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MYC-INDUCED VULNERABILITIES/THORACIC CANCER RESEARCH

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Oncogenic signalling profoundly alters how cells respond to their environment, typically putting tumour cells under tremendous pressure to reconcile conflicting cues. For example, tumour cells must re-organise their metabolic pathways to balance competing needs for biosynthetic precursors with energetic homeostasis, commonly while surviving in a milieu of limiting oxygen and nutrients.

We use genetically engineered mouse models, primarily of lung cancer and mesothelioma, to understand how developing tumours cope with conflicting cues in their natural environment. Our overarching hypothesis is that oncogene-induced biological perturbations can be exploited for cancer therapy, even in the absence of direct suppression of driver oncogenes. We use deregulated MYC as our paradigm oncogene coupled with a mixture of candidate and RNAi-based approaches to identify induced vulnerabilities *in vivo* and *in vitro*, and are actively exploring several strategies for selective elimination of cells that overexpress MYC.

MYC in cancer

Overexpression of the transcription factor MYC occurs in a vast number of human cancers. The overexpression may arise from focal or broad chromosomal amplification, gene translocation, enhanced mRNA and protein stability, or indeed increased signalling through upstream regulatory factors such as Ras, Notch, or ß-catenin. In many *in vivo* settings, MYC overexpression is sufficient to initiate or exacerbate tumorigenesis and MYC is moreover typically required to sustain the cancerous phenotype. A successful therapeutic strategy that exploits MYC expression would likely have a tremendous impact on human health. To facilitate investigation of physiologically relevant levels of deregulated MYC expression in any tissue, we have generated and characterised Rosa26$^{DM\text{-}lsl\text{-}MYC}$ mice and deposited them with Jaxmice for unrestricted distribution to the broader scientific community.

MYC and KRAS drive immune evasion

How tumours evade detection by the immune system defines the underlying principle behind the therapeutic success of immunotherapy across a spectrum of cancer types. MYC is known to induce expression of PD-L1, which inactivates cytotoxic T cells upon binding to PD1, but new data from multiple labs, including ours, indicated that PD-L1 expression is not the sole immune evasion strategy deployed by MYC. In 2020, we showed that MYC and KRAS combined to suppress multiple cascades involved in cell communication with the immune system, with downregulation of the Type I Interferon pathway and of MHC I-dependent antigen processing & presentation forefront in these transcriptional responses. The transcriptional changes occur immediately upon acute activation of KRAS or modest overexpression of MYC in cell culture, and importantly, persist throughout tumour progression *in vivo*. Mechanistically, we identified repressive transcriptional complexes comprising MYC and MIZ1 binding directly to multiple key regulators of Type I Interferons in pancreatic ductal adenocarcinoma (PDAC). Genetic suppression of MYC or MIZ1 restored Interferon signalling, enabling PDAC tumours to elicit CXCL13 production in nearby macrophages and thereby recruit anti-tumour effector immune cells to limit tumour progression, resulting in extended survival. In the year since publication, this provocative finding of active suppression of the Type I Interferon cascade by the MYC/KRAS pathway has been reproduced in multiple cancer types, including lymphoma, breast, lung, ovarian and oesophageal cancers, indicating widespread use of this immune evasion strategy across many (all?) cancers. Pharmacological inhibition of MYC transcriptional repressive complexes may thus have benefit as a generic cancer therapy.

MYC-induced metabolic vulnerability

As part of a coordinated programme of cell growth required for cell division, MYC engages a number of biosynthetic programmes, such as ribosome assembly and protein translation, placing tremendous energetic demand upon the cell. In order to maintain energetic homeostasis, MYC upregulates glucose transporters and glycolytic enzymes, promoting the Warburg effect of limited glucose breakdown, and in parallel induces expression of glutamine transporters and exploits this pathway to maintain the citric acid cycle. The energetic strain that MYC deregulation thus places upon the cell is evident in progressive activation of the AMP-activated protein kinase AMPK, which plays a key role in maintaining energetic homeostasis. AMPK in turn inhibits TORC1 to attenuate the rate of macromolecular synthesis, effectively allowing cells to balance the rate of ATP consumption with ATP production. Importantly, the AMPK-related kinase ARK/NUAK1 is also required for maintenance of ATP homeostasis in cells wherein MYC is overexpressed. NUAK1 plays a specific role in MYC-dependent activation of AMPK and also maintains mitochondrial respiratory capacity. Suppression of NUAK1 thus impairs the ability of MYC-overexpressing cells to respond to declining ATP levels while simultaneously depriving cells of ATP-generating capacity, suggesting that suppression of NUAK1 may be an effective means to selectively kill cancer cells with high levels of MYC expression.

Oncogene cooperation during lung cancer progression

Lung cancer remains one of the deadliest forms of cancer worldwide, accounting for 18% of all cancer-related deaths, and the incidence of lung cancer is on the rise, especially in the increasingly industrialised and densely populated cities of emerging economies. Poor prognosis arises in large part from the combination of late disease detection and limited matching of patients with emerging targeted therapies. We have found that modestly elevating MYC levels in a KRAS-driven model of lung cancer is sufficient to drive progression to metastatic disease. This progression arises in part through increased transcription of promiscuous ERBB family ligands. We have identified an unexpected requirement for signal transduction through the ERBB receptor tyrosine kinase network for both establishment and maintenance of KRAS-mutant lung cancer. Our data suggest that KRAS-driven tumours actively seek ways to amplify signalling through the RAS pathway to sustain the tumour phenotype.

Inflammation and genetics of mesothelioma

Mesothelioma is a lethal cancer of the lining of the chest cavity that arises in people chronically exposed to asbestos. There are no effective therapies and patient survival is typically less than 18 months from diagnosis. My lab has teamed up with respiratory physician Kevin Blyth to build an international network of clinicians and researchers with the common goal of improving patient outcomes for this dreadful disease. We have developed a new mouse model of mesothelioma that will enable us to investigate the interplay between asbestos-driven chronic inflammation and the major recurring mutations that are commonly found in human mesothelioma. Significantly, intrapleural injection of asbestos dramatically accelerates onset and severity of mesothelioma in our mice, even after homozygous deletion of 3 major tumour suppressor genes, indicating that chronic inflammation continues to contribute to mesothelioma beyond the acquisition of rate limiting mutations. This startling observation suggests that patients may benefit from interventions that aim to reduce inflammation, in addition to those directly targeting the tumour population.

Major developments in 2022

Building upon our growing strength in mesothelioma research, the year saw further successful bids for competitive funding, in the form of co-leadership of a CRUK Discovery Programme "REMIT: Reconstructing the *in vivo* Evolution of Mesothelioma for Improved Therapy" and positioning of Mesothelioma as one of six cancer themes in the CRUK Scotland Centre. We commenced work on our ambitious early detection of mesothelioma programme, IAMMED-Meso, following the extension of postdoc Pooyeh Farahmand, and the recruitment of new PhD students Danielle McKinven & Xinya Hong, along with Research Assistant, Nicola Brady. As contributors to the MRC National Mouse Genetics Network - Cancer Cluster, we also continued our work on generating a new multi-drug inducible Tandem Arrayed Regulator (TAR) allele to enable modelling sequential genetic events in mice, in contrast to current all-at-once model systems, with postdoc Sarah Laing extending her tenure in the lab to continue her work on disease positioning of immune-visible models of Lung Adenocarcinoma. These next generation models will provide exceptional platforms for further investigation of tumour progression and the dynamic interactions with anti-cancer immune responses. Postdoc George Skalka's efforts were rewarded with a 1-year funded project extension from Merck in collaboration with Cancer Research Horizons.

The year saw a welcome return to in-person conference attendance and members of the lab presented posters and/or spoke at the European Workshop on Cell Death (Fiugi Italy), EACR (Seville, Spain), CSHL Mechanisms & Models of Cancer (USA), the 5th European Workshop on AMPK & related kinases (Clydebank), and the CRUK Lung Cancer Conference (Manchester). Mice generated in the lab featured in 1 pre-print and 1 publication arising from collaborations with the labs of Beatson colleagues, Tom Bird & Saverio Tardito, while our expertise on MYC contributed to a further 2 publications with colleagues Hing Leung (Uni Glasgow) and Daniel Schramek (Uni Toronto). Former PhD student Declan Whyte submitted his manuscript on centrosome regulation by NUAK1 for publication.

We finally wish to thank local charity, Action on Asbestos, for their generous donation of £10,000 towards the cost of ongoing work on Mesothelioma. Their support is very much appreciated.

Publications listed on page 108