Introduction

Cancer cell invasion has been traditionally studied in three-dimensional (3D) models composed of rat or mouse extracellular matrix (ECM) proteins such as type I collagen and laminin (1). Uterine leiomyoma (myoma) is a benign smooth muscle neoplasm which has authentic human ECM components and hypoxic surrounding providing an excellent human tumor microenvironment mimic for studying cancer cell invasion and metastasis (2,3). We have previously characterized a novel fully human organotypic model where HSC-3 tongue squamous carcinoma cells were cultured on myoma tissue (4).

Aim of the Study

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, subserous), (hormonal treatment), menstrual cycle, age of donor (fertile/menopausal/post-menopausal). The myomas were obtained 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 adenocarcinoma 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 myomas were fixed and included in paraffin and sectioned. The myoma sections were stained with Hematoxylin and Eosin and analyzed microscopically.

Deepest invasion and total cell area were determined from the images using the MetaMorph image analysis software (Molecular Devices, LLC, Sunnyvale, CA), with 120 deepest invasion and 24 total cell areas determined in each group. Culture medium was collected at day 14 and also during the media change. Carboxyterminal telopeptide of type I collagen (ICTP) was analyzed from media samples using the UniQ ICTP EIA kit (Orion Diagnostica, Espoo, Finland). Matrix metalloproteinase inhibitor 1,10-phenanthroline (1, 10 and 100 g/ml in cell culture media) was used as a reference compound of invasion inhibition.

Organotypic Invasion Model

![Figure 1: Schematic overview of the organotypic 3D invasion platform. Tumor cells grown on top of the myoma tissue were allowed to invade into the tissue. The myomas were collected, fixed, embedded to paraffin and sectioned. Slices were stained with Hematoxylin and Eosin and invasion of cells was determined histomorphometrically. Proliferated noninvasive cell layer and any artificial invasion were excluded from the deepest invasion analysis. The difference in invasion between treatment groups was quantified as deepest invasion and total cell area (including proliferated and invaded cells).](Image)

![Figure 2: The effects of 1,10-phenanthroline (PHEN) treatment on cancer cell invasion. A) Deepest invasion (mm); B) Total cell area (mm²); C) ICTP released from myoma tissue into the culture medium at day 14 (µg/L); D) Representative Hematoxylin and Eosin stainings. The invasion depth; total cell area and ICTP values in A), B) and C) are shown as boxplot figures (median IQR50%/min/max). Outliers are marked as floating points in the figure but they were not removed in the statistical analysis. Statistical analysis was performed using ANOVA followed by Tukey’s post hoc test for pairwise comparison between the vehicle and treatment group. *p<0.05 and ***p<0.001 compared to vehicle group.](Image)

Summary

- MDA-MB-231(SA) breast cancer cells, PC-3 prostate cancer cells and HSC-3 tongue cancer 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 I collagen degradation as measured by medium ICTP levels was decreased in the 1,10-phenanthroline treated groups

Conclusions

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.

Acknowledgements

We thank Prof. Theresa Guise for generously providing the MDA-MB-231(SA) cell line, and Riika Käyhämaa, Esa Alhoniemi and Natalia Hablaining-Kirillov for their skilful technical assistance.

References