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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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)