Characterization and Pharmacological Intervention of Tumor-Microenvironmental Factors and Cancer Cell Growth in Co-Cultures of Fresh Human Lung Tissue and Patient-Derived, Disseminated Cancer Cells

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Introduction

Metastasis is being listed as the main cause of death in most cancers. Its complexity makes a challenge for most preclinical models as metastatic processes within human organotypic tissue are not reflected sufficiently. Human organotypic ex vivo models and their combination of predictivity and economic procedures attempt to remedy this difficulty.

Here, tumor growth is modulated within its natural microenvironment by adding cancer cells to human living Precision-Cut Lung Slices (PCLS).

Materials & Methods

**PCLS Preparation**
- AdGFP virus infection of cancer cells
- Seeding of AdGFP MDA-MB-231 melanoma DTC cells on PCLS

**Viability Assays**
- Cytokine-Measurements
- Three-dimensional Confocal microscopy

**Timeline**
- 0 hours
- 5 hours - 144 hours (Kinetic)

Results

Cancer cells adhere to the lung tissue and proliferate there. Using confocal microscopy, the kinetics of the cancer cell growth can be investigated as depicted in Fig. 2 (right). Cancer cells integrate into the lung tissue and increase 6-fold within the first day of culture. Cells decrease until day 3 but recover after 5 days (5.4-fold increase). Macrophages infiltrate melanoma accumulations [19-fold increase] and interact with selected cells. Proliferating cancer cells can be shown through GFP-Ki67 co-localization. (Fig. 2, left).

Extrinsic VEGF levels were increased 3.5fold in cancer cell-invaded PCLS and 5.4fold in tumor slices after 48h. Invasion of endothelial cells into the supranatant of these samples was increased 5.7fold in supernatants of cancer cell-invaded tissue and 6.1fold in tumor slices. Treatment with bevacizumab suppressed VEGF-release up to 24fold in cancer cell-invaded tissue and up to 50fold in tumor slices, using the highest concentration of 200 µg/mL after 48h (Fig. 4). Supernatants of these treatments showed impaired invasion of endothelial cells by up to 81% in cancer-cell invaded tissue and up to 83% in tumor slices.

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Cisplatin treatment [50µM] led to a decline of viability and reduced cancer cell number in PCLS by up to 37.5% and in tumor slices up to 48.7% after 72h. Cancer cell-invaded PCLS and tumor slices were less sensitive in regards to their respective anti-cancer drug efficacy than the 2D culture while no effect was seen on control tissue. (Fig. 3)

Figure 1: Timeline for the experimental procedure of the human organotypic cancer model.

Figure 2: Cancer growth on fresh human lung tissue. Right: Immuno-staining of proliferation marker Ki67 shows proliferating cells through co-localization after 24 hours. Left: Detection and counting of disseminated melanoma cells over the course of the experiment through GFP intensity. Confocal microscope stacks were used to count cancer cells within the lung tissue.

Figure 3: Anti-cancer effects of cisplatin in tumor slices, cancer cell invaded tissue slices and control tissue after 48h.

Right, top: Treatment with Cisplatin reduces the number of vital cancer cell within the tissue significantly after 72h, detected through cell tracker fluorescence in the lysates of cancer cell invaded PCLS. Right, bottom: Extrinsic LDH activity was measured to determine tissue viability. Left: Cisplatin treatment leads to morphological changes of MDA-MB-231 cells within human tissue and to a decreased cell number. Statistical significance are indicated by ***p<0.001. N=3 in duplicates, human PCLS.

Figure 4: Effects of bevacizumab on extrinsic VEGF in tumor slices, cancer cell invaded tissue slices and control tissue after 48h. Treatment with bevacizumab leads to significant decreased VEGF in both slices directly from tumor tissue as well as the cancer cell invaded slices. Statistical significance are indicated by *p<0.05 and **p<0.001. N=3 in duplicates, human PCLS.

To address patient-specific genetic disparities, co-cultures of lung tissue and disseminated melanoma cells were performed with both patient-derived cells that carried the driver mutation V600E of the BRAF gene and non-mutated melanoma cells. Treatment with vemurafenib [50µM], which interrupts the B-Raf/MEK/ERK pathway in V600E mutants resulting in programmed cell death, led to a 71% decrease of V600E cancer cells after 48 hours whereas non-mutated cells showed no significant cancer cell decrease (Fig. 5).

Figure 5: Patient-specific effects of Vemurafenib.

Left: Treatment with Vemurafenib reduces the number of vital cancer cell within the tissue after 48h, detected through GFP fluorescence in the lysates of cancer cell invaded PCLS. Right: Through sigmoidal curve fit of the dose response of the mutated melanoma cells towards Vemurafenib treatment, an IC50 value of 6.02 µM could be calculated. GFP-labeled, disseminated melanoma cells are thus more resistant towards Vemurafenib treatment in 3D ex vivo culture than they are in 2D cell culture (IC50: 0.88 µM). N=3 in duplicates, human PCLS.