3D Lung Tumor Spheroids for Oncoimmunological Assays

Commonly used acronym: *Lung tumor spheroids*
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SCOPE OF THE METHOD

The Method relates to	Animal health, Human health
The Method is situated in	Basic Research, Translational - Applied Research
Type of method	In vitro - Ex vivo
This method makes use of	Human derived cells / tissues / organs
Specify the type of cells/tissues/organs	murine lung cancer and fibroblast cell lines or human lung cancer and fibroblast cell lines

DESCRIPTION

Method keywords

lung cancer spheroids

mammary cells

tumour

mice
macrophage polarization
human cell-based model
fluorescence microscope
3D in vitro model
lentiviral reprogramming
immune cell migration
immunology
Immunotherapy
antigen specific T cell killing
cancer-associated fibroblasts
histocompatible

Scientific area keywords

Immunology
Lung cancer
T cell immunity
macrophage polarization
spheroids
immune checkpoint inhibitors
non-small cell lung cancer
tumor stroma
cancer-associated fibroblasts
immunotherapy

Method description

Lung cancer thrives in a complex multicellular tumor microenvironment (TME) that impacts tumor growth, metastasis, response, and resistance to therapy. While orthotopic murine lung cancer models can partly recapitulate this complexity, they do not resonate with high-throughput immunotherapeutic drug screening assays. To

address the current need for relevant and easy-to-use lung tumor models, a protocol is established to generate and evaluate fully histocompatible murine and human lung tumor spheroids, generated by co-culturing lung fibroblasts with tumor cells in ultra-low adherence 96-well plates. A spheroid generation protocol with the murine KrasG12D-p53ko (KP) and Lewis Lung Carcinoma (LLC) cell lines is delivered next to the human lung H1650 adenocarcinoma line. In addition, their application potential to study tumor-stroma organization, T-cell motility, and infiltration as well as distinct macrophage subsets' behavior using confocal microscopy is described. Finally, a 3D target-specific T-cell killing assay that allows spatiotemporal assessment of different tumor to T-cell ratios and immune checkpoint blockade regimens using flow cytometry and live cell imaging is described. This 3D lung tumor spheroid platform can serve as a blueprint for other solid cancer types to comply with the need for straightforward murine and human oncoimmunology assays.

Lab equipment

For generation:
- Laminar air flow;
- Ultra-low adherence (ULA) or cell-repellent U-shaped 96-well plates;
- Biosafety level 2 facility for lentiviral vector production and handling of patient-derived T cells;
- Irradiator for stimulation of T cell feeder cells.

For evaluation:
- Flow cytometry;
- Microscopy (confocal, incucyte technology,…);
- NGS methods,…

Method status

Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

- Straightforward murine and human multicellular lung tumor spheroid platform that recapitulates the characteristic tumor islet in stroma architecture found in human non-small lung cancer biopsies;
- Platform suitable for macrophage and T-cell infiltration studies;
- Platform suitable for target tumor cell-specific killing evaluation in framework of fundamental and translational immunotherapy research;
- In 96-well format for evaluation on conventional imaging instruments;
- Possibility to perform high-throughput spatiotemporal gene, cell, and/or drug screening;
- Can serve as a blueprint for other solid cancer types to comply with the need for straightforward murine and human oncoimmunology assays.

Challenges

- Generation or access to histocompatible fluorescently-labelled tumor cell lines and tissue-specific fibroblasts;
- Access to histocompatible target specific T cell clones and/or myeloid cells;
- Not every lung tumor cell line is suitable for generation of lung tumor spheroids with relevant tumor-stroma islet architecture.

Future & Other applications

Our 3D lung tumor spheroids can serve as a blueprint for other solid cancer types to comply with the need for straightforward murine and human oncoimmunology assays. Furthermore, we are currently expanding our expertise by optimizing the generation of lung cancer patient-derived organotypic cultures.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

Associated documents

De Ridder K and Tung N et al_Novel3Dlungtumorspheroids_AdvNanbioRes_2021.pdf

Links

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