Systematic Three-Dimensional Coculture Rapidly Recapitulates Interactions between Human Neurons and Astrocytes

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Introduction

We have developed improved experimental human cell culture systems for investigating intercellular signaling dysfunction between neurons and astrocytes in various models of neuropathy. These can then be utilized as screening platforms for strategies to induce neural circuit regeneration towards normative homeostasis. We are focused on astrocytes as they display unique features in humans that cannot be recapitulated in animal models. Current methods are insufficient considering the long time requirements with three-dimensional (3D) organoids developed from human pluripotent stem cells (hPSCs) and the loss of intimate interactions produced by 2D cultures from dissociated primary tissue. Our systematic methods rapidly produce structurally complex hAstros and synapses in high-density coculture with iNeurons in precise numbers, allowing for improved studies of neural circuit function, disease modeling, and drug screening. We conclude that these bioengineered neural circuit model systems are reliable and scalable tools to accurately study aspects of human astrocyte-neuron functional properties while being easily accessible for cell-type-specific manipulations and observations.
Methods

To address these barriers, we have devised novel coculture systems utilizing 3D spheres, termed Asteroids, by combining pre-differentiated hPSC-derived astrocytes (hAstros) with neurons generated from neural stem cells or from those directly induced via transgenic Neurogenin 2 overexpression (iNeurons). Circuit maturation was assessed by analyzing morphological complexity and synaptic density via immunochemistry and confocal microscopy.
Results

Figure 1. 2D Coculture of iNeurons and Pre-matured hAstros. (A) Example images showing the continued hPSC colony structure in the iNeuron line within 1 day after induction of NGN2, and the subsequent drastic change toward neuronal morphology by day 6. hAstros could be added to the iNeurons to be used for coculture studies. (B) Cocultures of iNeurons (TUBB3-positive, nuclei are DAPI positive) with pre-matured hAstros (GFAP positive) are stable at 2 weeks. hAstro morphology becomes branched and elongated after 2 weeks of coculture, although not similar to that of astrocytes in the brain. (C) Example of high-density neuronal fibers projecting out of iNeuron seeds (yellow arrows). (D) hAstros (S100- and mGFP-positive) on high-density neuronal fibers induces morphological rearrangement that increases the number of branches and size compared with dissociated hAstros without iNeurons. n = 10 technical replicates for hAstros only, n = 11 for cocultures. Error bars represent SEM. Scale bars are 50 μm.
Figure 2. Organotypic-like Coculture Recapitulates Human Astrocyte Complexity. (A) Image of cultured tissue (yellow arrow) in a well of a six-well plate on a PTFE membrane insert. (B) Comparison of organoid spheres plated on either Matrigel or PTFE, with or without NGN2 induction, demonstrating the presence or absence of rosette structures, respectively, at the same image magnification. (C) (Left) Merge of bright-field and GFP fluorescence of mGFP astrospheres plated on top of organotypic-like iNeurons. (Middle) A single-plane confocal image of hAstros after 4 weeks of coculture. (Right) Flattened z series of a single hAstro. (D) Time series representing the dynamic morphological complexity of cocultured hAstros. Scale bars, 50 μm.
Results

Figure 3. Analysis of 3D Asteroid Cocultures. (A) Bright-field examples and illustration of asteroids in the absence (left panel) or presence of hAstros with (right panel) or without induction of NGN2. (B) Progenitor cells, as rosette structures, are not present with NGN2 induction (right panel). (C) After 35 days, hAstros are dispersed throughout the asteroids. (D) mGFP reveals the fine-membrane processes emanating from GFAP-positive hAstro branches. (E) The addition of hAstros significantly increases the number of opposed pre- and postsynaptic markers compared with iNeurons without hAstros; the combination of iNeurons with hAstros increases synaptic density to a further extent. *p < 0.05; **p < 0.01. (F) Costello syndrome-model hAstros (HRASG12S) significantly increase synaptic density within asteroids compared with wild-type control (HRASWT) and no hAstro addition. Insets are magnified examples of synapses, including perisynaptic astrocyte processes. ***p < 0.001. (E and F) Data obtained by random sampling of 3–4 different spheres from each of 3 different independent biological replicates. Error bars represent SEM. Scale bar, 50 μm.
Figure 4. Bioengineering and Assembly of 3D Spheres. (A) Bioreactor spinner flasks enable long-term separation of spheres, whereas close physical contact promotes merging of multiple spheres. (B) Morphologically complex hAstros are evenly, yet seemingly randomly, dispersed throughout the asteroids. Neurons are MAP2 positive. (C and D) Examples of 3D imaging demonstrate the macroscale composition of integrated spheres.
Discussion

hAstro branching complexity and protein abundance of maturation markers increased when cocultured with high density neurons. Three biological replicate astrocyte lines, compared to control groups, induced a heightened synaptic density of cocultured iNeurons. As proof of principle for non-cell autonomous disease studies, cell type-specific calcium transients of astrocytes were observed via a transgenic reporter and synaptic structures of iNeurons were increased in the presence of HRAS mutant astrocytes compared to wild-type.

We conclude that these bioengineered neural circuit model systems are reliable and scalable tools to accurately study aspects of human neural circuit functional properties while being easily accessible for cell type-specific manipulations and assays. The macroscale 3D setting reproduces aspects of the neurosurgical environment such as allowing for physical manipulations. With addition of various other cell types, these systems can be further utilized for posing various questions about precise cell-cell interactions.
Summary Points

• Novel three-dimensional “asteroid” coculture system is described

• Complex human astrocyte-specific morphology is generated in vitro

• This system produces rapid and tight association of astrocytes with synapses