A mouse mammary gland organoid protocol to mimic breast morphology in vitro

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Contact person

Beaunelle de Bruijn

Organisation

Name of the organisation Oncology - KU Leuven

Department Oncology

Country Belgium

SCOPE OF THE METHOD

The Method relates to	Animal health
The Method is situated in	Basic Research
Type of method	In vitro - Ex vivo
Species from which cells/tissues/organs are derived	mouse
Type of cells/tissues/organs	mammary gland

DESCRIPTION

Method keywords

Mouse mammary gland organoids

primary material

branched morphology

ECM composition

growth factor supplementation
tumor initiation
pubertal development

Scientific area keywords

Developmental biology
Oncology
3D organoid models
stem cell biology

Method description

The protocol is aimed at developing primary mammary gland organoids that have a morphology similar to the one of the *in vivo* breast, which is organized as a complex network of interconnected branches. The organoids are derived from the mouse mammary gland by mechanical dissociation and enzymatic digestion of the tissue to obtain small mammary tissue fragments that spontaneously organize in sphere-shaped organoids. The sphere-shaped organoids are then transferred in mixed Basement Membrane Extract (BME) and collagen gels, supplemented with growth factors to induce an elongated and branched morphology. By combination of the right ECM stiffness (mixed collagen: Matrigel gels) and growth factor supplementation, we managed to obtain complex organoids, up to 1.2 mm in length and with branches up to the 5th level. This will allow to study how the branching process works in branched organs in our body with reduced use of animal models. Also, mutations can be introduced in the model, which can be used to study tumor initiation and progression.

Method status

History of use
Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

- Organoids are derived from primary material so they are not transformed,
- They show a complex morphology similar to the *in vivo* gland,

- Relatively fast procedures,
- No need of special equipment.

Challenges

- Relatively low throughput,
- Technically challenging,
- Long culture time (15-20 days),
- Primary material cannot be expanded indefinitely so it still requires use of animals.

Modifications

We are currently testing additional factors on the organoids to mimic *in vitro* the remodeling stages of the adult breast during pregnancy, lactation and involution upon weaning to have a model that can reduce the use of animals to study these developmental processes.

Future & Other applications

The model can be used to study the impact of breast remodeling on tumor predisposition. The concept of modulating the matrix stiffness and providing a growth factor alternation may apply to induce branching also in organoid models of other branched organs.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

Caruso M, Huang S, Mourao L, Scheele CLGJ. A Mammary Organoid Model to Study Branching Morphogenesis. Front Physiol. 2022 Mar 16;13:826107. doi: 10.3389/fphys.2022.826107. PMID: 35399282; PMCID: PMC8988230.
Caruso M, Saberiseyedabad K., Mourao L, Scheele CLGJ. A guide towards 3D mammary and breast organotypic cultures. Methods Mol Biol. 2023, Springer Nature (submitted).

Associated documents

fphys-13-826107.pdf

Links

A Mammary Organoid Model to Study Branching Morphogenesis.

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