**PO-306**

**TARGETING CD70-POSITIVE CANCER ASSOCIATED FIBROBLASTS TO TACKLE THE IMMUNE SUPPRESSIVE TUMOUR MICROENVIRONMENT IN COLORECTAL CANCER.**

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**Introduction**

Tumour progression and invasiveness are determined not only by the malignant cancer cells themselves but also by the surrounding tumour microenvironment, comprising of cancer-associated fibroblasts (CAFs). CAFs represent a heterogeneous population with both cancer-promoting and cancer-restraining actions, lacking specific markers to target them. Expression of the immune checkpoint CD70 is normally tightly regulated and limited to cells of the lymphoid lineage. Instead, tumours hijack CD70 to facilitate immune evasion by increasing the amount of suppressive regulatory T cells (Tregs), inducing T cell apoptosis and skewing T cells towards T cell exhaustion. In this study, we aimed at exploring the expression patterns of CD70 in colorectal cancer (CRC), not merely focusing on the tumour cells, but also taking the tumour microenvironment into account.

**Material and methods**

We have analysed the prognostic value of CD70 expression by immunohistochemistry in CRC specimens and studied its relationship with well-known fibroblast markers, microsatellite instability and Tregs. Furthermore, primary CAF cell lines were used to study the role of CD70 on migration and immune escape.

**Results and discussions**

We revealed prominent expression of CD70 on a specific subset of CAFs in invasive CRC specimens. Cancer cells show almost no expression of CD70. CD70+ CAFs proved to be an independent adverse prognostic marker. Functionally, CD70+ CAFs stimulated migration and significantly increased the frequency of naturally occurring Tregs. Finally, experiments aimed at therapeutically targeting these CD70-positive CAFs are currently being analysed using 2D and 3D models.

**Conclusion**

We have identified the expression of CD70 on CAFs as a novel prognostic marker for CRC. Performing this research, we found evidence of a cross talk between CD70+ CAFs and naturally occurring Tregs, paving the way towards immune escape. As such, this study provides a strong rationale for our ongoing exploration of CD70-targeting antibodies in CRC, especially in light of the limited immunotherapeutic options available in CRC.

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**PO-307**

**DIFFERENTIAL SECRETOME ANALYSIS OF CANCER-ASSOCIATED ADIPOCYTES (CAA) AND MATURE ADIPOCYTES TO IDENTIFY ADIPOCYTE-DRIVEN MICROENVIRONMENTAL REGULATORS OF BREAST CANCER PROGRESSION.**

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**Introduction**

In breast cancer, obesity is linked to invasive tumours that respond poorly to chemotherapy. We have explored relationships between breast cancer cells and adipocytes in the tumour microenvironment using a transwell coculture system. Human breast adipocytes induced chemo-resistance in both hormone receptor positive (MCF-7) and triple negative (MDA-MB-231) breast cancer cells. In addition, cancer-associated adipocytes (CAA) induced a partial epithelial-mesenchymal transition, creating a population of breast cancer cells that were highly migratory. In this study, we used discovery mass spectrometry and antibody arrays to analyse the proteins secreted by CAA to identify proteins that may contribute to breast cancer progression.

**Material and methods**

Breast adipose tissue samples were collected from patients undergoing breast surgery at Christchurch Hospital. Pre-adipocytes were isolated and differentiated into mature adipocytes, followed by co-culture with MCF-7 and MDA-MB-231 cancer cells for 3 days in a transwell co-culture system. Breast cancer cells were removed and adipocytes were washed and serum free media was added for 24 hours. This conditioned media was collected from pre-adipocytes, mature differentiated adipocytes and CAA co-cultured with either MCF-7 or MDA-MB-231 cells. Secretomes were compared using antibody arrays and iTRAQ labelling with LC-coupled LTQ-Orbitrap tandem mass spectrometry. Validation of iTRAQ results was performed using ELISA.

**Results and discussions**

Of the 327 proteins identified using iTRAQ-MS, 11.3% were intracellular and thus excluded; the remainder were either extracellular or secreted proteins. Of the 183 secreted proteins, 45% of these were secreted at higher levels by adipocytes after co-culture with breast cancer cells compared with adipocytes alone. These included proteins associated with inflammation and matrix remodelling. Chitinase 3-like 1 (Chi3L1) levels were increased according to both iTRAQ-MS and antibody array analysis. Recombinant Chi3L1 treatment (4 ng/ml) increased viability, proliferation, migration and resistance to chemotherapy in both breast cancer cell lines.

**Conclusion**

Our study describes for the first time, the secretome profile from CAA and provides a comprehensive platform for further research investigating local interactions between breast cancer cells and CAA. Our data identified CAA derived Chi3L1 as a key player in the interactions between adipocytes and cancer cells within the tumour microenvironment.

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**PO-308**

**HAF INHIBITION IN GLIOMA CELLS REDUCES TUMOUR GROWTH WITHOUT MODIFYING THE EFFICACY OF CHEMO- AND RADIOTHERAPY.**

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**Introduction**

Glioblastoma (GB) are known to be highly hypoxic and both hypoxia inducible factors, HIF-1 and HIF-2, have been implicated in their growth and resistance to treatments. Recently, it was proposed that a severe chronic hypoxia would enhance HIF-2 expression at the expense of HIF-1 through HAF (hypoxia-associated factor). HAF is expressed in many cancers including high and low grade glioma. However, its implication in GB growth and its treatments is poorly documented (Koh et al., 2011). In this context, we conducted in vivo and in vitro studies to identify whether HAF...
expressed by glioma cells modulates tumour growth and efficacy of GB conventional treatments, i.e. chemotherapy (temozolomide, TMZ) and radiotherapy (X-rays).

**Material and methods** Stable inhibition of HAF expression was established in human glioblastoma cells by RNA interference (U251shHAF). Orthotopic GB models were developed in mice (8/group) for U251shHAF and U251Sc cells (scrambled-shRNA infected cells) as control. Tumour development was assessed with 7T MRI (T2w sequence). At the end of the experiments, an immunohistology study was performed to characterise the vascularisation (PECAM), glial (GFAP) and inflammatory (CD68) reactions. In vitro, the radio- and chemosensitivity of U251shHAF were studied by clonogenic assay and cell cycle analysis following X-rays irradiation (X-RAD 225Cx) or TMZ exposition. Annexin-V binding and propidium iodure uptake followed by flow cytometry was used to quantify apoptotic and necrotic cells.

**Results and discussions** The stable inhibition of HAF expression in U251 cells leads to around 70% of its extinction in either normoxia or hypoxia (1% O2). Accordingly, the expression of VEGFA and CAIX, both known as HIF-1 and HIF-2 dependent genes, was decreased in U251shHAF cultured in hypoxia (1% O2) compared to U251Sc cells. Loss of function of HAF leads to a significant growth delay of U251shHAF tumours of 3 weeks compared to U251Sc tumours, although both tumours display similar vascularisation, glial and inflammation reactions.

In other hand, HAF silencing in glioma cells does not modify their sensitivity to X-rays or TMZ as suggested by the similar results obtained for both U251shHAF and U251Sc cells, through clonogenic assay, cell cycle and apoptosis analyses.

**Conclusion** Our results suggest that HAF might be of poor prognosis for GB since its inhibition in glioma cells reduces tumour growth without alleviating glioma cell chemoresistance.

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**Tumour Angiogenesis**

**PO-310** MIRNAS IN TUMOR-DERIVED MICROVESICLES PROMOTE ANGIOGENESIS AND TUMOUR CELL GROWTH

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**Introduction** Intercellular communication between cells and their microenvironment is important for tumour growth. Tumor-derived microvesicles (MVs) have recently received a great deal of attention because of their ability to induce an aggressive phenotype, immune escape, angiogenesis and drug resistance through the horizontal transfer of cellular macromolecules between cancer cells. We evaluated the role of miRNAs in tumor-derived MVs on angiogenesis and tumour growth in addition to searching for its target genes.

**Material and methods** MVs were isolated by ultracentrifugation in A549, H460 and BEAS2B cell lines. Candidate miRNAs in MVs were selected by miRNA array. RNA sequencing with barcodes for downstream quantitation on the NanoString nCounter technology.

**Results and discussions** Comparing colorectal tumours characterised by Microsatellite stable (MSS), DSP was able to differentiate immune hot and cold tumours despite MSS status. Since there is a subset of patients with MSS colorectal cancer that still responds to immunotherapy, this suggests DSP could ultimately be used to identify unique spatial biology and immune characteristics that might further expand beyond MSS and MSI status to help predict patients’ response to therapy.

**Conclusion** Using this novel approach, we demonstrate multiplexed protein analysis of defined micro-regions within a tumour enabling systematic interrogation of the immune microenvironment within the tumour. We demonstrate the ability of this technology to reveal immune biology that can point to novel biomarkers or therapeutic targets.

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**PO-309** CHARACTERISATION OF THE IMMUNE MICROENVIRONMENT OF COLORECTAL CANCER USING A NOVEL HIGH-PLEX PROTEIN ANALYSIS TECHNOLOGY

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**Introduction** Spatial characterisation of the immune microenvironment within tumours enables a better understanding of immunology and oncology. However, it has proven difficult to perform such studies in a highly multiplexed manner using limited samples. To address this unmet need, we have developed an imaging and tissue-sampling platform designed to simultaneously analyse up to hundreds of tumour and immune proteins in a single FFPE tissue section with spatial resolution. This novel technology, called Digital Spatial Profiling (DSP) was applied to the characterisation of the therapeutic response of colorectal cancer patients to immunotherapy.

**Material and methods** FFPE colorectal tumour specimens were subjected to DSP to determine the spatial expression of 30+immune related proteins. Following antigen retrieval, sections were stained with a cocktail of 30+DNA barcoded antibodies in combination with fluorescently labelled antibodies which were used to define the immune-enriched regions of the tumour. Using the fluorescent signal as a guide, regions of interest (ROI) were delineated followed by UV excitation of the defined ROI’s, which releases the antibody-bound DNA barcodes for downstream quantitation on the NanoString nCounter technology.

**Results and discussions** Comparing colorectal tumours characterised by Microsatellite stable (MSS), DSP was able to differentiate immune hot and cold tumours despite MSS status. Since there is a subset of patients with MSS colorectal cancer that still responds to immunotherapy, this suggests DSP could ultimately be used to identify unique spatial biology and immune characteristics that might further expand beyond MSS and MSI status to help predict patients' response to therapy.

**Conclusion** Using this novel approach, we demonstrate multiplexed protein analysis of defined micro-regions within a tumour enabling systematic interrogation of the immune microenvironment within the tumour. We demonstrate the ability of this technology to reveal immune biology that can point to novel biomarkers or therapeutic targets.