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ONCOGENE-INDUCED VULNERABILITIES

Group Leader

Daniel J. Murphy

Research Scientists
Katarina Gyuraszova
Bjorn Kruspig
Nathiya Muthalagu[1]

Graduate Students
Sarah Laing
Tiziana Monteverde
Jennifer Port
Declan Whyte[2]

[1]Worldwide Cancer Research, with Owen Sansom
[2]Prostate Cancer UK Future Leaders Academy

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.

Our overarching hypothesis is that such 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 screening 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 huge number of human cancers arising from almost every tissue type. MYC 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 β-catenin. In a number of *in vivo* settings, MYC overexpression is sufficient to initiate or exacerbate tumourigenesis and moreover is typically required to sustain the cancerous phenotype. A successful therapeutic strategy that exploits MYC overexpression would likely have a tremendous impact on human health.

MYC-induced metabolic vulnerability

As part of a coordinated programme of cell growth required for cell division, MYC engages a number of biosynthetic programmes, prominently including 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 that of ATP production. Importantly, the AMPK-related kinase ARK5/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.

Additionally, we have now found that NUAK1 plays a key role in protecting cells from toxic levels of reactive oxygen species (ROS). ROS are naturally produced as by-products of mitochondrial electron transport chain activity, and the elevated metabolic demand of cancer cells can thus increase ROS production. Paradoxically, hypoxia can also elevate ROS production and is moreover a common feature of most cancers. Tumour cells cope with the threat posed by ROS in part by diverting glucose away from the mitochondria but also by increasing pathways that detoxify ROS. We have found that suppression of NUAK1 impairs this latter response, thereby exposing an intrinsic vulnerability in cancer cells. We have determined that acute inhibition of the antioxidant response pathway, via targeted suppression of NUAK1, eradicates MYC-driven adenomas in a genetically engineered mouse model of colorectal cancer. All well as providing strong evidence to support targeting NUAK1 in human colorectal cancer, this observation challenges dietary advice commonly given to patients who already have cancer, in that popular consumption of antioxidant supplements may actually benefit the cancer cells more than the patient.

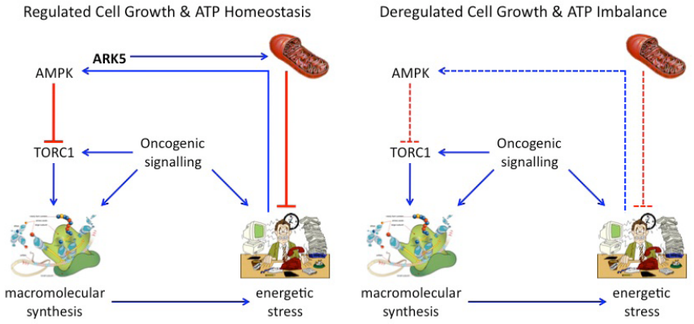

Figure 1
Induced dependencies need not reflect direct molecular interactions. Oncogene-induced cell growth, typically requiring signal transduction via the mechanistic target of rapamycin (mTOR) pathway, drives rampant ATP consumption, which must be compensated for through increased cellular intake of fuel (e.g. glucose, amino acids and fatty acids) combined with AMPK-mediated attenuation of macromolecular synthesis. Upon suppression of Ark5, this feedback mechanism is impaired, leading to ATP depletion and bioenergetic catastrophe. In principle, any intervention that similarly impairs bioenergetic homeostasis may selectively kill tumour cells.

Paradoxically, canonical activation of NUAK1 requires STK11 (aka LKB1), an erstwhile tumour suppressor associated with Peutz-Jehger's syndrome and a frequently mutated gene in sporadic lung cancer. Curiously, we have found that NUAK1 remains active in LKB1-deficient cells, indicating LKB1-independent mechanisms of NUAK1 regulation. Similar to AMPK, we have found that calcium signalling is required for NUAK1 activity in the absence of LKB1 and have shown that calcium-dependent activation of PKC increases NUAK1 activity. The precise mechanism of activation is as yet unclear and may involve ROS-dependent modification of NUAK1 cysteines. Notably, MYC deregulation sensitises cells to calcium-dependent signalling, in part via transcriptional regulation of multiple proteins involved in the calcium signal transduction pathway. It thus appears that MYC indirectly activates NUAK1 (and potentially other AMPK-related kinases) by enhancing cellular sensitivity to calcium.

Oncogene cooperation during lung cancer progression

Lung cancer remains one of the deadliest forms of cancer worldwide, accounting for some 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 in order to sustain the tumour phenotype. As there are presently no clinically proven small molecule inhibitors of KRAS, our observation raises the exciting possibility that simultaneously inhibiting signalling components upstream and downstream of KRAS with existing therapeutic agents may benefit the very large number of lung cancer patients whose disease is driven by mutant KRAS.

Oncogene cooperation in pancreatic cancer

Activating mutations in KRAS initiate almost all cases of pancreatic ductal adenocarcinoma (PDAC), the deadliest form of pancreatic cancer. MYC is an obligate effector of RAS's oncogenic output, and genetic ablation of even one copy of MYC can dramatically extend the lifespan of KPC mice. In collaboration with Rosalie Sears (Oregon Health Sciences University) and Jennifer Morton, we are examining the role of MYC during pancreatic development to explore potential MYC-induced vulnerabilities that might reveal new therapeutic opportunities. We have shown that a modest elevation of MYC above physiological expression dramatically accelerates onset of PDAC and drives lineage plasticity that is strongly implicated in the severity of this debilitating disease.

Major developments in 2017

A major development was a successful application, spearheaded by our colleague Jennifer Morton, to establish a Pancreatic Cancer UK–funded 'future leaders in pancreatic cancer' postgraduate academy of five PhD students, resulting in Declan Whyte commencing his studies in my group. At the other end of the PhD journey, Jennifer Port and Tiziana Montevderde both completed their studies and submitted their respective theses in September. Tiziana subsequently moved to the CRUK Manchester Institute, joining the group of Michela Garofalo, while Jennifer secured a scientific writing position in the Netherlands. We embarked on an exciting new direction to develop new *in vivo* models for mesothelioma, buttressed by collaboration with the MRC Toxicology groups in Leicester, along with support from clinicians in Edinburgh and Glasgow. This programme aims to recapitulate the inflammatory ecosystem associated with asbestos exposure, combined with state-of-the-art manipulation of genes associated with human mesothelioma, in order to shed much-needed light on early-stage disease progression. New work performed primarily in our own group was published in *Oncogene*, while we made significant contributions to other works published in *Nature Communications* and *Nature Scientific Reports*. We additionally published a translational review on the subject of mesothelioma along with two invited commentaries on important new discoveries in mesothelioma and lung cancer.

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