Enhancers

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Background

Aging related diseases are primarily metabolic, leading to long term inflammation, but in drug discovery immunometabolism remains difficult to assess and often relies on expensive and low throughput methods. **This project will establish real-time fluorescence assays that model and measure neuroinflammatory response to stimuli in human microglial cells (HMC3).** The HMC3 cell line is a robust model for human microglial cells, and the pro-inflammatory response of this cell line has been established [1]. This cell line has been shown to activate NF-kB expression when exposed to bacterial lipopolysaccharide (LPS)[2] and is said to be an effective model for drug therapy development [3]. Two transcription factors known to play a role in inflammation are nuclear factor kappa B (NF-kB) and peroxisome proliferator-activated receptor (PPAR) [4]. Enhancer sites of these transcription factors have been coupled with fluorescent proteins to create a real-time reporter of inflammatory pathway activation. Fluorescence will be analyzed and used to quantify cell inflammatory response to stimuli.

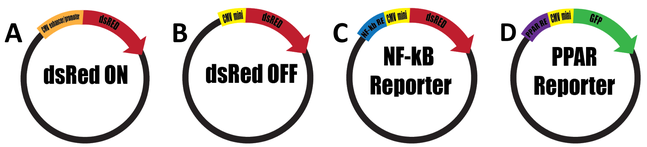

Figure 1: Plasmids developed for reporter assay. *Constitutively active reporter (dsRed ON) with cytomegalovirus (CMV) enhancer/promoter upstream dsRed (**A**). Constitutively inactive promoter (dsRed OFF) contains CMV minimal promoter (CMVmini) upstream dsRed – expected **no** fluorescence (**B**). NF-kB responsive element upstream CMVmini driving dsRed expression – expected fluorescence when NF-kB pathway is activated (**C**). PPAR responsive element upstream CMVmini driving expression of GFP – expected fluorescence when PPAR pathway is activated (**D**).*

Methods

Reporter plasmids were constructed using standard cloning methods using materials and kits from NEB. The pLenti backbone is adapted from plasmid pLenti CMV GFP puro (658-5) [5]. Our reporter plasmids use dsRed and nano luciferase as a reporters downstream of a CMV minimal promoter. Enhancer sequences were identified from previous literature [6,7]. HMC3 cells were transfected using Lipofectamine 3000 and imaged using standard fluorescence microscope and Keyance BZ-x800.

dsRed Expression in HMC3 cells

Aim 1: Verify efficacy of dsRed expression in HMC3 cells under constitutively active cytomegalovirus (CMV) enhancer and promoter.

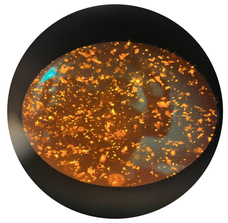

Figure 2: High efficiency transformation of HMC3 cells with constitutively active dsRed reporter. *HMC3 cells expressing dsRed ~48 hours post transfection with constitutively active reporter plasmid. High efficiency transformation (~80%) seen under 20x magnification in standard fluorescence microscope.*

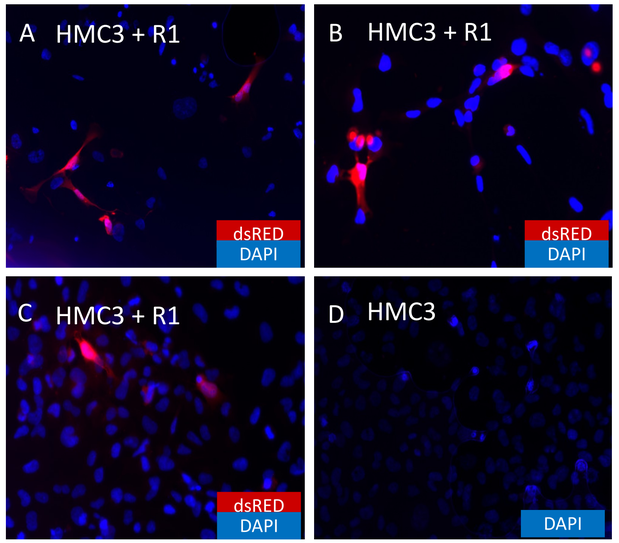

Figure 3: Fluorescence imaging of HMC3 cells with and without dsRedON HMC3 cells transfected with dsRedON reporter stained with DAPI viewed under 20x magnification show expression of dsRed (**A-C**). Untransfected HMC3 cells viewed under the same conditions do not fluoresce (**D**)

Next Steps – LPS dependent dsRed expression

Aim 2: Develop a reliable reporter assay for NF-kB in HMC3 cells.

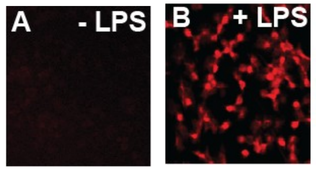

Figure 4: Human microglial cells engineered as cell reporter assay for NF-kB*. Human microglial precursor cells (hMPCs) with an NF-kB signaling reporter do not express dsRed under standard conditions (**A**). NF-kB reporter gene is activated, causing fluorescence in the presence of LPS (**B**). [2]*

Future Applications

Aim 3: Develop a library of reporters for transcription factors indicated by transcriptomic analysis of microglial states

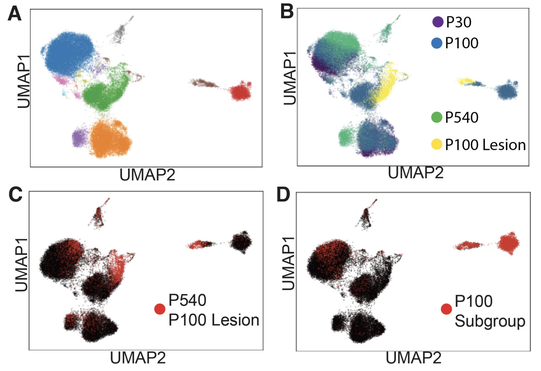

Figure 1: Transcriptomic features for improved analysis of microglial states. *Sparse features separate microglial cells into finer groups than previously possible (**A**). Separation of data into cohorts reveals clusters unique to P100, P540, and Lesioned brains (**B**). Individual sparse features provide explicit transcriptomic signatures of potentially related clusters like P540 and Lesions (**C**)(**D**). [8]*

Aim 4: Modify existing plasmids for directed evolution of Zika Virus

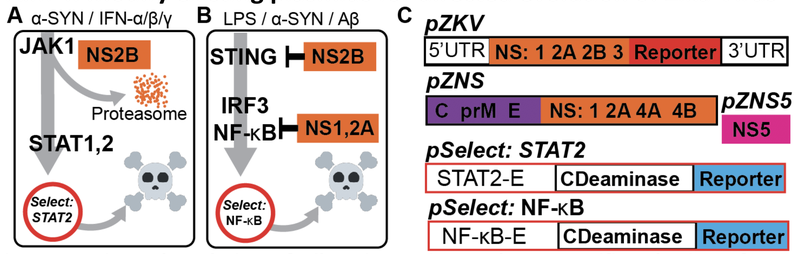

Figure 6: Directed evolution of Zika Virus using Pathway specific selection of ZIKV immunosuppressive mechanisms. *Schematic of a JAK/STAT pathway, a known ZIKV inhibitory mechanism (A). Activators and ZIKV inhibitors of NF-κB (B). Plasmids for pathway specific DE of ZIKV (C)*

References

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