Establishment of 3D Neurosphere Cultures from Human iPSC-derived Neural Progenitor Cells

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Abstract

Human induced pluripotent stem cells (iPSCs)–derived neurospheres provide an advanced in vitro system for modeling the human brain. This system can be used to investigate areas of research focused on brain development, disease pathology, drug screening, and the toxic effects of environmental chemicals. Here, we use ATCC iPSC-derived neural progenitor cells (NPCs) to generate 3D neurospheres *in vitro*. We describe a straightforward method of generating neurospheres and further support their utilization for experimental applications such as dopaminergic differentiation and drug toxicity assays. We found that NPC-derived neurospheres grew exponentially and maintained their progenitor state for up to two weeks in culture. Further we found that neurospheres were able to successfully differentiate to multiple brain lineage cells including dopaminergic neurons in 3D and displayed higher tyrosine hydroxylase (TH)-positive cells compared to NPCs in 2D cultures. Neurospheres from normal donor cells successfully differentiated and expressed the TH marker uniformly however, neurospheres from Parkinson's disease donor cells displayed different patterning post differentiation than the neurospheres from normal donor cells. Neurospheres treated with various chemotherapeutic agents in multiple doses gave differential responses between healthy and disease cells. Viability of healthy neurospheres were significantly affected by paclitaxel and vincristine at all three dosages as compared to the control whereas no significant differences were seen for neurospheres from disease donor cells. Similar difference in sensitivity to paclitaxel and vincristine was observed in the two different NPCs. Interestingly, NPCs from healthy donors demonstrated greater sensitivity to paclitaxel and vincristine as compared to the neurospheres derived from the same cells. And this trend was also seen for NPCs and neurospheres from the diseased donor. Responses to amiodarone and chlorhexidine were found to be similar between both neurospheres from healthy and diseased. However, NPCs were slightly more sensitive to amiodarone and chlorhexidine as compared to their neurosphere counterparts. These data demonstrate that iPSCs-derived neurospheres are a powerful tool for developmental studies, drug screening, and toxicity testing compared to 2D NPC cultures.

Results

Table 1. iPSC-derived cells and media used in this study

ATCC® No.	Designation	Comments
ACS-5003™	Neural Progenitor Cell; Normal	Derived from ATCC-BXS0117 Human iPSC (ACS-1031™)
ACS-5001™	Neural Progenitor Cells; Parkinson's Disease	Derived from ATCC-DYS0530 Human iPSC (ACS-1013™)
ACS-3003™	Growth Kit for Neural Progenitor Cell Expansion	Specially designed for the expansion of NPCs; add to DMEM: F-12
ACS-3004™	Neural Progenitor Cell Dopaminergic Differentiation Kit	Specially designed for the dopaminergic differentiation of NPCs; add to DMEM: F-12
30-2006™	DMEM: F-12 Medium	Base medium
ACS-3035™	Cell Basement Membrane Gel	Feeder-free substrate for culture of NPCs and neurons

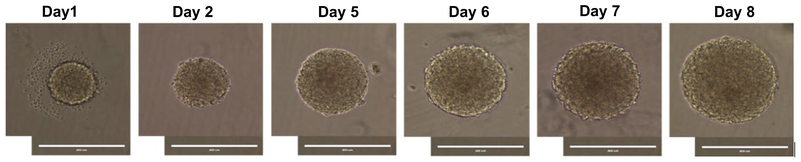

Figure 1. ACS-5003 neurosphere growth in culture. ACS-5003 NPCs seeded at 2000 cells/well. Day 1 represents 24 hours after initial plating of NPCs in U shaped ultra low attachment (ULA) 96 well culture vessels in NPC expansion medium. Scale bar 400µm.

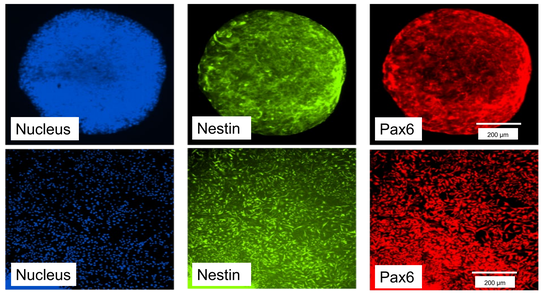

Figure 2. ACS-5003 neurosphere and 2D NPC cultures derived from 3D neurospheres maintain NPC-specific markers. Top panel shows sectioned day 14 ACS-5003 neurosphere with individual channels representing nucleus in blue, Nestin in green, and Pax6 in red. Bottom panel shows 2D ACS-5003 culture with individual channels representing nucleus in blue, Nestin in green, and Pax6 in red.

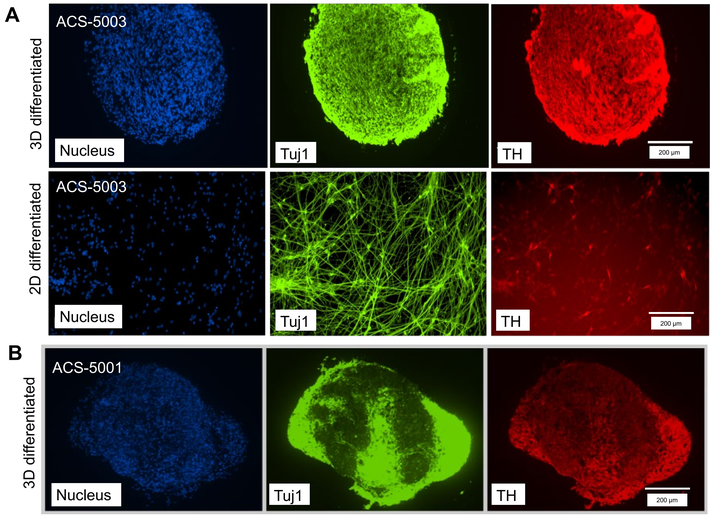

Figure 3. A) ACS-5003 neurospheres successfully differentiate in 3D and show higher TH positivity compared to 2D ACS-5003 culture in dopaminergic differentiation medium (ACS-3004™). Top panel shows day 21 culture of sectioned ACS-5003 dopaminergic differentiated neurosphere and bottom panel shows 2D ACS-5003 culture with individual channels representing nucleus in blue, Tuj1 in green, and TH in red. **B) ACS-5001 neurospheres from Parkinson's disease donor cells displayed different patterning after dopaminergic differentiation.** Individual channels representing nucleus in blue, Tuj1 in green, and TH in red.

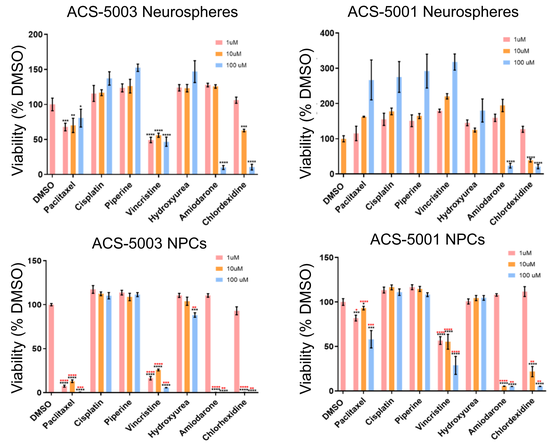

Figure 4. ACS-5003 and ACS-5001 neurospheres showed differential response to various chemotherapeutic drug treatments and these responses varied with their 2D NPC counterparts. Top panel shows drug toxicity responses for ACS-5003 and ACS-5001 neurospheres, 24-hour post-drug exposure. Bottom panel shows drug toxicity responses for ACS-5003 and ACS-5001 NPCs, 24-hour post-drug exposure. Cell viability was assayed using resazurin reduction assay. X-axis represents the drugs used for the assay, and Y-axis represents change in percentage compared to DMSO control. Colored bars represent drug dosages.

Summary

- ATCC Neural Progenitor Cells can convert to neurospheres with 100% efficiency by using ultra-low attachment culture vessels and NPC expansion medium.
- NPC-derived neurospheres formed solid circular spheres without the formation of any hollow cavities and maintained their non-differentiated state for more than 2 weeks.
- 3D NPC neurospheres can be converted to 2D NPCs with out losing marker expression.
- Normal NPC neurospheres differentiated into dopaminergic neurons had more tyrosine hydroxylase (TH) positivity as compared to the differentiated 2D monolayer culture.
- 3D neurospheres are less sensitive to chemotherapeutic drugs compared to 2D NPC cultures. For example, NPCs in 2D culture were more sensitive to paclitaxel than 3D neurospheres at all three doses tested.
- Normal neurospheres were sensitive to vincristine but Parkinson's neurospheres were not.
- Here we have demonstrated that human neural progenitor cells can be cultured as 3D neurospheres while maintaining critical markers, exhibit enhanced differentiation to dopaminergic neurons and are amenable to drug toxicity studies using standard cell viability assays.

References

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