Colorectal cancer (CRC) 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 underpinning CRC. Our overarching goals are to identify early-stage diagnostic biomarkers and develop stage- and subtype-specific targeted therapies.

Most colorectal tumours 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 oncogenic and tumour-suppressive pathways, including KRAS, TP53, and TGFβ. In this past year, we have developed tractable models of CRC ranging from early-stage adenomas through to treatment-refractory, KRAS-mutant CRCs, with in vivo organoid cultures adding value to our suite of in vivo models.

The Wnt-antagonist NOTUM is a druggable mediator of cell competition in early-stage CRC. Widespread screening can detect tumours at early stages amenable to therapeutic intervention, rendering an urgent need for the validation of early-detection biomarkers and the identification of druggable targets to prevent progression of early-stage disease. We therefore sought to understand how common-initiating mutations impact the dynamics of adenoma formation.

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 (Flanagan et al., 2021, Nature). Using gene expression profiling, we found that APC-deficient adenomas expressed an abundance of transcripts for several Wnt antagonists, relative to APC-proficient tissues, with the most highly upregulated gene, NOTUM, encoding a secreted Wnt inhibitor that disrupted Wnt signalling (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 ViCReAPCfl/fl 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. Secreting NOTUM could therefore act in a paracrine fashion to inhibit Wnt signalling in non-neighbouring transformed wild-type ISC-environment, inducing their differentiation and withdrawal from the stem cell pool (Figure 1D). By contrast, Wnt-gang-independent, APC-deficient, super-competing cells could expand unabated with their progeny taking over the entire intestinal crypt.

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

Epithelial TGFβ/ALK5 engages growth-factor signalling to drive intestinal tumourigenesis with aggressive features

Building on our work with early-stage CRCs, we sought to develop means to identify so-called born-to-be-bad CRCs that are endowed with inherent metastatic potential, which enables metastasis-founder cells to disseminate before the primary tumour is clinically detectable. Indeed, histological assessment alone fell short of reliably identifying early-stage aggressive lesions destined to progress to metastatic spread. By transcriptionally profiling an early-stage human CRC cohort, enriched for born-to-be-bad lesions that went on to relapse, we correlated aggressive traits with elevated epithelial cell-intrinsic—rather than stromal—TGFβ signalling (Figures 2A–2C), alongside oncogenic KRAS mutations and APC deficiency (Flanagan et al., 2022, Nature Comms).

We therefore generated ViCReAPCfl/fl;KrasG12D;Apcfl/ﬂ mice, where the AKSCA allele encoded a constitutively active form of the TGFβ/ALK5 receptor, which instigated downstream TGFβ signalling in the intestinal epithelium. We found that, in the presence of concurrent Apc and Kras mutations, epithelial-specific activation of TGFβ signalling elicited rampant acceleration of intestinal tumourigenesis, engendering dissemination-prone tumours with born-to-be-bad transcriptional features. Mechanistically, epithelial TGFβ signalling induced a growth-promoting EGFR-signaling module that synergised with mutant APC and KRAS to drive MAPK signalling, sensitising tumour cells to MEK inhibitors and significantly prolonging survival (Figure 2D). Our data suggested that the convergence of activated Wnt, MAPK, and TGFβ/ALK5 signalling drove mitogenic and survival pathways that could be targeted therapeutically to slow the progression of intestinal tumours with aggressive behavioural traits.

Whereas tumour-suppressive roles are often ascribed to epithelial TGFβ signalling in CRC, our study found that epithelial cell-intrinsic TGFβ/ALK5 activation synergised with Wnt and MAPK signalling to drive intestinal tumourogenesis. Indeed, we identified epithelial TGFβ/ALK5 signalling as a potentially actionable, predictive biomarker in poor-prognosis, dissemination-prone early-stage CRCs that could reliably identify at-risk patients, offering an opportunity for early therapeutic intervention at a potentially curable stage. These findings were in line with the ‘Big Bang’ model of CRC progression, which predicts that pro-invasive behaviour could be installed early in the disease trajectory. Overall, we identified epithelial TGFβ/ALK5 signalling both as a determinant of early dissemination and a potential therapeutic vulnerability of CRCs with born-to-be-bad traits.
Kras allelic imbalance drives MAPK-dependent tumour initiation but sensitizes to MEK inhibition treatment to early metastasis.

Towards this aim, we developed genetically engineered mouse models, which allowed the detection of wild-type Kras in the context of oncogenic KrasG12D in the phenotypically normal premalignant intestinal epithelium, the crypt-progenitor phenotype induced by acute Apc loss, long-term APC-deficient tumour development, and the metastatic setting. In the homeostatic small-intestinal epithelium, we found that mutant KrasG12D increased MAPK signalling, promoting enterocyte proliferation and suppressing Paneth-cell differentiation, with the deletion of the wild-type allele exacerbating these phenotypes and additionally increasing the abundance of secretory goblet cells, suggesting that wild-type Kras restrains the activity of oncogenic KrasG12D in the premalignant setting. We further found that deletion of wild-type Kras potentiated KRASG12D activity and downstream MAPK signalling, increasing the capacity of KRASG12D-mutant APC-deficient cells to dedifferentiate and initiate tumourigenesis, rendering tumours addicted to oncogenic KrasG12D. In turn, however, this rendered tumours addicted to oncogenic KRASG12D and KRASG12D-driven tumours resistant to MEK1/2 inhibition (Figure 3A) by dampening their dependence on MAPK signalling, posing a major clinical challenge. Importantly, deletion of wild-type Kras in oncogenic KrasG12D-driven, p53-mutant, aggressive tumours promoted initiation but significantly perturbed tumour progression and metastasis, reducing emoted morphological features, compromising invasiveness, and altering the tumour microenvironment. Furthermore, the loss of wild-type Kras significantly accelerated tumourigenesis and reduced survival (Figure 3B) in our aggressive NOTCH1-driven, KRAS-mutant intestinal adenocarcinoma model that metastasised to the liver (VilCreERKrasG12DTrp53fl/fl mice, designated KPN). These findings further suggested that in addition to screening CRC-patients for KRAS-mutation status, stratifying patients for KRAS allelic status might discern those who would derive benefit from inhibition of downstream effector signalling.

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