A Human Pluripotent Stem Cell-Derived Organoid Model for Recapitulation of Central Nervous System (CNS) Barrier and Fluid Secretion Functions of the Choroid Plexus

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INTRODUCTION

The choroid plexus (ChP) plays a critical role in producing cerebrospinal fluid (CSF) and forming the blood-CSF barrier in the central nervous system (CNS). Recently, a human pluripotent stem cell (hPSC)-derived three-dimensional organoid model of the choroid plexus was developed (Pellegrini et al., Science 2020). Proof of concept experiments highlighted the potential of ChP organoids to be used in biomarker discovery and blood-CSF permeability assays. Here we demonstrate that the recently developed STEMdiff™ Choroid Plexus Organoid Differentiation Kit generates cyst-forming organoids that express appropriate markers of the choroid plexus. We further characterized CSF-like fluid extracted from the lumen of the cysts and investigated the barrier-forming capacity of ChP organoids.

METHODS

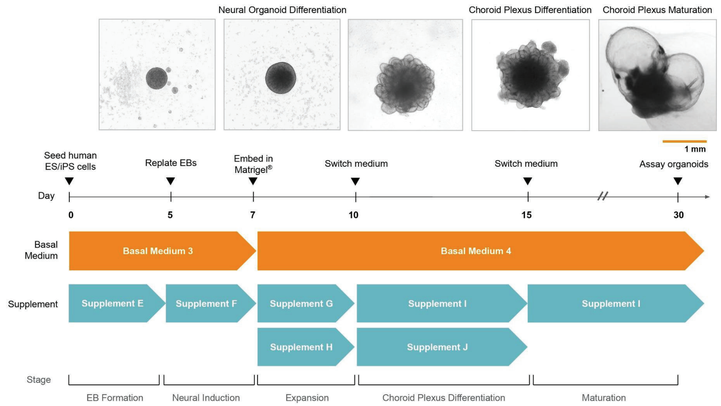

FIGURE 1. Protocol for the STEMdiff™ Choroid Plexus Organoid Differentiation Kit

Choroid plexus organoid differentiation: The STEMdiff™ Choroid Plexus Organoid Differentiation and Maturation Kits (Catalog #100-0824/100-0825) were used to generate ChP organoids. Briefly, single-cell suspensions of hPSCs (6 cell lines maintained in either mTeSR™1 or mTeSR™ Plus) were cultured at 9,000 cells/well in Corning® 96-well round-bottom ultra-low attachment microplates in embryoid body (EB) formation medium for 5 days at 37°C. The aggregates were then switched to an induction medium for 2 days (days 5 - 7). On day 7, neural aggregates were embedded in Corning® Matrigel® droplets and grown in expansion medium for 3 days (days 7 - 10). On day 10, organoids were grown in a choroid plexus differentiation medium for 5 days (days 10 - 15) on an orbital shaker. On day 15, culture medium was switched to a maturation medium with feeding every 2 - 3 days. On the specified day of culture, ChP organoids were harvested for RNA or fixed in 4% paraformaldehyde for immunostaining and whole organoid clearing (Masselink et al., Development 2019)

Extraction and western blot analysis of CSF-like fluid: On day 50, ChP organoids were transferred to a 1.5 mL centrifuge tube and washed 3 times with sterile phosphate-buffered saline (PBS). A 28 gauge needle attached to a syringe was used to pierce the cyst and extract the CSF-like fluid. CSF-like fluid was analyzed on a gradient (4 - 20%) SDS-PAGE gel by western blot. Blots were incubated with either primary anti-IGF2 antibody followed by secondary goat anti-rabbit-HRP or HRP anti-Clusterin antibody. Cell lysate from ChP Organoids, fresh ChP maturation medium and spent medium were used as controls. Protein bands were detected using Bio-Rad ECL substrate and visualized on a Bio-Rad Gel Doc XR system.

Assessment of choroid plexus organoid barrier integrity: Day 40 and day 100 ChP organoids were incubated with low-molecular weight FITC-dextran at 1 mg/mL in BrainPhys™ Imaging Optimized Medium (Catalog #05796) with STEMdiff™ Neural Organoid Supplement I (Catalog #100-0832) for 16 hours. Organoids were then treated with 2 mM EDTA for 2 hours before being imaged on a Zeiss AXIO Observer Z1. Fluorescence intensity was measured over a cross-section of a cyst.

RESULTS

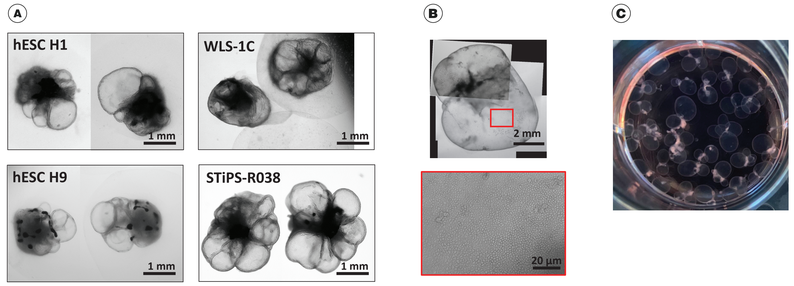

FIGURE 2. Morphology of ChP Organoids

(A) Representative morphology of day 30 ChP organoids derived from multiple cell lines displaying fluid-filled cysts. **(B)** Tiled image of a day 50 STiPS-M001 ChP organoid with a magnified image (lower panel, red box) showing tightly-packed cuboidal ependymal cell morphology. **(C)** Image of day 50 STiPS-R038 ChP organoids in a 6-well plate displaying large fluid-filled cysts.

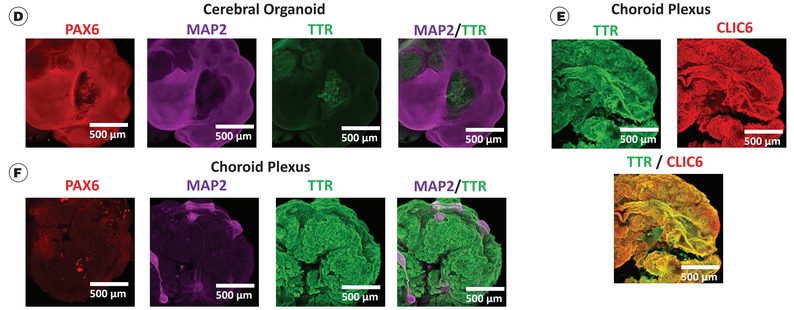

FIGURE 3. Immunostaining of ChP Organoids

(A) Day 50 cerebral organoids displayed a higher proportion of PAX6+ and MAP2+ cells, but a lower level of TTR+ regions. **(B, C)** ChP organoids displayed regions of high TTR+ and CLIC6+ expression, but had lower expression of **(C)** PAX6 and MAP2.

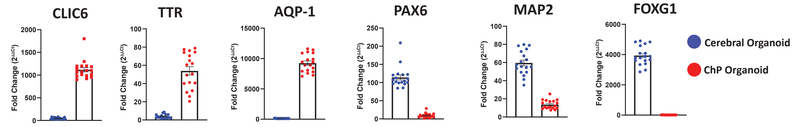

FIGURE 4. RT-qPCR Analysis of ChP Organoids

RT-qPCR analysis of day 50 ChP organoids showed upregulation of CLIC6, TTR, and AQP-1 in ChP organoids, while expression of PAX6, MAP2, and FOXG1 was lower compared to cerebral organoids (average ± SEM; n = 6 cell lines, 3 experiments per cell line). Each data point is an average of data from 3 organoids. Data were normalized to 18S/TBP and compared to an undifferentiated hPSC control. All genes exhibited a significant difference in expression between cerebral and ChP organoids (T-test, $p < 0.0001$).

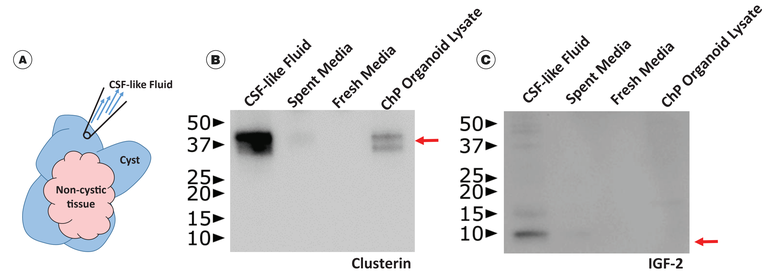

FIGURE 5. Western Blot Analysis of CSF-Like Fluid

(A) CSF-like fluid was extracted from the cyst of a day STiPS-R038 ChP organoid (n=1) using a needle and syringe. CSF-like fluid was analyzed by western blot for **(B)** Clusterin and **(C)** IGF-2, two proteins found in abundance in human CSF (Pellegrini et al., Science 2020). Molecular weight ladder indicating protein size in kDa is labeled on the left. Red arrows indicate expected size of target protein on the blot.

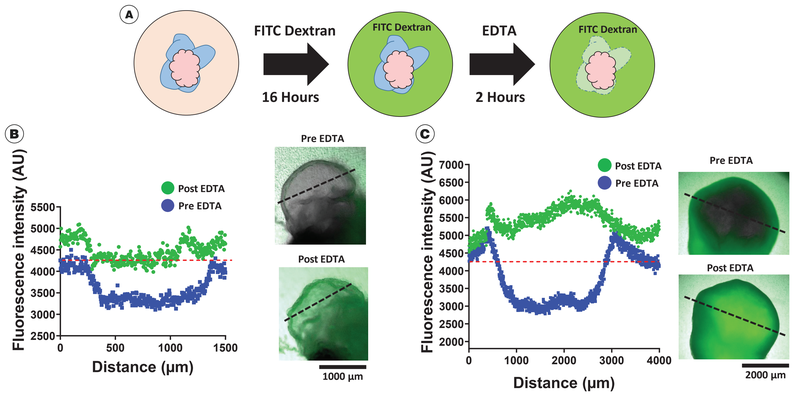

FIGURE 6. Assessment of Barrier Integrity Using FITC-Dextran

(A) Day 40 and 100 WLS-1C ChP organoids (n=1 per timepoint) were incubated with FITC-dextran in BrainPhys™ Imaging Optimized Medium for 16 hours, followed by treatment with EDTA for 2 hours. **(B)** Day 40 and **(C)** day 100 ChP organoids were imaged before and after EDTA treatment with fluorescence intensity measured over a cross section of a cyst (black dotted line). Red dotted line indicates baseline fluorescence in the medium with FITC-dextran alone.

Summary

- STEMdiff™ Choroid Plexus Differentiation Kit generates organoids that contain large fluid-filled cysts and express choroid plexus markers TTR, CLIC6, and AQP-1.
- CSF-like fluid extracted from ChP organoids contains Clusterin and IGF-2 proteins, as measured through western blot analyses.
- ChP organoid cysts form a barrier which is impermeable to low-molecular weight dextran.

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References
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- Masselink W, et al. Broad applicability of a streamlined ethyl cinnamate-based clearing procedure. Development. 2019 Feb 1;146(3):dev166884.