

Material and Methods: Tumour tissue slices from colorectal cancer and breast cancer patients were prepared and cultured according to Individuum's standard operating protocols. For antibody diffusion assays tissue slices were incubated with different commercially available antibodies followed by immunofluorescence staining and microscopy. Functional effects of Trastuzumab treatment were examined in breast cancer cell lines (BT474 and MCF-7 cells) and breast cancer tissue slices using the expression level of pAkt as readout, shown by western blotting. Her-2 expression levels of cells and tissue slices were analyzed by immunohistochemistry.

Results: The antibody diffusion assays showed a time-dependent penetration of antibodies into and through 400 µm thick cultured tissue slices within 24 hours. Treatment of breast cancer cell lines and breast cancer tissue slices with different concentrations of Trastuzumab revealed a dose-dependent reduction of pAkt only in Her-2 positive cells and tissues. Thus, drug effects of therapeutic antibody Trastuzumab could be demonstrated in secondary cell lines and verified in organoid cultures.

Conclusions: Overall the data revealed that antibodies diffuse into 400 µm thick cultured tissue slices reaching their target within 24 hours. Furthermore, we demonstrated that functional drug effects of therapeutic antibodies could be validated in organoid cultures. Therefore, the preclinical model based on cultured cancer tissue slices developed by Individuum is suitable to examine not only the effects of classical chemotherapeutics, as we have shown recently, but also of larger molecules, such as antibodies. Thus, this model representing the natural tumour environment is a promising and important tool to prioritise drugs, support dose finding and to individualise therapy.

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POSTER

Frequency of Mitochondrial Point Mutations and Deletions in Late Stage Colorectal Cancer Patients

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Objective & Background: Defects in mitochondrial function have been proposed to contribute to progression of cancer. This dysfunction may result from impairment in cellular respiration, forcing the cell to revert to glycolysis for energy. This switch causes cells to become undifferentiated and cancerous. This study examined the frequency of random mitochondrial mutations and deletions in late stage colorectal cancer tumours and their corresponding normal tissue. This analysis was performed using a novel random mutation capture assay which has the sensitivity to detect one mutation in one hundred million base pairs.

Methods: Mitochondrial DNA was extracted from 20 patients with late stage colon cancer and adjacent normal tissue. This DNA was and digested with TAQ 1α for 10 hours replacing the enzyme every hour. QPCR was performed across the TAQ1α restriction sites. The primer sequences used for identifying random mitochondrial mutations are 5'-ACAGTTTATGTAGCTTACCTCC-3' and 5'-TTGCTGCGTGCTTGATGCTTGT-3'. The primer sequences used to determine mitochondrial DNA copy number are 5'-ACAGTTTATGTAGCTTACCTCC-3' and 5'-TTGCTGCGTGCTTGATGCTTGT-3'. PCR was performed to assess single nucleotide polymorphisms. We will assess mutation frequency of TAQ1 restriction site (TCGA) located in the gene encoding the 12S rRNA subunit (bp 634-637). By flanking multiple TAQ1 restriction sites with a primer pair, the detection prevalence of the RMC assay can be skewed towards DNA deletions. As a result, the expected frequency of a PCR product due to mtDNA point mutations drops exponentially to 1×10^{-15} . In contrast, mtDNA deletions occur at a higher rate, and hence, every mutation detected with these primer pairs will be a deletion.

Results: A statistically significant increase in the frequency of deletions was detected in normal versus tumour tissue ($p = 0.021$), however levels of point mutations did not differ between tumour and normal.

Conclusions: The lower rate of deletions seen in tumour tissue versus adjacent normal may be explained by the Warburg theory where anaerobic metabolism is predominant in cancer. In contrast to normal cells, which generate energy by the oxidative phosphorylation, tumours and cancer cells generate energy through glycolysis which could result in lower levels of mitochondrial deletions in tumours.

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POSTER

A Therapeutic Sphingosine 1-phosphate Antibody Inhibits Intratumoral Hypoxia and Sensitizes to Standard Chemotherapy in a Preclinical Model of Prostate Cancer

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Background: Hypoxia promotes neovascularization, metastasis, growth and resistance to treatments. The activation of HIF-1α has been identified as the master mechanism of adaptation to hypoxia. We recently identified the sphingosine kinase 1/sphingosine 1-phosphate (SphK1/S1P) pathway as a new modulator of HIF-1α activity under hypoxia in multiple cancer cell models (Ader et al, Cancer Res, 2008). S1P elicits proliferation, survival, or angiogenesis, and is believed to exert most of its actions as a ligand for a family of specific GPCRs to elicit paracrine or autocrine signaling. We have suggested that inhibiting SphK1/S1P signaling, which is up-regulated under hypoxia, may help normalizing the tumour microenvironment and increase sensitivity to chemotherapy, in the broader concept of "normalization of tumour vessels" as tumour oxygenation is known to enhance response to chemotherapy (Ader et al., Cancer Res, 2009).

Methods: Quantitation of hypoxia and angiogenesis, and treatment efficacy using an orthotopic (o.t.) xenograft model of fluorescent HRPc cells.

Results: We provide *in vitro* evidence that inhibiting the S1P exogenous signaling, through pharmacological inhibition of its receptors or by taking advantage of a monoclonal antibody neutralizing S1P, blocks HIF-1α accumulation and its activity in prostate cancer cells under hypoxia. Second, using an o.t. model of prostate cancer, we show that an anti-S1P antibody inhibits intratumoral hypoxia, modifies vessel architecture and improves tumour perfusion within 5 days of treatment. Third, we demonstrate that an anti-S1P strategy sensitizes to docetaxel, the 'gold standard' treatment for HRPc. A 5-day anti-S1P antibody pretreatment markedly sensitizes to docetaxel in an o.t. PC-3/GFP model established in nude mice. The combination anti-S1P antibody together with docetaxel was not only accompanied by a smaller primary tumour volume compared to docetaxel alone, but also significantly reduced the occurrence and number of metastases.

Conclusions: These data establish the proof-of-concept that blocking the exogenous action of S1P reduces intratumoral hypoxia and sensitizes to chemotherapy in prostate cancer animal model.

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POSTER

The Impact of Ionizing Radiation on the Motility and Matrix Remodelling Properties of Carcinoma-associated Fibroblasts

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Purpose: Carcinoma-associated fibroblasts (CAFs) are key components of solid malignancies and play central roles in cancer sustainability. In this work we have investigated the invasive capacity and matrix remodelling properties of CAFs after ionizing radiation (IR).

Methods: CAFs were isolated from fresh lung tumour specimens from 14 different donors. Initially, radiation protocols were established by monitoring cellular viability at different doses of radiation. For further analyses, the migrative, invasive and adhesive capacities of CAFs were determined after a single dose of 18 Gy. Additionally, protein levels of secreted major matrix modulators represented by matrix metalloproteinases (MMPs)-1, -2, -3, -7, -8, -9, -13 and their endogenous inhibitors (TIMPs)-1, -2, -3, -4 were measured 4 to 6 days post-irradiation, as well as cell surface expression of various integrins.

Results: IR resulted in premature cellular senescence and caused moderate but significant inhibition of the proliferative, migrative and invasive capacity in CAFs. IR also promoted MMP-3 and inhibited MMP-1 appearance, whereas expression and activity of the gelatinases MMP-2 and MMP-9 were unchanged. Furthermore, the levels of TIMPs were not affected. Surface expression of integrins α2, β1, α5 was consistently enhanced.

Conclusions: Our data indicate that therapeutic doses of IR exert advantageous inhibitory effects on the proliferative, migratory and invasive capacity of lung CAFs, along with a prominent reduction of MMP-1 expression. However, the observed enhancement of MMP-3 could represent a negative outcome from radiation. Also, the altered surface expression of integrins in