Colorectal cancer is the third most common cancer in the UK and the second most common cause of cancer mortality. The focus of our group is to understand the early changes associated with intestinal neoplasia in order to identify novel markers of the disease as well as new targets for therapy. The key intestinal tumour suppressor is the APC gene, which is mutated in approximately 80% of sporadic cancers but rarely in other cancers. This year our group was part of the SpeciCancer Cancer Research UK Grand Challenge team funded to understand the basis of tissue specificity of the driver mutations in cancer. Central to our work is the use of novel inducible models of intestinal tumourigenesis that allow us to study the functions of specific tumour suppressor genes.

Elucidating the cell-of-origin for colorectal cancer

Loss of APC is the most common mechanism of deregulated Wnt signalling in colorectal cancer (80% of cancers carry this mutation). However, in liver cancers, APC is rarely mutated and instead the Wnt pathway is activated through mutation of β-catenin. While our laboratory has identified significant differences between APC and β-catenin mutations in terms of the level of Wnt signalling they promote, one question that still puzzled us was why activating mutations in β-catenin within an intestinal stem cell, which would be long lived and should allow ample time for accumulation of β-catenin, fail to transform the intestine. For many years, the rapid turnover of the intestine (4–6 days) had suggested that the intestinal stem cell was the most likely cell-of-origin, and our work had shown that targeted Apc loss to stem cells, mice readily develop cancer. Non-stem cell could be transformed but with much less efficiency. To examine this further, we modelled the model of cancer comparing a single activating mutation of β-catenin versus bi-allelic APC mutation. We took into account the likelihood of the mutation, the requirement for two APC mutations and the fact that it took much longer for a β-catenin mutation to produce a phenotype. Using these parameters, the model predicted that if the stem cell was the cell-of-origin, one would expect β-catenin mutations, but if you include in addition transit amplifying (TA) cells then bi-allelic APC mutations were much more likely. Interestingly, the human colon has many more TA cells than the mouse, which may explain why an Apc mutation leads to small intestinal tumours in the mouse and colonic tumours in man.

Inhibiting Wnt signalling in vivo in cancers that have lost APC, deleting BCL9/9L

Different thresholds of Wnt pathway activation are thought to be required for stem cell maintenance, regeneration, differentiation and cancer. However, the principle that oncogenic Wnt signalling could be specifically targeted remains controversial. Here, we examined the requirement of BCL9/9L, constituent members of the Wnt-enhancerosome, for intestinal transformation following loss of the tumour suppressor APC. While these genes were required for stem cell maintenance of Lgr5+ intestinal stem cells and intestinal regeneration, BCL9/9L deletion had no impact upon normal intestinal homeostasis. Loss of BCL9/9L suppressed many features of acute APC loss and the subsequent deregulation of the Wnt pathway in vivo. This resulted in a level of Wnt pathway activation that favoured tumourogenesis in the proximal small intestine and blocked tumour growth in the colon. Furthermore, BCL9/9L deletion completely abrogated β-catenin-driven intestinal and hepatic transformation. We speculate these results support the right hypothesis of Wnt-driven tumour formation. Importantly, loss of BCL9/9L is particularly effective at blocking colorectal tumourigenesis and suppressing the impact of mutations that most resemble those found in human cancer (Gay et al., Nat. Comms. 2019: 10, 723).

Inhibiting Wnt Signalling when the Wnt pathway is not mutated – RAL GTPases

Alongside loss of the tumour suppressor APC, colorectal cancers are commonly driven by activating mutations in the oncoprotein KRAS. The Ral GTPases are effectors of RAS signalling and can be seen by implication as potential therapeutic targets for KRAS mutant colorectal cancers and for RAS mutant cancers more broadly. As part of a collaborative effort with Julia Corden’s group (University of Glasgow), we identified the Ral GTPases as critical regulators of Wnt signalling and maintenance of the intestinal stem (ISC) and enteroendocrine populations of the Drosophila midgut and intestinal stem cell compartment in the mammalian intestine. In Drosophila, Rala was required within ISCs for efficient regeneration downstream of Wingless (Wg) signalling, while in the murine intestine, genetic deletion of either mammalian orthologue (Rala or Ralb) resulted in both reduced ISC function and Lgr5 positivity, hypersensitivity to Wnt inhibition and impaired tissue regeneration following damage. Ablation of both mammalian orthologues resulted in rapid crypt death. Mechanistically, we found that the Ral GTPases were required for efficient potentiation of the Wnt signalling pathway through participation in the internalisation of the Wnt receptor Fz/7. As a result, ligand-dependent Wnt signalling required Ral GTPase function for efficient activation, implicating Ral GTPase function in definition and maintenance of the intestinal stem cell pool. Intriguingly, as a result of their association with Wnt receptor internalisation, where activation of the Wnt signalling pathway is uncoupled from a dependence on upregulation of secreted Wnt ligands, our ongoing research in this area focuses upon potential cross-talk between the RAS and Wnt signalling pathways.

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