Generation of human cortical organoids

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Organisation

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Country Belgium

Geographical Area Flemish Region

SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Basic Research, Translational - Applied Research
Type of method	In vitro - Ex vivo
Specify the type of cells/tissues/organs	Induced Pluripotent Stem Cells/Embryonic Stem Cells

DESCRIPTION

Method keywords

HUman brain organoids

Cortex

Embryonic development

human embryonic stem cell derived organoid model

Scientific area keywords

Developmental neurobiology

neurobiology

neurodevelopmental disorders

3D organoid models

Microcephaly

Method description

This method generates cortical brain organoids from embryonic stem cells or induced pluripotent stem cells. The protocol involves neural induction of 3D aggregates (embryoid bodies) which are then directed towards the cortical lineage in the presence of small molecules. SMAD inhibition (dorsomorphin + SB-431542) will result in neuronalization, while prolonged exposure to EGF/FGF-2 allows proliferation and corticogenesis progression. Subsequent maturation occurs in the presence of NT-3. The cortical organoids can be kept in culture for extended periods of time and will eventually develop additional differentiated neuronal (including interneurons) and glial (e.g. astrocytes) cell types. These organoids are particularly useful to study neurodevelopment and neurodevelopmental diseases. The protocol was first published by Sloan et al., Nat Protoc 2018. In our slightly adapted protocol we use Aggrewell 800 plates to generate spheroids that are more homogeneous in size compared to the original protocol.

Lab equipment

- Laminar flow,

- Centrifuge,

- Incubator,
- Aggrewell 800 plates,
- General cell culture equipment.

Method status

Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

- The protocol is fairly easy and the use of Aggrewell plates ensures the generation of large batches of organoids with minimal variability in size.
- Organoids can be kept in culture for extended periods of time (years).
- These organoids mimic important aspects of human fetal corticogenesis.

Challenges

- Although the majority of cell types are present, correct layering is lost at later stages of organoid development.
- The extended culture has the disadvantage that it takes a long time before organoids reach certain stages of development.
- As organoids get larger, they have a tendency to fuse.

Modifications

We are currently working on cryopreservation protocols to allow the generation of batches of organoids of specific developmental stages.

Future & Other applications

We are currently developing assembloid models for glioblastoma research.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

Sloan SA, Andersen J, Pa?ca AM, Birey F, Pa?ca SP. Generation and assembly of human brain region-specific three-dimensional cultures. Nat Protoc. 2018 Sep;13(9):2062-2085. doi: 10.1038/s41596-018-0032-7.

Ribeiro JH, Etlioglu E, Buset J, Janssen A, Puype H, Berden L, Mbouombouo Mfossa AC, De Vos WH, Vermeirssen V, Baatout S, Rajan N, Quintens R. A human-specific, concerted repression of microcephaly genes contributes to radiation-induced growth defects in cortical organoids. iScience. 2025 Jan 20;28(2):111853. doi: 10.1016/j.isci.2025.111853.

Associated documents

Ribeiro, iScience 2025 - A human-specific, concerted repression of MCPH genes contributes to radiation-induced growth defects in cortical organoids.pdf

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