Colorectal cancers (CRC) are a heterogeneous group of diseases that arise from the epithelial cells lining the gut. They exhibit a range of phenotypes and behave in a variety of ways, from early-stage adenomas through to metastatic disease. Understanding the molecular mechanisms underlying CRC development is crucial for developing effective treatments.

**Colorectal Cancer and Wnt Signalling**

Colorectal cancer is a heterogeneous disease comprising distinct molecular subgroups that differ in their histopathological features, prognosis, metastatic propensity, and response to therapy. Utilising state-of-the-art preclinical models harbouring key driver mutations, our group is interrogating the molecular mechanisms underlying CRC development. Our overarching goals are to identify early-stage diagnostic biomarkers and develop stage- and subtype-specific targeted therapies.

Colorectal tumours that arise left or right to the splenic flexure differ profoundly in their epidemiology, histopathogenesis, and molecular landscapes. Left-sided CRCs develop from benign adenomas through the adenoma-carcinoma pathway, typically entailing aberrant activation of Wnt signalling, with loss-of-function mutations in the negative Wnt regulator APC sufficient for adenoma formation. Progression to adenocarcinoma is underpinned by the accumulation of compounding mutations in oncogenes and tumour suppressors, such as KRAS, TP53, and SMAD4, as well as the acquisition of chromosomal instability. Right-sided CRCs arise through an alternative serrated neoplasia pathway, so-called because the precursor lesions harbour a distinctive saw-tooth mucin morphology. Right-sided CRCs carry a worse prognosis than left-sided CRCs, with ex vivo organoid cultures adding value to our suite of in vivo models.

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**The Wnt-antagonist NOTUM is a druggable target for the treatment of CRC.**

Given that the inactivation of the tumour suppressor APC is a frequent early event in adenoma initiation, we sought to identify how APC-mutant intestinal stem cells (ISCs) compete with their wild-type neighbours to achieve clonal dominance and fixation (Fangaran et al., 2021). Using gene expression profiling, we found that APC-deficient adenomas expressed an abundance of transcripts for several secreted Wnt-antagonists, relative to APC-proficient tissues, with the most highly upregulated gene, Notum, encoding a secreted WNT deacylase that disrupts WNT ligand-binding (Figure 1A). Culture of wild-type organoids in conditioned medium, collected from Apc-mutant cells, curtailed growth (Figure 1B), decreased the expression of ISC-associated genes, and induced differentiation. Addition of a NOTUM inhibitor (Figure 1B), or genetic deletion of Notum in Apc-mutant organoids, abolished the effects of the conditioned medium.

In ViliCreERAPlexCre/+, mice, genetic or pharmacological inhibition of NOTUM compromised the ability of Apc-mutant cells to expand and form intestinal adenomas, significantly prolonging survival (Figure 1C). Deletion of Notum in Apc-mutant Lgr5+ISCs impaired their ability to outcompete wild-type counterparts. Interestingly, wild-type Lgr5-ISCs in the vicinity of Apc-mutant cells exhibited reduced expression of the Wnt-regulated stemness marker Sox9, whereas cells adjacent to Apc-mutant Notum+/− cells retained robust levels of Sox9, consistent with a role for secreted NOTUM in driving the differentiation of wild-type Lgr5-ISCs. Secreted NOTUM could therefore act in a paracrine fashion to inhibit Wnt signalling in neighbouring non-transformed wild-type ISC populations, inducing their differentiation and withdrawal from the cell cycle, and ultimately driving their removal from the stem cell pool (Figure 1D). By contrast, Wnt (androgen-independent, APC-deficient, super-competitor cells could expand unabated with their progeny taking over the entire intestinal crypt.

Our findings identify NOTUM as a druggable mediator of cell competition and mutation fixation during the early stages of adenoma development. Bolstering the fitness of wild-type ISCs by inhibiting NOTUM might serve as a viable approach for preventing progression of early-stage disease in high-risk individuals with hereditary CRC.

**Oncogenic KRAS-driven metabolic reprogramming unveils novel therapeutic vulnerabilities.**

To delineate how oncogenic KRAS alters the molecular landscape of APC-deficient cells and identify actionable therapeutic vulnerabilities, we performed transcriptomic and metabolomic profiling of ViIcreAPC+/+KrasG12D/+ intestinal tissues, compared with ViIcreAPC−/−Kras−/− controls. We found a significant enrichment of pathways associated with mRNA translation and metabolism in ViIcreAPC+/+KrasG12D/+ mice, manifesting as elevated rates of cell proliferation and protein synthesis, and extensive metabolic reprogramming. Concomitant deletion of APC and oncogenic activation of KRAS in the mouse intestinal epithelium increased glutamine consumption through a pronounced upregulation of genes associated with glutamine transport and metabolism (Najumudeen et al., 2021). Genetic scarcity imaging to map the spatial distribution of glutamine in intestinal tissues in situ, we detected paradoxically reduced levels of intracellular glutamine in ViIcreAPC+/+KrasG12D/+ relative to ViIcreAPC+/+Kras−/− counterparts, and decreased channeling of 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 antipporter SLC7A5/LAT1, which exchanges intracellular glutamine for essential amino acids (such as leucine, isoleucine, histidine, and lysine), that stimulate mTOR signalling and fuel protein synthesis.

Targeted deletion of SLC7A5 in the intestinal epithelium of ViIcreAPC+/+KrasG12D/+ mice restored intracellular glutamine levels and decreased the translocation of essential amino acids (Figure 2A), suppressing mTOR signalling, protein synthesis, and the hyperproliferative state of the crypt-progenitor phenotype. Consequently, SLC7A5 deletion attenuated polyp formation and sensitised tumours to mTOR inhibition, prolonging the survival of ViIcreAPC+/+KrasG12D/+ mice (Figure 2B). Deletion of SLC7A5 also compromised tumour formation and metastasis (Figure 2C and 2D) in the aggressive metastasis-prone, KiRA-driven ‘KiP’ model of CRC. (ViIcreAPC+/+KrasG12D/+Slc7a5−/−) mice. These CRCs, with ex vivo organoid cultures adding value to our suite of in vivo models.
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 KRAS mutations, but also for the highly aggressive CMS5 class.

Modelling and targeting right-sided colon cancers

We developed a new mouse model of right-sided colon cancer, driven by oncogenic BRAF (BRAFV600E) and loss of the epithelial β-catenin (Alk5/β-catenin) and displaying membranous, not nuclear, β-catenin localisation. We further implicated the transcriptional coactivator YAP as the driver of the Wnt-low, foetal-like signature, and found that microbial-driven inflammation supported the initiation and progression of these tumours, consistent with their preference for the microbe-rich right colon and their responsiveness to antibiotic treatment. Going forward, we will use this model to evaluate how the different environmental contexts of right- and left-sided tumours influence tumour evolution and response to therapy, and in particular interrogate the impact of microbiota and bacterial biofilms on the stem cell subpopulations of right-sided tumours. Building on the findings from the BEACON clinical trial, whereby doublet and triplet combinations targeting the EGFR/MAPK pathway showed modest efficacy in BRAF-mutant CRC, we will use our right-sided colon cancer model to evaluate novel targeted therapies.

Figure 3

Combined mutation of Braf and Aki5 drives patient-relevant right-sided colon cancer in mice. (A) Immunohistochemistry for β-catenin in intestinal tissues from VilCreERKrasG12D/+Trp53fl/fl, Slc7a5+/+, and VilCreERKrasG12D/+Trp53fl/fl, Slc7a5+/+, mice. Scale bar, 100 µm. (B) Gene set enrichment analysis showed suppression of this light (ISC signature) and positive enrichment of the foetal (spheroid signature) in right panel of BA-right sided colorectal tissues relative to WT control tissues. (C) Expression of the BA signature in right vs left-sided tumours from CRC patients, showing that the BA tumour model aligned closely to human right-sided disease. (D) Expression of the BA signature was associated with a shorter survival after relapse.

Overall, these approaches will further inform our understanding of CRC pathogenesis, and provide a platform for the development of novel stage- and subtype-specific therapies.

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