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IN VIVO CANCER BIOLOGY

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Our lab uses preclinical models to study cancer processes, interrogating the role of cancer-related pathways within a biological context. By validating *in vitro* discoveries in physiologically relevant models we hope to expedite novel therapeutic approaches to the clinic. The group has expertise in modelling different cancer types and co-leads the MRC National Mouse Genetic Network *Cancer Cluster.* Specific projects in the lab focus on how the RUNX/CBFβ transcriptional complex and the BCL-2 family of apoptotic regulators contribute to tumour progression, metastasis and recurrence in breast, prostate and other cancers.

Deciphering the role of the RUNX/CBFβ transcriptional complex in breast cancer

Our lab has a long-standing interest in the RUNX/CBFβ transcriptional complex, an essential regulator of mammalian development which is often found dysregulated in cancer. It is not surprising that this family of genes is altered in cancer considering the pathways regulated by this complex (Figure 1), yet there is often a dichotomy on how these proteins manifest their effects in a cancer setting. Genetic aberrations of the *RUNX1* and *CBFB* genes are particularly prevalent in breast cancer, and two PhD students, Kerri Sweeney and Adiba Khan, recently submitted and successfully defended their theses exploring the enigmatic role of these genes.

In collaboration with Prof Ewan Cameron (University of Glasgow) and funded in part by Breast Cancer Now, Kerri's thesis was titled *'Investigating the tumour suppressor function of RUNX1 in breast cancer'.* Deletion of *Runx1* in two independent *in vivo* models of breast cancer accelerated disease onset and led to emergence of multifocal and multicentric tumours. RNAseq analysis revealed an increased stem-like transcriptional signature in *Runx1*-deficient tumours, while loss of *Runx1* predisposed to increased stem/progenitor-like behaviour in functional mammosphere assays. Adiba's studies revealed that while loss of *Cbfb* did not overtly alter normal development of the mammary gland, when combined with oncogenic WNT signalling it dramatically accelerated onset of mammary tumours, providing the first *in vivo* evidence that CBFβ has a tumour suppressor role in a mouse model of breast cancer. Adiba, along with Masters Student Nimrit Kaur, showed however that loss of *Cbfb* did not promote tumour susceptibility within the MMTV-*PyMT* model. Thus, as observed in patients, CBFβ played a context-dependent role in breast cancer. Profiling of RUNX/CBFβ-deleted mammary tumours has revealed that loss of the complex evoked changes to the tumour microenvironment where an important aspect of RUNX/CBFβ activity might be to orchestrate the immune microenvironment, a hypothesis we are pursuing further.

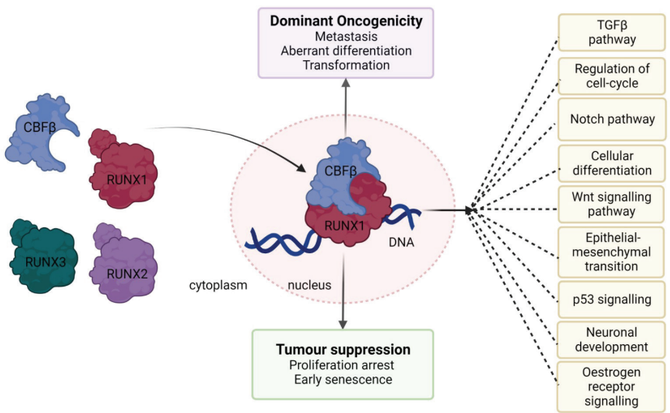
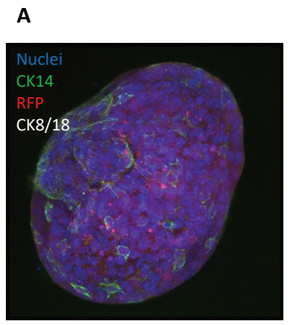


Figure 1
Schematic representation of the RUNX/CBFβ complex and its role in regulation of cell fate processes. RUNX proteins (RUNX1, RUNX2 and RUNX3) interact with their binding partner CBFβ to form a heterodimeric complex which translocates to the nucleus where RUNX can bind to DNA (RUNX1 shown here) to regulate transcription of various target genes involved in a multitude of signalling pathways (yellow boxes). Depending on context, the RUNX/CBFβ complex can support either suppression of cell proliferation (green box) or promotion of growth enhancing signals (purple box).



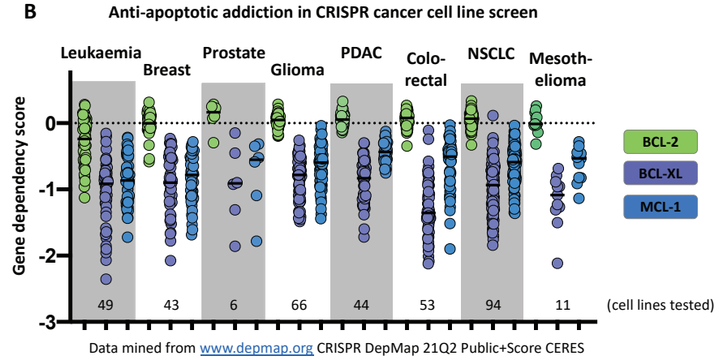


Figure 2
Investigating tumour cell dependency on BCL-2 family proteins.
A Colony forming cell assays conducted on mouse mammary epithelial cells. Red fluorescent protein (RFP)- expressing mammary cells are resuspended in a basement membrane matrix and establish 3D structures to investigate the dependency of tumour-initiating cells on BCL-2 family proteins. 3D structures are stained for basal (anti- CK4/14) **[green]**- and luminal (anti-CK8/18) **[white]**- cell specific cytokeratin antibodies and imaged using confocal microscopy. **B** Frequent genetic requirement for BCL-XL (purple), MCL-1 (blue) but not BCL-2 (green) genes for viability across a panel of cancer cell lines is evident in a CRISPR cancer cell line screen. Gene dependency scores below 0 indicate dependence on that gene for survival, PDAC (pancreatic ductal adenocarcinoma), NSCLC (non-small cell lung cancer). Data mined from www.depmap.org dataset CRISPR DepMAp 21Q2 Public+Score CERES.

Investigating the function of MCL-1 in tumour development and targeting of MCL-1 to improve cancer therapy

MCL-1 is a protein best known for its role in cancers of the blood, but we have found a key role for MCL-1 in breast cancer showing that MCL-1 is required for tumour development and maintenance of established tumours (Campbell et al., *Cell Death Dis* 2018 9:19). In collaboration with Prof Stephen Tait, our experiments revealed that the anti-apoptotic function of MCL-1 was required in breast cancer cells (Campbell *et al., Cell Death Diff* 2021 28:2585-600). Interestingly, while thought to be responsible for tumour initiation, metastasis and treatment resistance, we have found that breast cancer stem cells were particularly dependent on MCL-1 and were effectively killed by MCL-1 inhibiting drugs. A focus of PhD student Matthew Winder's work is to further define the requirement for MCL-1 in breast cancer stem cells and unravel the role of MCL-1 at the time of tumour initiation (Figure 2A). We hope that understanding the requirement for MCL-1 at this early stage of tumour evolution may allow the development of cancer preventative treatment approaches. At the same time, we are interested in the role of MCL-1 in treatment resistance where Masters Student Vibhuti Aggarwal's project combined MCL-1 inhibition with novel drug combinations to enhance tumour cell death in models of triple negative breast cancer.

Our data suggested that MCL-1 also has a role in prostate cancer where it could act as a barrier to tumour cell elimination by prostate cancer therapies. Advanced prostate cancer, where the tumour has spread to distant sites around the body, is a lethal diagnosis. Furthermore, bone metastases is a particularly painful and debilitating condition. MCL-1 seems preferentially increased in advanced prostate cancer and bone metastases. Funded by a Prostate Cancer Research grant, Dr Laura Martinez Escardo is investigating whether targeting MCL-1 can improve response to hormone or chemotherapy in advanced prostate cancer. New treatments for prostate and breast cancer are urgently required as these diseases account for over 23,000 deaths in the UK each year. We hope to prove MCL-1 as a valid target in prostate and breast cancer and expedite the use of MCL-1 inhibitors in these cancer types.

Targeting the BCL-2 family to induce radiosenistisation

Pro-survival members of the BCL-2 family such as MCL-1, BCL-2 and BCL-XL are frequently upregulated in cancer where elevated expression acts as a barrier to efficient cell death induction by cancer therapies. In addition to our studies in breast and prostate cancer, we noted that many tumour types show dependence on MCL-1, or it's close relative, BCL-XL (Figure 2B). More than half of cancer patients receive radiotherapy and so, Masters Student Rosie Willis investigated whether inhibition of pro-survival BCL-2 proteins with a class of drugs called BH3-mimetics, could sensitise to radiotherapy and lead to more efficient cancer cell elimination. This project is co-led by Dr Kirsteen Campbell and Dr Joanna Birch who, in collaboration with Prof Karen Blyth and other Glasgow RadNet colleagues, secured a Cancer Research UK RadNet Pump Priming Grant to characterise radiosensitisation by BH3-mimetics in pancreatic cancer and glioblastoma.

MRC National Mouse Genetic Network (NMGN) Cancer Cluster

The lab are excited to co-lead the *Cancer Cluster* within the highly interactive MRC National Mouse Genetic Network (https://nmgn.mrc.ukri.org/). With colleagues in Glasgow, Belfast, London, Oxford, and the Mary Lyon Centre at Harwell, we will utilise state-of-the-art technologies such as spatial phenotyping to study complex cancer-host interactions and position models that recapitulate the human disease. We will also refine models to mirror human tumour evolution more accurately and through robust patient-relevant mouse models, assess responses to novel therapies with improved predictability. To find out more please visit https://nmgn.mrc.ukri.org/clusters/cancer/.

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