Cancer cell invasion has been traditionally studied in three-dimensional (3D) models composed of rat or mouse extracellul ar matrix (ECM) proteins such as type collagen and laminin (1). Uterine leiomyoma (myoma) is a benign smooth muscle neoplasm which provides an optimal microenvironment for studying cancer cell invasion and metastasis (2). We have previously characterized a novel fully human organotypic model where HSC-3 tongue squamous carcinoma cells were cultured on myoma tissue (3).

To further characterize the myoma model with human breast and prostate cancer cell lines, reference compounds and more advanced analysis methods

Materials and Methods

Myoma selection criteria included size, location (intramural/submucous), medication (hormonal treatment), menstrual cycle, age of donor (fertile/menopausal/postmenopausal). The myoma tissues were provided after informed consent of the patients from routine surgery at the Department of Obstetrics and Gynaecology, Oulu University Hospital. The study was performed using discs from the same dissected myoma.

Human MDA-MB-231(SA) breast cancer cells, PC-3 prostate adenocarcinoma cells and HSC-3 tongue squamous carcinoma cells were added on top of macroscopically heterogeneous 8-mm myoma biopsy discs. The cells were attached overnight and the myoma discs were transferred onto nylon discs resting on curved steel grids in 24-well plates with sufficient volume of media as described earlier (2). After 14 days of culture, the myomae were fixed and embedded in paraffin and sectioned. The myoma sections were stained with Hematoxylin and Eosin and analyzed microscopically.

Deepwell invasion and total cell area were determined from the images using the MetaMorph image analysis software (Molecular Devices, LLC. Sunnyvale, CA). Each myoma disc with 120 deepest invasion and 24 total cell areas determined in each group. Culture medium was exchanged every 3 days, and every 2 days the disc was fixed, stained, and examined. Carboxyterminal telopeptide of type I collagen (ICTP) was analyzed from media samples using the Leu ICTP EIA kit (CisBio International, Denmark). Matrix metalloproteinase inhibitor 1-10-phenanthroline (1, 10 and 100 µmL, in cell culture media) was used as a reference compound of invasion inhibition.

Organotypic Invasion Model

Deepwell invasion and total cell area

A) Deepwell invasion (µm) on Day 14

B) Summary

- MDA-MB-231(SA) breast cancer cells, PC-3 prostate cancer cells and HSC-3 tongue carcinoma cells were highly invasive in the myoma tissue
- Treatment with the matrix metalloproteinase inhibitor 1,10-phenanthroline inhibited the deepest invasion and total cell area of cancer cells as measured by histomorphometry
- Type 1 collagen degradation as measured by medium ICTP levels was decreased in the 1,10-phenanthroline treated groups

C) Figure 1: Schematic overview of the organotypic 3D invasion platform. Cancer cell invasion of the myoma tissue in 3D is shown in red. The invasion depth (µm) for each cell line was measured by manual counting of invading cells from multiple sections. The invasion depth was measured as the distance from the top of the invader to the bottom of the myoma tissue.

D) Figure 2: The effects of 1,10-phenanthroline (PHEN) treatment on cancer cell invasion. A) Deepwell invasion (µm) on Day 14. B) Total cell area (µm²) on Day 14. C) ICTP released from the myoma tissue incubated with different concentrations of PHEN. D) Images show that the treated myoma tissue samples are invaded by cancer cells. E) The invasion depth, total cell area and ICTP values in A) and B) are shown as box plots (median, 1st and 3rd quartiles). Outliers are marked as floating points in the figure but they were not retrieved in the statistical analysis. Statistical analysis was performed using R and followed by Tukey’s post hoc test to pairwise comparison between the vehicle and treatment group. *p<0.05 and ***p<0.001 compared to vehicle group.

References

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Culturing Cancer Cells on Myoma Tissue: Development of a Novel Fully Human Organotypic 3D Invasion Model Applicable as a Preclinical Tool for Cancer Drug Development

Jukka P. Rissanen1, Katja M. Fagerlund1, Johanna M. Tuomela1, Mari I. Suominen1, Jussi M. Halleen1, Tuula A. Salo2.
1Pharmatest Services Ltd, Turku, Finland
2University of Oulu and Oulu University Hospital, Institute of Dentistry, Oulu, Finland

Summary

Our results demonstrate that the established fully human organotypic 3D model is a reliable tool for demonstrating the efficacy of known reference compounds capable of inhibiting cancer cell invasion and metastasis. We conclude that the myoma model has the potential to be developed as a clinically predictive large scale method for identifying novel cancer drug candidates and confirming their efficacy in early preclinical phase of cancer drug development.

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