3D Organoids from primary melanoma cell lines and from iPSc-derived neural crest stem cells

Created on: 18-02-2020 - Last modified on: 25-02-2020

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Organisation

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Department Department of Brain and Disease Rearch
Specific Research Group or Service Lab of Computational Biology
Country Belgium
Geographical Area Flemish Region

SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Basic Research
Type of method	In vitro - Ex vivo
Specify the type of cells/tissues/organs	Human iPSC; Primary melanoma cells

DESCRIPTION

Method keywords

3D culture
organoid
single-cell
ECM
hydrogel
PEG

Scientific area keywords

melanoma
enhancer
single-cell RNA seq
single-cell ATAC seq

Method description

We propose to generate three-dimensional tumoroids from the primary melanoma cell lines, as well as 3D organoids from the iPSc-derived neural crest stem cells. We will use the AggreWell system (STEMCELL Technologies) to generate uniform, size-controlled

three-dimensional spheroids. After 5 days in the AggreWell plate, the spheroids are moved to a PEG-based artificial ECM hydrogel (Gjorevski et al.; Nature Protocols 2017). The organoids can be cultured for weeks in these PEG-droplets. At different time points during organoid culture, organoids will be used for immunostaining and/or for single-cell sequencing. We will dissociate the PEG gel to obtain single cells by use of the cell-dissociation enzyme TrypLE.

Lab equipment

Biosafety cabinet ;
Cell incubator CO2-connected ;
Centrifuge for plates.

Method status

Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

3D organoids mimic tissue architecture heterogenous cell culture to study cellular differentiation enhancer testing.

Challenges

Not every cell type/tissue can be studied.

Modifications

Different cell types are studied to form organoids.

Future & Other applications

Drug application: concentration and activity can be tested.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

Gjorevski N & Lutolf MP. Synthesis and characterization of well-defined hydrogel matrices and their application to intestinal stem cell and organoid culture. Nature Protocols 12 (11); 2263-2274 (2017)

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Financed by