Alzheimer's disease modeling using hiPSC neurons and microglia to screen for neurotoxic and/or neuroprotective compounds

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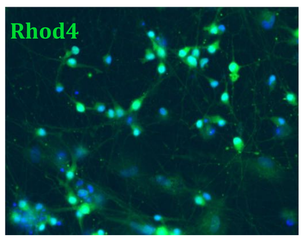
Introduction

Microglia, the neuroimmune cell, can play a neuroprotective or a neurotoxic role depending on the tissue microenvironment. In this study, we developed human induced pluripotent stem cell (hiPSC) co-culture systems to study the role of genetic background in neuroinflammation and neurodegenerative diseases. We used Vala Sciences' imaging platform, the IC200 Kinetic Image Cytometer® (KIC), to characterize hiPSC-microglia alone or in co-culture with hiPSC-neurons. We developed custom algorithms for our CyteSeer® image analysis software that automate neurite tracing, nucleus mapping, and analysis of calcium activity in single cells in a co-culture system. We characterized microglia derived from hiPSCs by immunofluorescence of the biomarkers TREM2, PU.1, IBA-1, CX3CR1, TMEM119, CD11b, and P2RY12. Next, we assessed hiPSC-microglia function by measuring engulfment of pHrodoRed Zymosan beads. Increased engulfment after IL-4 stimulation, but not after lipopolysaccharide (LPS) stimulation, demonstrated polarizability to different microglial activation states (M2 vs. M1, respectively). We also demonstrated differential engulfment of β-amyloid 1-40 and β-amyloid 1-42 (the Alzheimer's-relevant β-amyloid fragment) by hiPSC-microglia. Adding hiPSC-microglia to a glutamatergic neuron cultures doubled neurite outgrowth, suggesting a protective and supportive role for microglia in this system. We are performing neurite outgrowth and live calcium imaging assays on neuron-microglia co-culture systems treated with ApoE and β-amyloid to determine if microglia protect neurons from or sensitize neurons to these Alzheimer's-specific stressors.

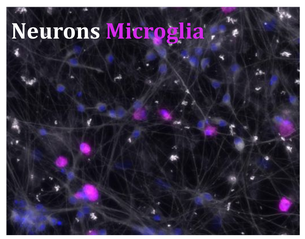
IC200-KIC® and CyteSeer®

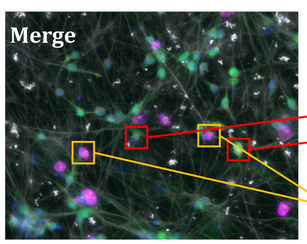
Vala's **IC200-KIC®** combines ultra-fast videography with high-throughput analytics. It is a benchtop (21" x 21" x 22") automated digital microscope with an environmental chamber and is capable of acquiring digital movies from live cells at rates of up to 100 frames per second at full frame size. The IC200-KIC® supports multiple well plates, objectives, and filter sets. It can acquire images in up to 5 optical channels for high content analysis.

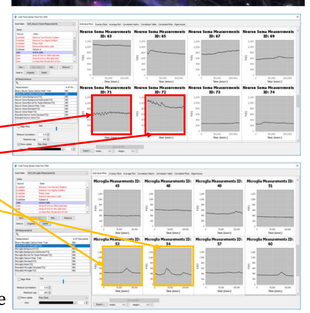
Live calcium KIC imaging



Post-KIC immunostain







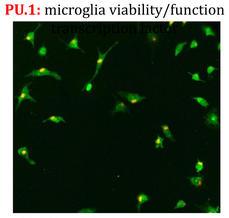
CyteSeer®, Vala's powerful high-content imaging analysis software, can analyze single cells from static images and kinetic time series.

Together, the IC200-KIC® platform and CyteSeer® can measure compound effects on up to thousands of cells simultaneously to discern curative or detrimental effects with high sensitivity.

For these studies, the IC200-KIC® was equipped with a 20X/0.75 NA objective to acquire both live movies and single-timepoint images from fixed cells.

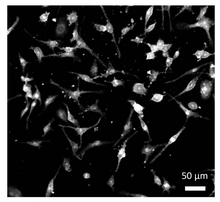
hiPSC-Microglia Express Biomarkers

TREM2: non-inflammatory engulfment of Aβ plaques & apoptotic neurons
PU.1: microglia viability/function

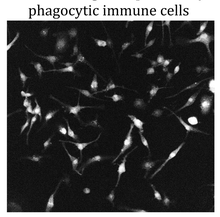


IBA-1: expressed by microglia, upregulated upon activation

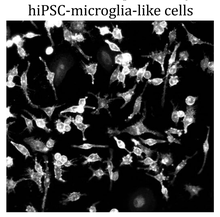
CX3CR1: chemokine receptor activated during immune response



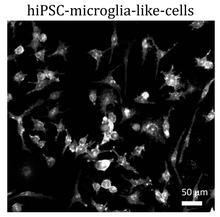
CD11b: integrin expressed by phagocytic immune cells



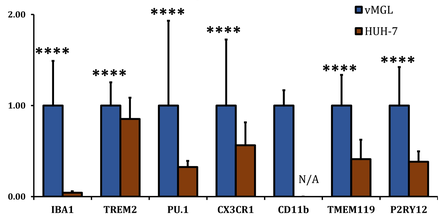
TMEM119: expressed by hiPSC-microglia-like cells



P2RY12: biomarker for hiPSC-microglia-like-cells



Normalized Biomarker Expression vMGL vs HUH-7 Liver Cells



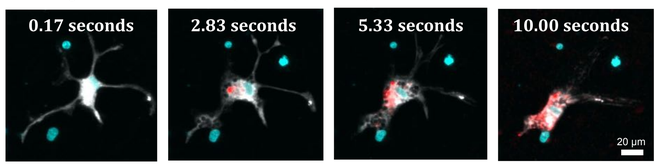
In-house hiPSC-derived microglia were plated on 384 well Greiner plates and fixed with 4% PFA. Cells were imaged on the IC200-KIC® using a 20X air objective to visualize stained biomarkers. CyteSeer® analysis software was used to quantify the biomarker APIs (n=3 wells) compared to the HUH-7 liver cell line. Statistical analyses by unpaired two-tailed t-tests.

hiPSC Microglia Engulf Beads

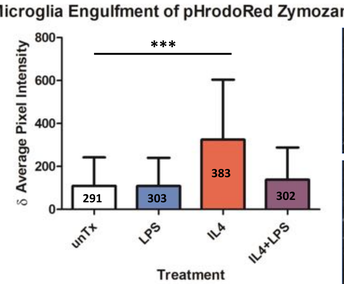
hiPSC-derived microglia were stained with CellTracker Deep Red and plated in 384-well Greiner plates for time-lapse imaging using the IC-200 KIC®. Cells were treated with pHrodo-Red Zymosan beads (ThermoFisher) directly before imaging. Alternating images were acquired on the IC-200 KIC for 5 hours for the CellTracker and pHrodo-Red. CyteSeer® analysis software was used to quantify the increase in API of the pHrodo-Red fluorescence in each microglia, indicating engulfment of Zymosan particles.

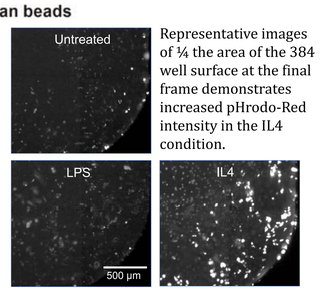
Time-lapse of Zymozan Bead Engulfment

hiPSC-microglia in neuron co-culture (neurons not shown).



Vala Microglia Engulfment of pHrodoRed Zymozan beads



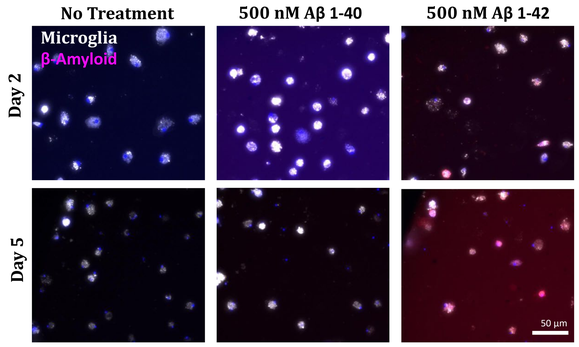


Representative images of ¼ the area of the 384 well surface at the final frame demonstrates increased pHrodo-Red intensity in the IL4 condition.

hiPSC-microglia mono-culture was treated with LPS (100ng/mL), IL4 (200ng/mL), or LPS+IL4 for 1 hour (N=3 per condition) before Zymozan particle addition/imaging. Increased engulfment with IL4 demonstrates microglial induction to M2 anti-inflammatory state. (***p<0.001 by one-way ANOVA followed by Dunnet's post-test)

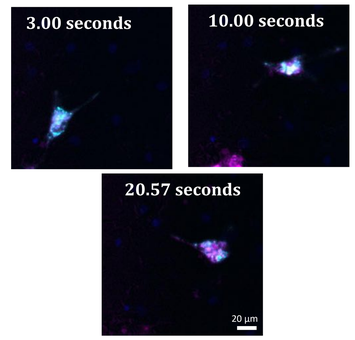
hiPSC Microglia Engulf β-Amyloid 1-42

hiPSC-derived microglia were stained with CellTracker Deep Red and plated in 384-well Greiner plates for time-lapse imaging using the IC-200 KIC®. Cells were treated with Tamra-labled amyloid beta 1-40 or 1-42 fragments directly before imaging. Images were acquired on the IC-200 KIC the CellTracker and Tamra fluorescence. Images were captured once per timepoint (left) or every 5 minutes for 12 hours (right).

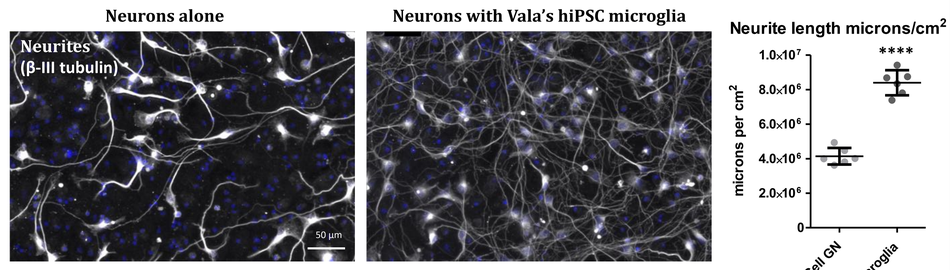


Time-lapse of β-Amyloid Engulfment

hiPSC-microglia in neuron co-culture (neurons not shown) engulfing pre-fibrilized Tamra-Aβ 1-42.



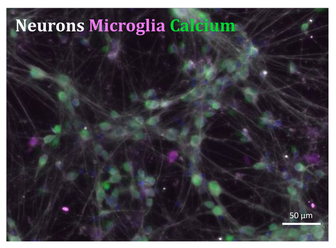
hiPSC Microglia Enhance Neurite Outgrowth

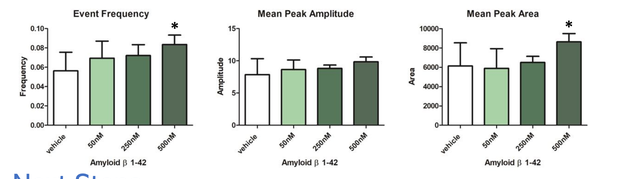


iCell GlutaNeurons (Fujifilm Cellular Dynamics) were plated at 90K cells/cm² on 384 well Greiner plates coated with 0.1% PEI and 3ug/mL laminin with or without in house-derived microglia (18,000 cells/cm²). Cultures were maintained for 14 days with media changes every 2-3 days. Cells were fixed and stained for anti-β-III tubulin (neurites) and with Hoescht (nuclei). Plates were scanned using the IC-200 KIC® with the 20X air objective. CyteSeer® analysis software was used to quantify neurite outgrowth. Graphs show mean and SD for n=6 wells per condition (p<0.0001; unpaired two-tailed t-test).

High Content Analysis in Co-Culture

iCell GlutaNeurons (Fujifilm Cellular Dynamics) were plated at 90K cell/cm² with astrocytes (10K cells/cm2). Microglia were added at 9K cells/cm² on day 7 of culture. Aβ was added after 12 days for 48 hours. Cultures were loaded with Rhod-4 calcium dye on day 14 and the IC-200 KIC® with the 20X air objective was used to acquire 120-second image series at 4 frames per second for each well (n=6). Plates were fixed and stained for Tuj1 (neurons) and IBA1 (microglia). CyteSeer® overlaid live calcium images with fixed images to isolate neuronal calcium signals. 500 nM Aβ induces hyperactivity, both increasing frequency and magnitude of calcium transients. Statistic: ANOVA and Tukey's post hoc test.





Next Steps

Other Alzheimer's-associated stressors, such as ApoE4 fragments and Kainic Acid (a model of excitotoxicity) are being tested in this system to demonstrate disease relevance in our model. High content analysis in co-culture is being applied for Parkinson's Disease modeling with hiPSC-derived dopaminergic neurons.

Contact

For more information on the IC200-KIC®, CyteSeer®, or high content screening contract/collaborative research opportunities, please visit www.valasciences.com.

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Acknowledgements

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