Colorectal cancer (CRC) — the third most common cancer in the UK and the second leading cause of cancer mortality — is a heterogeneous disease comprising distinct molecular subgroups that differ in their histopathological features, prognosis, metastatic proclivity and response to therapy. Despite advances in the detection and treatment of early-stage disease, patients with advanced, recurrent or metastatic CRCs have few therapeutic options and a dismal prognosis. Utilising state-of-the-art preclinical models harbouring key driver mutations, our group is interrogating the molecular mechanisms underpinning CRC initiation, progression, metastasis and response to therapy. Our overarching goals are to identify early-stage diagnostic biomarkers and develop stage- and subtype-specific targeted therapies.

Deegeneration of Wnt signalling is a hallmark of most CRCs, with loss-of-function mutations in the negative Wnt-regulator APC sufficient for adenoma formation. Progression to adenocarcinoma, however, requires the acquisition of additional compounding mutations such as in the KRAS proto-oncogene. Accordingly, approximately 35% of all human CRCs harbour concurrent oncogenic mutations in KRA S alongside APC deficiency, resulting in the constitutive activation of the pro-proliferative MAPK signalling cascade. In a background of APC loss, the acquisition of an oncogenic K RAS mutation not only fuels aberrant cell proliferation, but also confers intrinsic resistance to inhibitors of the downstream MAP-kinase MEK and metabolic sensor mTOR, as well as intrinsic and acquired resistance to EGFR-targeted agents, thus posing a major therapeutic challenge. Furthermore, KRAS has been widely deemed un-druggable as multiple attempts to directly target its oncogenic function have met with failure, underscored by the need to develop innovative therapeutic strategies for treatment-refractory KRAS-mutant CRCs. Using a suite of preclinical mouse and organoid models, we aim to delineate how oncogenic KRAS alters the molecular landscape of APC-deficient cells with a view to identifying actionable therapeutic vulnerabilities while also sparing normal intestinal homeostasis and Wnt-driven stem cells in bystander tissues.

To probe the mechanisms underlying the cooperation between APC loss and oncogenic KRAS during adenocarcinoma progression, we crossed mice expressing a tamoxifen-inducible, intestine-specific C rebbinase, under the control of the villin promoter (villinCre), with mice harbouring conditional alleles of Apc (Apcfl) either alone or in combination with oncogenic K rass (KrasG12D). Using transcriptional, translational and metabolomic profiling, we found a significant enrichment of pathways associated with mRNA translation and metabolism in Apcfl/flKrasG12D/+ intestinal tissues, relative to Apcfl/flKrasG12D++ counterparts. Dashed lines denote adenoma boundaries. Scale bars, 50 μm. (A) Schematic depicting serine/threonine-protein kinases interacting with cap-binding protein eIF4E by the MAPK-driven MNK1/2 (at left) and the MNK1/2-inhibitor eFT508 (Figure 1E) restored phosphorylation in colonic epithelial cells, relative to Apc−/−Kras−/− counterparts. Dashed lines denote adenoma boundaries. Scale bars, 50 μm. (B) Schematic of treatment timeline. Mice were killed at each time point coinciding with the acquisition of metastatic disease. Intestines were fixed, paraffin-embedded and sectioned. Intestinal sections were stained with haematoxylin and eosin (H&E). Scale bars, 50 μm. (C) Schematic depicting the Warburg effect. Concomitant deletion of Apc and oncogenic KRAS in the mouse intestinal epithelium additionally increased glutamine consumption through a pronounced upregulation of genes associated with glutamine transport and metabolism. Using mass spectrometric imaging to map the spatial distribution of glutamine in intestinal tissues in situ, we detected paradoxically reduced levels of intracellular glutamine in Apcfl/flKrasG12D/+ intestinal tissues, relative to Apcfl/flKrasG12D++ counterparts, and decreased channeling of isotopically labelled glutamine derivatives through the tricarboxylic acid cycle. These findings suggested a metabolic fate other than glutaminolysis for glutamine in this molecular setting. Indeed, we found selective upregulation of the glutamine transporter SLC7A5/LAT1, which exchanges intracellular glutamine for neutral essential amino acids, such as leucine, which further augments the global mRNA translation capacity associated with Apc loss, rendering APC-deficient cells resistant to rapamycin. Mechanistically, oncogenic KRAS drives increased phosphorylation of the mRNA cap-binding protein eIF4E by the MAPK-interacting serine/threonine-protein kinases MNK1 and MNK2 (Figure 1A). This results in enhanced translation of a subset of pre-oncogenic mRNAs, including transcripts encoding c-MYC, driving cell proliferation, oncogenic transformation and resistance to rapamycin (Figure 1B and 1C). Intrastestinal-specific deletion of MNK1/2 (Figure 1D) or treatment with the MNK1/2-inhibitor eFT508 (Figure 1E) restored sensitivity to rapamycin, curtailing tumour growth and extending survival of APC-deficient, Kras-mutant mice. Importantly, the MNK1/2-eIF4E axis is not required for normal development and eft508 is well-tolerated, lending hope for approximately 20% of poor prognosis CRC patients whose tumours display elevated mTOR and MNK activity.

Oncogenic KRAS-driven metabolic reprogramming unveils novel therapeutic vulnerabilities (‘Rosetta’ CRUK Grand Challenge) KRAS activation alone elicits a metabolic shift towards aerobic glycolysis (commonly known as the Warburg effect). Concomitant deletion of Apc and oncogenic activation of KRAS in the mouse intestinal epithelium additionally increased glutamine consumption through a pronounced upregulation of genes associated with glutamine transport and metabolism. Using mass spectrometric imaging to map the spatial distribution of glutamine in intestinal tissues in situ, we detected paradoxically reduced levels of intracellular glutamine in Apc−/−Kras+/+ intestinal tissues, relative to Apc−/−Kras+/+ counterparts, and decreased channeling of isotopically labelled glutamine derivatives through the tricarboxylic acid cycle. These findings suggested a metabolic fate other than glutaminolysis for glutamine in this molecular setting. Indeed, we found selective upregulation of the glutamine transporter SLC7A5/LAT1, which exchanges intracellular glutamine for neutral essential amino acids, such as leucine, which further augments the global mRNA translation capacity associated with Apc loss, rendering APC-deficient cells resistant to rapamycin. Mechanistically, oncogenic KRAS drives increased phosphorylation of the mRNA cap-binding protein eIF4E by the MAPK-interacting serine/threonine-protein kinases MNK1 and MNK2 (Figure 1A). This results in enhanced translation of a subset of pre-oncogenic mRNAs, including transcripts encoding c-MYC, driving cell proliferation, oncogenic transformation and resistance to rapamycin (Figure 1B and 1C). Intrastestinal-specific deletion of MNK1/2 (Figure 1D) or treatment with the MNK1/2-inhibitor eFT508 (Figure 1E) restored sensitivity to rapamycin, curtailing tumour growth and extending survival of APC-deficient, Kras-mutant mice. Importantly, the MNK1/2-eIF4E axis is not required for normal development and eft508 is well-tolerated, lending hope for approximately 20% of poor prognosis CRC patients whose tumours display elevated mTOR and MNK activity.

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Targeting the glutamine antiporter SLC7A5 alters amino acid dynamics and extends survival of tumour-prone mice. (A) Haematoxylin and eosin (H&E) staining (bottom) and ion intensity distribution of glutamine, glutamate and alanine (top) in intestinal tissues from Apc\(^{−/−}\)Kras\(^{G12D/+}\)Slc7a5\(^{+/+}\) and Apc\(^{−/−}\)Kras\(^{G12D/+}\)Slc7a5\(^{−/−}\) mice. Ion intensities, determined by mass spectrometric imaging (MSI), are shown in the red-green-blue (R-G-B) spectrum. (B) Kaplan–Meier survival curves for vehicle- and rapamycin-treated Apc\(^{−/−}\)Kras\(^{G12D/+}\)Slc7a5\(^{−/−}\) mice. Mice were aged until they developed symptoms of intestinal disease and, subsequently, treated with vehicle or rapamycin until clinical endpoint. (C) Kaplan–Meier survival curves for Apc\(^{−/−}\)Kras\(^{G12D/+}\)Slc7a5\(^{+/+}\) and Apc\(^{−/−}\)Kras\(^{G12D/+}\)Slc7a5\(^{−/−}\) mice. Ion intensities, determined by mass spectrometric imaging (MSI), are shown in the red-green-blue (R-G-B) spectrum. (D) Incidence of metastasis (mets) in Kras\(^{G12D/+}\) mice. Ion intensities, determined by mass spectrometric imaging (MSI), are shown in the red-green-blue (R-G-B) spectrum.

These findings advocate the development of combinatorial therapeutic strategies targeting SLC7A5 and mTOR, and hold promise particularly for CRCs of the metabolic CMS3 subtype that are enriched for a neutrophil gene expression signature. Moreover, elevated expression of Notch-pathway components and TGFB2 correlates with poor patient survival. Targeting Ly6G+ neutrophil populations, using a small-molecule CXCR2-inhibitor, an ALK5-inhibitor, a TGFB ligand-trap, or anti-Ly6G antibodies, attracts cytotoxic T-cells to the pre-metastatic niche and abrogates metastasis without, intriguingly, impacting primary tumour burden [Jackstadt et al. Cancer Cell 2019, 36(3): 319–336]. We will next assess how epithelial tumour cell-intrinsic signalling rewire the tumour microenvironment and identify key stromal determinants of immune evasion.

Using our suite of preclinical models, we are evaluating emerging therapeutics that target the altered translational dynamics and metabolite dependencies of transformed cells and partnering with leading industry innovators (CRT/Celgene Translational Alliance; Novartis) to accelerate the path from bench to bedside. Overall, these approaches will inform our understanding of CRC pathogenesis and metastatic competence, and provide a platform for the development of novel stage- and subtype-specific therapies.

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