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IN VIVO CANCER BIOLOGY

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Our group uses *in vivo* models to study cancer processes, interrogating aspects of the disease and cancer-related pathways within a biological context. By validating *in vitro* discoveries in physiologically relevant models, we hope to expedite novel therapeutic approaches for patient benefit. The group has expertise in modelling different cancer types but has a specific interest in breast and prostate cancer and how certain signalling nodes such as the RUNX/CBFβ transcriptional complex and pro-survival factor MCL-1 contribute to tumour progression and metastasis.

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

The *RUNX* genes are key transcription factors which play important roles in mammalian development, being for example indispensable for normal haematopoiesis and osteogenesis. However, these proteins, along with their obligate DNA binding partner CBFβ, are also implicated in various types of cancer, most notably in haematological malignancies but also in epithelial cancers. Indeed, *RUNX1* and *CBFβ* are among the most recurring genetic anomalies in breast cancer, with around 13% of all breast cancers exhibiting alterations in this gene family. Intriguingly, both loss and gain of RUNX function have been linked with different subtypes of breast cancer (Riggio & Blyth, *FEBS J.*, 2017; Rooney et al, *Adv Exp Med Biol.*, 2017). Our group have therefore been exploring this enigmatic role of the RUNX/CBFβ complex using *in vivo* models of breast cancer.

Loss of *Runx1* in the *MMTV-PyMT* mouse model results in early tumour onset. However, there is no difference in overall survival, and indeed there is selection against cells having lost RUNX1 at later stages in the disease progression, suggesting this model may exhibit both the tumour suppressor and pro-oncogenic features of RUNX1 function. Deletion of *Runx1* in an oncogenic β-catenin setting also significantly accelerates disease onset, and this phenotype is even more pronounced if both *Runx1* and *Runx2* are deleted. Loss of RUNX function with activated β-catenin elicits an abnormal expansion of a stem/progenitor population with a skewed enrichment of basal-like colonies in organoid culture, potentially expanding a population of cells exquisitely sensitive to the transforming properties of WNT signalling. Furthermore, by modulating *Runx1* in a murine epithelial cell line, we have conclusively shown that RUNX1 regulates mammary stemness, whereby loss of *Runx1* potentiates mammosphere capability (Figure 1A). Complementary to this, overexpressing *Runx1* seems to constrain the ability of the cells to form mammospheres (Figure 1B). It is notable these effects only happen in 3D cultures, whereas modulating *Runx1* does not affect mammary cell proliferation when cultured in 2D, thus providing rationale for the importance of investigating these genes in a physiologically relevant model.

Given that CBFβ plays a crucial role in the regulation of RUNX proteins, it is exciting that loss of *CBFβ in vivo* phenocopies the combined loss of *Runx1* and *Runx2* with accelerated disease onset in the same β-catenin driven mouse model of breast cancer. Whether CBFβ also regulates mammary stemness is currently being investigated. We are also profiling the transcriptional signature of RUNX/CBFβ deleted mammary tumours to unravel the mechanism/vulnerabilities of RUNX pathway alteration in cancer.

MCL-1 is a clinically actionable vulnerability in breast cancer

Around 11,500 women die each year in the UK from breast cancer and new treatments are required to help prevent these deaths. MCL-1 is a protein best known for its role in cancers of the blood, but we have found that high MCL-1 predicts poor prognosis in breast cancer (Campbell *et al, Cell Death Disease*, 2018). This is particularly relevant in triple negative breast cancer where treatment resistance and disease recurrence remain a major challenge. Using mouse models of breast cancer, we have found that MCL-1 is required for both tumour development and for maintenance of established tumours (Figure 2A).

Many functions for MCL-1 have been reported. In collaboration with Professor Stephen Tait, we have found that it is the anti-apoptotic function of MCL-1 that is key in breast cancer. This is important because a new class of drugs specifically targeting MCL-1 anti-apoptotic function, known as BH3-mimetics, have been developed and are in clinical trials for haematopoietic malignancies. In our experiments, targeting MCL-1 pharmaceutically, or genetically, in human breast cancer cell lines and in mouse models of breast cancer restricted growth and induced tumour regression *in vivo*. This highlights the potential for BH3 mimetic drugs targeting MCL-1 to be used to treat breast cancer.

We have also found that breast cancer stem cells, the cells thought to be responsible for metastasis and treatment resistance, are particularly dependent on MCL-1 and treatment with BH3-mimetics targeting MCL-1 effectively kills these cells (Figure 2). We believe that targeting MCL-1 could be particularly important in the context of advanced disease, treatment resistance and recurrence, and that targeting MCL-1 could offer a new therapeutic axis in breast cancer.

Challenges and Achievements

It has been an unusual and challenging year! But we are incredibly proud of the lab achievements under the circumstances, not least how everyone adapted to home working, lab meetings over Zoom, online seminars and courses, and carrying out essential research under COVID-secure conditions when access to the labs was permitted. We were delighted to publish our study describing a novel role for *RUNX1* and *RUNX2* as drivers of renal cell carcinoma and how these genes correlate with poor clinical outcome (Rooney *et al*, Cancer Research, 2020). Congratulations also go to our student Narisa Phinichkusolchit, who spent most of lockdown writing her PhD thesis '*ROCK1-mediated apoptotic blebbing in genetic models of tissue homeostasis and tumourigenesis*', which she successfully defended in November. It was very disappointing that we couldn't celebrate Narisa's success, due to COVID regulations, and while we will miss Narisa we wish her well in her new position in London. Meanwhile, we welcomed PhD student Matthew Winder to the group in October. Matthew will be further investigating the role of MCL-1 in breast cancer stem cells.

Publications listed on page 97

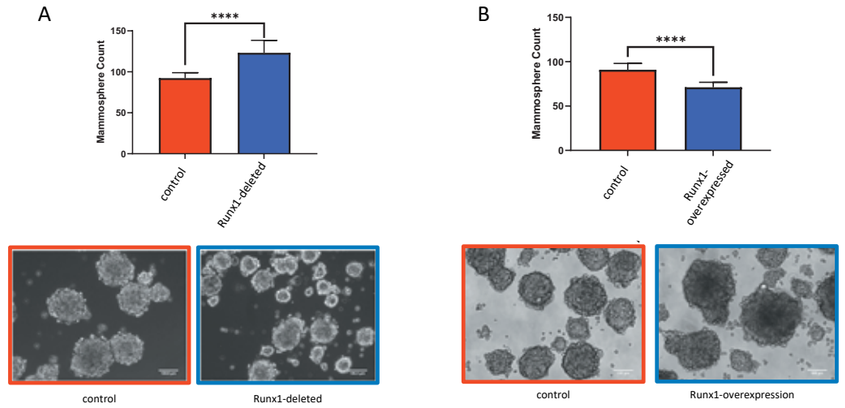

Figure 1
Runx1 regulates stemness in mammary cells. A) CRISPR/Cas9-based genome editing was used to delete *Runx1* in HC11 mammary cells. *Runx1*-deleted cells formed significantly more mammospheres than control cells in a 7-day assay, as shown in the graph and representative images. B) HC11 mammary epithelial cells with overexpression of *Runx1* resulted in significantly less mammospheres being formed compared to control cells.

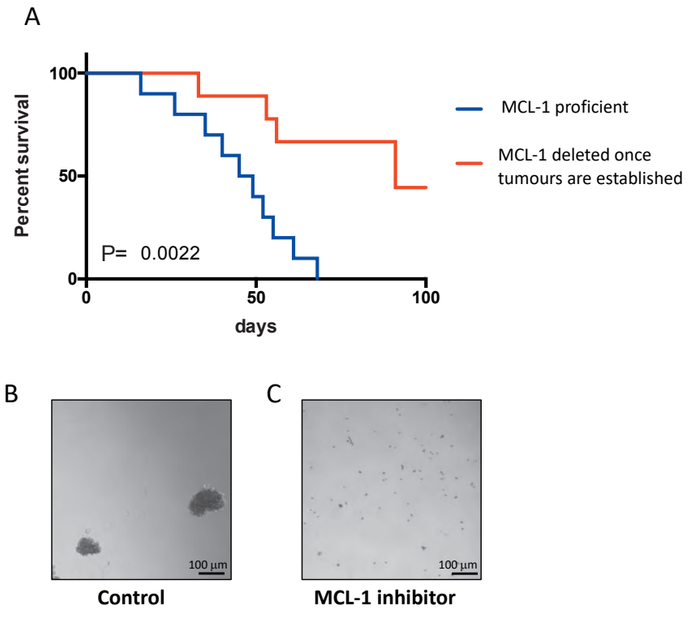

Figure 2
A. Genetic deletion of MCL-1 in mammary tumours prolongs survival. Tumour-related survival of mice whose tumours were allowed to grow to 5mm prior to *in situ* deletion of MCL-1 (red line, n=9 mice) or control (blue line, n=10 mice). Median survival increased from 47 to 91 days upon MCL-1 deletion with 4/9 mice showing complete tumour regression and long-term tumour-free survival. B-C. Tumoursphere assay reveals requirement for MCL-1 in breast cancer stem cells. Plating of breast cancer stem cells in non-adherent culture conditions gives rise to tumoursphere formation (**B**). Addition of a BH3-mimetic drug to target MCL-1 inhibits tumoursphere formation (**C**).