13C Tracer Analysis and Metabolomics in 3D Cultured Cancer Cells

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Contact person

Dorien Broekaert

Organisation

Name of the organisation VIB - KU Leuven

Department Center for Cancer Biology - Oncology

Country Belgium

Name of the organisation Katholieke Universiteit Leuven (KUL)

Department Department of Oncology

Country Belgium

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 cancer cell lines

DESCRIPTION

Method keywords

3D Cell culture

Cancer cells

Metabolism

Metabolic quenching
spheroids
metabolomics

Scientific area keywords

Cancer metabolism
13C tracer analysis
3D
spheroids
extracellular matrix
cancer
metastasis

Method description

Metabolomics and 13C tracer analysis are state-of-the-art techniques that allow determining the concentration of metabolites and the activity of metabolic pathways, respectively. Three dimensional (3D) cultures of cancer cells constitute an enriched *in vitro* environment that can be used to assay anchorage-independent growth, spheroid formation, and extracellular matrix production by (cancer) cells. Here, we describe how to perform metabolomics and 13C tracer analysis in 3D cultures of cancer cells. Intracellular metabolites are extracted from these quenched cells, and 13C-label incorporation patterns and metabolite levels are determined via MS-based analysis.

Lab equipment

- Humidified temperature and CO2-controlled cell culture incubator ;
- Vacuum aspirator ;
- Biological safety cabinet ;
- Cell counter ;
- Thermometer (-40°C to 0°C) ;
- Chemical fume hood ;
- Tissue Lyser ;
- Heating block ;
- Centrifuge fitting 15 and 50 mL tubes ;
- Vortex ;

- Refrigerated centrifugal vacuum concentrator ;
- Mass Spectrometer with gas or liquid chromatographic technique.

Method status

Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

A key feature of the method lies in the possibility to study metabolism in cells growing in an anchorage-independent manner which might be more representative of the physiological status of metastatic cancer cells. This allows us to recapitulate some cancer phenotypes of disseminated cancer cells during the metastatic process. In addition, the described quenching process enables to rapidly stop the metabolism of cancer cell spheroids as well as to measure the concentration and labeling patterns of metabolites in a reliable manner.

Challenges

To obtain accurate metabolic measurements you need to have an adequate amount of cells to extract. When growing cells in this system, the number of spheroids is limited per well to avoid a group of cells to grow as a single spheroid (which will not recapitulate the metabolism of colonizing cells). Therefore, it is necessary to optimize the number of wells to pool together according to cell type and condition to test to achieve the proper amount of cell extracts.
Metabolic quenching of spheroids that grow in suspension is a more time-consuming protocol compared to the quenching of attached cells. Since metabolism is a rapidly adapting process, it is critical to work as fast as possible and maintain low temperature during the process to minimize the chance of perturbations in cellular metabolism.

Modifications

This protocol can be adapted for metabolic measurements of alternative systems of cells growing in suspension.

Future & Other applications

This protocol can be adapted for metabolic measurements of alternative systems of cells growing in suspension.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

Links

13C Tracer Analysis and Metabolomics in 3D Cultured Cancer Cells

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