Establishment of relevant human iPSC-derived models in the context of neurobiology and neurodegeneration

Ncardia
Stem cell experts

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The development of physiological relevant models for neurodegenerative diseases remains a challenge, with an unmet need in translatable human-based platforms. With the emergence of iPSC technology, it is now possible to generate brain-derived cell types, namely neurons, astrocytes and microglia, which are key players in multiple disease pathogenesis.

Cortical neurons derived from human iPSCs were recently developed by Ncardia that are post mitotic, cortical layer committed and display branching phenotype (figure 1).

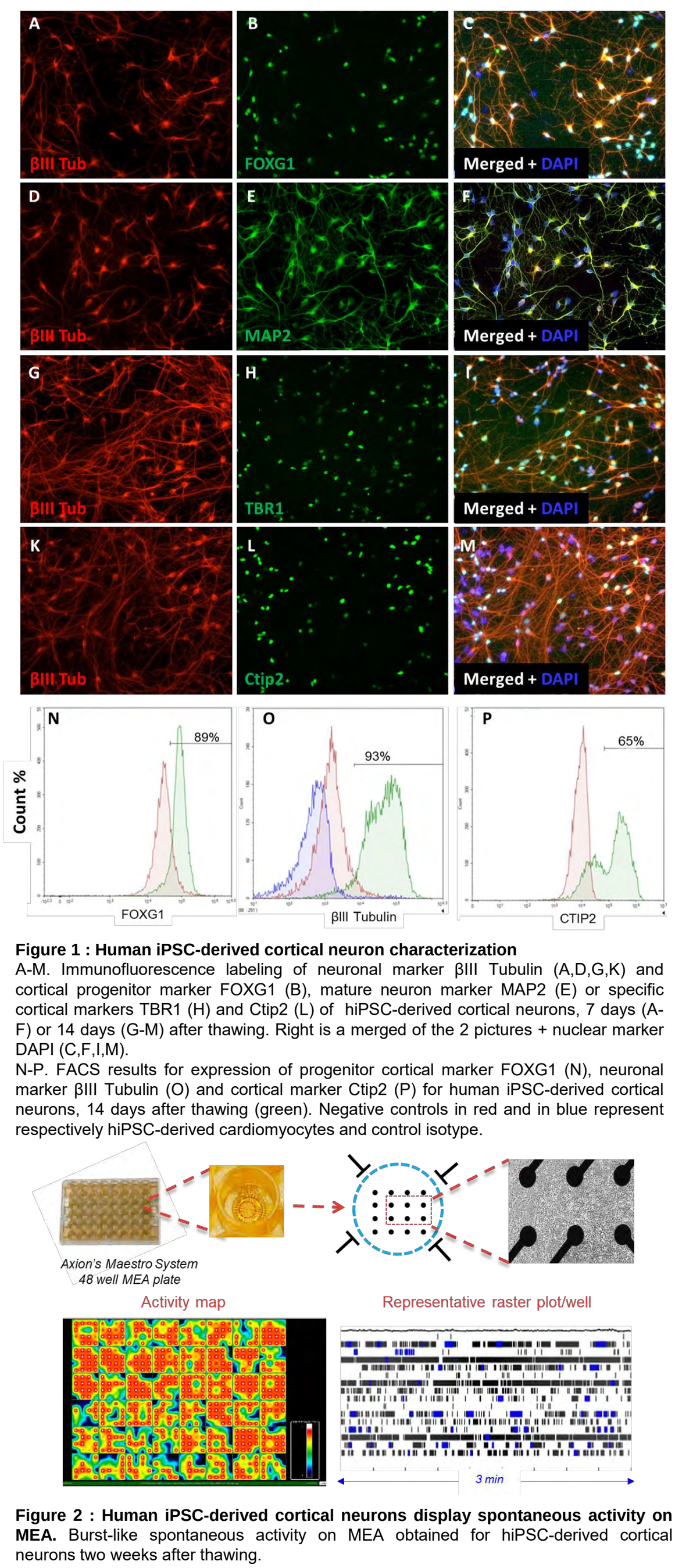

Figure 1 : Human iPSC-derived cortical neuron characterization
A-M. Immunofluorescence labeling of neuronal marker βIII Tubulin (A,D,G,K) and cortical progenitor marker FOXG1 (B), mature neuron marker MAP2 (E) or specific cortical markers TBR1 (H) and Ctip2 (L) of hiPSC-derived cortical neurons, 7 days (A-F) or 14 days (G-M) after thawing. Right is a merged of the 2 pictures + nuclear marker DAPI (C,F,I,M).
N-P. FACS results for expression of progenitor cortical marker FOXG1 (N), neuronal marker βIII Tubulin (O) and cortical marker Ctip2 (P) for human iPSC-derived cortical neurons, 14 days after thawing (green). Negative controls in red and in blue represent respectively hiPSC-derived cardiomyocytes and control isotype.

Figure 2 : Human iPSC-derived cortical neurons display spontaneous activity on MEA. Burst-like spontaneous activity on MEA obtained for hiPSC-derived cortical neurons two weeks after thawing.

The neurons exhibit spontaneous activity on MEA, presenting firing and bursting properties as early as day 7 and evolve into more complex structured patterns of activity (figure 2).

To better understand the role of neuroinflammation in early stages of neuropathology, Ncardia developed a new protocol for the generation of microglia based on the differentiation steps that occur during human development. The monocyte precursors exhibit common identity markers such as CD45+/CD11b+/CD14+ and are generated in high purity and viability, making them a reliable source to produce microglia in a later stage of development (figure 3).

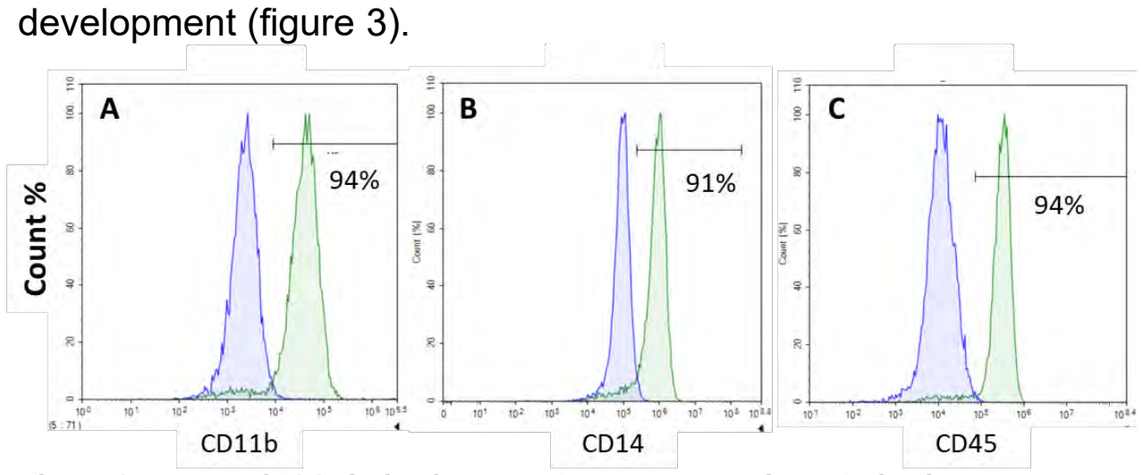

Figure 3 : Human iPSC-derived monocyte precursors characterization
FACS results for expression of common identity markers CD11b (A), CD14 (B) and CD45 (C) for human iPSC-derived monocyte precursors. Negative controls in blue represent control isotype.

The resulting microglia cells have typical dynamic behavior and dual morphology (ramified/ameboid) and express the microglia-specific marker Iba1 (figure 4).

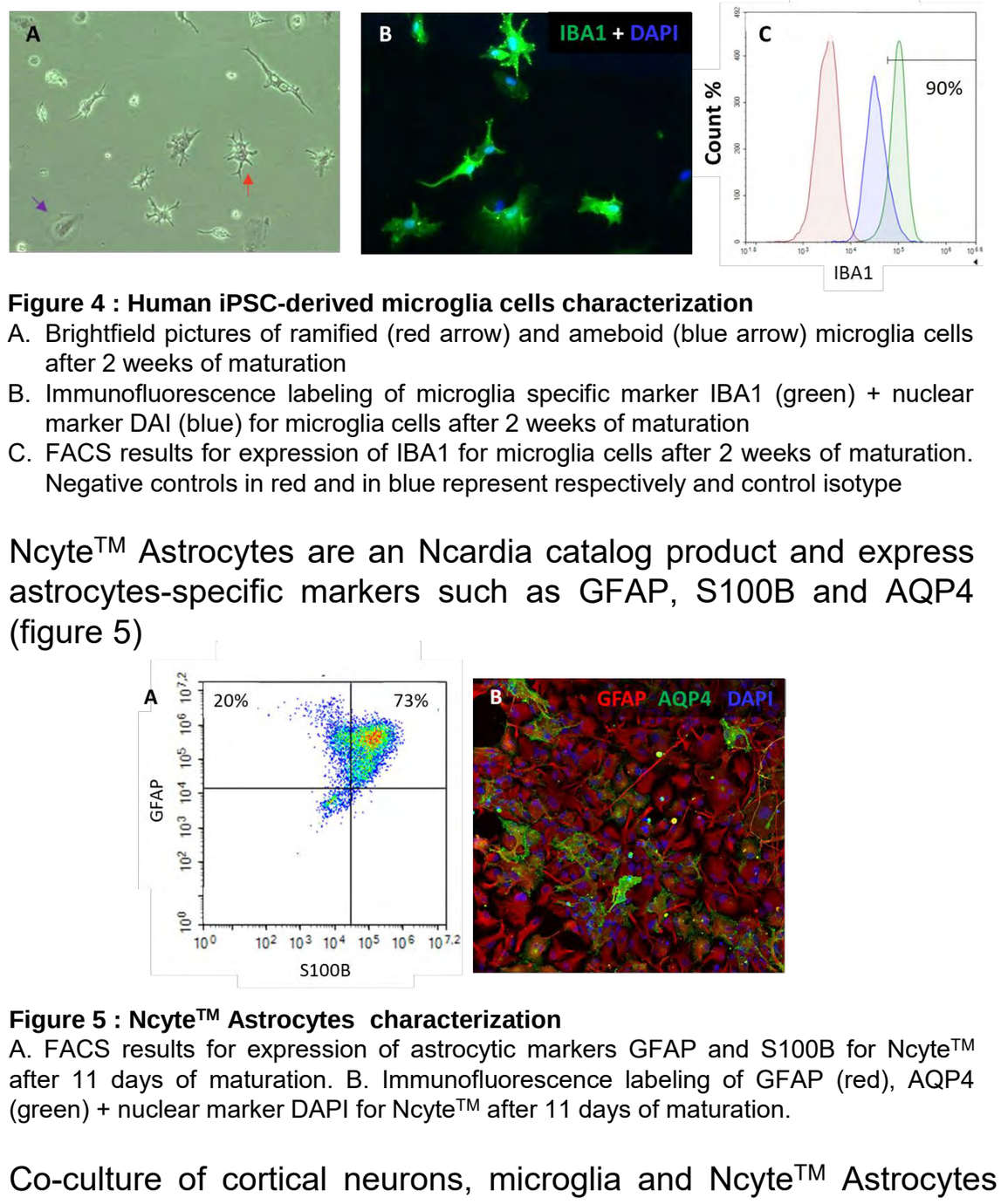

Figure 4 : Human iPSC-derived microglia cells characterization
A. Brightfield pictures of ramified (red arrow) and ameboid (blue arrow) microglia cells after 2 weeks of maturation
B. Immunofluorescence labeling of microglia specific marker IBA1 (green) + nuclear marker DAI (blue) for microglia cells after 2 weeks of maturation
C. FACS results for expression of IBA1 for microglia cells after 2 weeks of maturation. Negative controls in red and in blue represent respectively and control isotype

Ncyte™ Astrocytes are an Ncardia catalog product and express astrocytes-specific markers such as GFAP, S100B and AQP4 (figure 5)

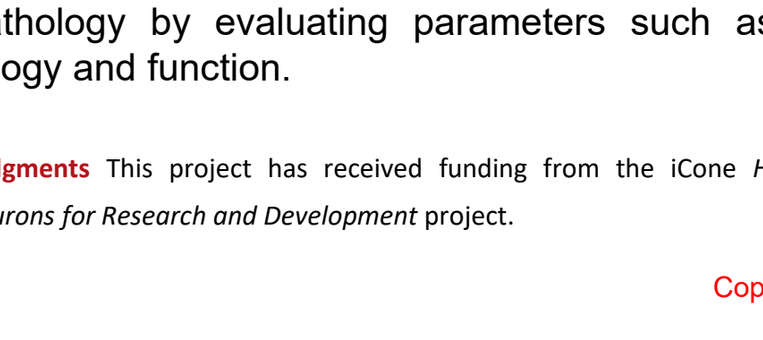

Figure 5 : Ncyte™ Astrocytes characterization
A. FACS results for expression of astrocytic markers GFAP and S100B for Ncyte™ after 11 days of maturation. B. Immunofluorescence labeling of GFAP (red), AQP4 (green) + nuclear marker DAPI for Ncyte™ after 11 days of maturation.

Co-culture of cortical neurons, microglia and Ncyte™ Astrocytes present a translatable and versatile system, incorporating both neuronal and support glial cells generated from the same donor.

High content imaging coupled with long-term functional assays developed at Ncardia provide insight into neurobiology and neuropathology by evaluating parameters such as cell health, morphology and function.

Acknowledgments This project has received funding from the iCone *Human iPSC-derived Cortical Neurons for Research and Development* project.