CANCER II

TIME: 11.30 – 12.30 LOCATION: BERWICK ROOM

INVESTIGATING THE CORRELATION BETWEEN MT1-MMP EXPRESSION AND THE INVASIVE CAPACITY OF MULTIPLE CANCER CELL TYPES

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Background: Membrane Type-1 Matrix Metalloproteinase (MT1-MMP) plays a central role in the degradation of the extracellular matrix (ECM), which is composed mainly of fibrillar type 1 collagen. In order for cancer cells to metastasise, they must first invade through the ECM. MT1-MMP has been shown to play a key role in this process and its overexpression has been associated with poor outcomes in multiple solid cancer types.
Aims: To determine the role of MT1-MMP in the invasive potential of multiple cancer cell types.
Methods: Sarcoma cell lines HT1080 and U2OS underwent MT1-MMP knockout (KO) using CRISPR Cas9. Knocked out populations were established from single cells and induced mutations were Sanger sequenced. The carcinoma cell lines MDA-MB-231, MCF-7, PC3 and LNCaP were also studied, and MT1-MMP expression levels across the panel of cell lines were established using western blot, qRT-PCR and flow cytometry. A 3D type 1 collagen gel assay was used to quantify cell invasion over 14 days using time-lapse microscopy.
Results: MT1-MMP was variably expressed across the panel of cell lines. MT1-MMP expression positively correlated with cancer cell invasion through 3D type 1 collagen and was associated with an invasive spindle-like phenotype. CRISPR KO of MT1-MMP significantly reduced ($P<0.0001$), but did not prevent, the invasion of HT1080 cells through 3D collagen. However, MT1-MMP KO completely abrogated the invasion of U2OS cells.
Conclusions: MT1-MMP is a key facilitator of cancer cell invasion through 3D type 1 collagen, and its KO significantly reduced the invasive potential of cancer cells.

GLUCOCORTICOID RECEPTOR REGULATED TRANSCRIPTION FACTOR NETWORKS IN TRIPLE NEGATIVE BREAST CANCER

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Triple negative breast cancer (TNBC) accounts for ~15% of breast cancer diagnoses. TNBCs do not express the estrogen receptor (ER), progesterone receptor (PR) or human epidermal growth factor 2 (HER2). These receptors are the major targets for endocrine and biological therapies, meaning TNBC patients are left with limited treatment options and a poor prognosis. To identify novel therapeutic options, it is important to understand the signalling pathways that drive tumour growth in TNBC.
The glucocorticoid receptor (GR) is a nuclear receptor related to ER and PR. Normally GR has anti-inflammatory and anti-tumorigenic roles. However, high expression of GR in TNBC is correlated with poor relapse-free survival, suggesting a functional switch in GR behaviour in these tumours. It is therefore hypothesised that modulation of GR-regulated signalling could provide a novel therapeutic option for patients with TNBC.
GR crosstalk between Hippo, HIF1A and NFκB signalling was evaluated by bioinformatic analysis. These pathways were selected as not only are they known to modulate GR but are also implicated in poor prognosis in TNBC. Gene set enrichment analysis (GSEA) was undertaken using publicly available ChIP-seq data sets for each of these pathways in TNBC. This analysis confirms links between each of the four pathways suggesting extensive crosstalk and common regulatory mechanisms. In vitro assays using TNBC cell lines

will now be used to investigate the cellular processes that are coregulated by these pathways as identified by GSEA, including proliferation, apoptosis and metabolism of lipids and carbohydrates to elucidate the mechanisms driving TNBC growth.

GLIOBLASTOMA STEM CELLS FORM RADIORESTISTANT NETWORK FOLLOWING CHEMICALLY INHIBITED ROCK

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Introduction: Glioblastoma multiforme (GBM) is the most aggressive and fatal primary brain tumour in adults. Despite a relatively well-defined treatment course of surgery with radiochemotherapy patients, high rates of relapse experienced by >90% of patients, and consequently tumours that are resistant to therapy, contribute to a poor prognosis of 12-15 months. Tumour cells and malignant multicellular networks have been separately implicated in the therapeutic resistance of GBM. We show that small molecule inhibition of Rho-associated serine/threonine kinase (ROCKi) significantly promotes the outgrowth of neurite-like cell projections in cultures of heterogeneous patient-derived GBM stem-like cells.
Methods: We assessed cellular behaviour through live cell imaging, immunocytochemistry, image analysis as well as live cell metabolic assays.
Results: The chemical inhibition of the ROCK pathway resulted in a reversible neurite-like outgrowth phenotype. We observe that cells form an interactive cellular network, communicating and exchanging mitochondria and lysosomes, and provides a protective effect following radiation treatment. We have also found in ROCK inhibitor induced networks stimulates mitochondria transfer from neural progenitors to GBM stem cells.
Conclusion: These findings demonstrate a link between ROCKi-regulated cell projection dynamics and the formation of radiation-resistant multicellular GBM networks. Within these networks we have demonstrated the ability of cells in network to transfer organelles between GBM-GBM as well as normal and malignant cells.

ATR MODULATES LONG INTERSPERSED ELEMENT-1 (LINE1) RETROTRANSPOSITION IN HUMAN LIVER CANCER CELLS

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Liver cancer generally develops on the background of a chronic liver disease following the accumulation of genetic damage and epigenetic alterations of growth regulatory genes, leading to activation of oncogenes and loss of function of tumour suppressor genes. This includes dysregulation of repeat elements belonging to the Long Interspersed Nuclear Elements (LINE1 or L1) class. The LINE1 elements mobilize themselves via an RNA intermediate which then get reverse transcribed to DNA before integrating into new locations in the genome and are thus categorized as 'retrotransposons'. We have examined LINE1 retrotransposition efficiency of various liver cancer cell lines using a cell culture based retrotransposition assay and found that all the cell lines supported active retrotransposition, although to various extent. However, there was no correlation between level of active retrotransposition and the basal level of LINE1 expression in the cells. Since, active LINE1 retrotransposition through 'Target Primed Reverse Transcription' (TPRT) involves first DNA strand nicking by ORF2 endonuclease followed by second strand cleavage, we explored the DNA damage response pathways involved in regulating the process. For this purpose, we examined LINE1 retrotransposition in the cell lines in the presence and absence of DNA damage response suppression using small molecule inhibitors towards ATM (KU-55933), DNA-PK (NU-7441), ATR (VE-821), CHK1 (SRA737) and PARP (Rucaparib). Overall, we observed an increase in the retrotransposition efficiency of engineered human LINE1 in all the cell lines particularly upon inhibition of Ataxia

Telangiectasia And Rad3-Related Protein (ATR), a serine/threonine kinase involved in DNA replication stress and DNA damage signalling.