IMMUNOCOMPETENT HUMAN 3D BRAIN CELL CULTURES TO ADVANCE ATMP DEVELOPMENT

Catarina Brito, iBET, Instituto de Biologia Experimental e Tecnológica; Instituto de Tecnologia Química e Biológica António Xavier, Nova Universidade de Lisboa (ITQB-NOVA), Portugal
anabrito@ibet.pt
Catarina M Gomes, iBET and ITQB-NOVA
Daniel Simão, iBET
Gabriela Silva, iBET and ITQB-NOVA
Maria João C Sebastião, iBET
Patrícia Gomes-Alves, iBET and ITQB-NOVA
Paula M Alves, iBET and ITQB-NOVA

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One of the major challenges in ATMP development is to address the impact of the manufacturing process in the activation of host immune response to the therapeutic cells and gene therapy vectors. Human-relevant experimental assays in which the neuro-immune axis is depicted are therefore required to address safety and potency of advanced therapeutics. In the case of central nervous system (CNS) disorders, both astrocytes and the resident immune cells, microglia, can undergo activation and trigger inflammatory responses. Here, we present a 3D human CNS cell culture that recapitulates neuroinflammatory hallmarks and can be a useful tool in ATMP development.

We explored a neural cell culture methodology pioneered by our team, in which neuron-astrocyte interactions are recapitulated in hiPSC-derived 3D neural cell spheroids (neurospheroids). Neural progenitors derived from human induced pluripotent stem cells (hiPSC) were aggregated and differentiated in perfusion stirred-tank bioreactors (STB) into neurospheroids, that are composed of neurons, astrocytes, and oligodendrocytes. The bioprocess was successfully transferred to a miniaturized STB system and incorporation of microglial cells differentiated from hiPSC (iMGL) was achieved through optimization of culture medium and cell ratio. Neurospheroids with or without microglia were challenged with prototypical neuroinflammatory factors (TNF-α, IL-α, and C1q) and with viral vectors with known tropism towards the CNS, namely human adenovirus 5 (hAdv5) and adeno-associated virus (AAV) serotype 9, an interesting candidate for gene therapy due to its remarkable ability to cross the blood-brain barrier.

Quantitative transcriptomics (NGS) and proteomics (SWATH-MS), together with targeted protein detection showed that the inflammatory challenge modulated TNFα and NF-ƙB signaling pathways, leading to the upregulation of neuroinflammatory genes (e.g., SERPINA3, and C3), secretion of pro-inflammatory cytokines and chemokines (e.g., IL8 or CXCL8, and CCL2) and impairment of the astrocytic metabolic function. These are all hallmarks of neuroinflammation. iMGL efficiently infiltrated the neurospheroid 3D structure and adopted a physiological phenotype, maintaining the expression of typical microglia markers (e.g., TMEM119, TREM-2 and IBA-1) and exhibiting functional features (e.g., phagocytosis). To access viral vector immunogenicity and its impact in transgene expression, neurospheroids cultured in miniaturized STB were exposed to hAdv5 and AAV9, at different multiplicities of infection (MOI). Transduction was evaluated in terms of transgene expression and cell toxicity, as a function of the innate immune reaction elicited by the viral vectors with emphasis on microglial response.

We advocate that the hiPSC-derived neurospheroid cultures are suitable screening tools for addressing neuroinflammatory responses to ATMPs and can contribute to accelerate the development of emerging therapeutic modalities.

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