COLORECTAL CANCER AND WNT SIGNALLING

Group Leader
Owen Sansom FRSE, FMEDSci

Associate Scientist
Andrew Campbell

Research Scientists
Constantinos Alexandrou[1]
Dustin Flanagan[2]
Michael Hodder[3]
Rene Jackstadt
Joel Johansson[4]
Georgios Kanellos[1]
John Knight
Dana Koludrovic
Noha Mohamed[5]
Arafath Najumudeen[6]
Madelon Paauwe[6]
Alexander Raven[3]
Johan Vandevoorde[6]
Nikola Vlahov

Scientific Officers
Catriona Ford[6]
Tam Jamieson
Xavier Cortes Lavaud
Rachel Ridgway

Graduate Students
Sigrid Fey[7]
Joshua Leach[8]
Rachael Smith[7]
Lucas Zeiger[6]

[1]Celgene
[2]MRC
[3]CRUK Grand Challenge 'SpecifiCancer'
[4]Novartis
[5]AstraZeneca
[6]CRUK Grand Challenge 'Rosetta'
[7]Pancreatic Cancer UK
[8]MRC Clinical Research Training Fellowship

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 SpecifiCancer 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 potentiate, 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 if we targeted *APC* loss to stem cells, mice would rapidly develop cancer. Non-stem cells could be transformed but with much less efficiency. To examine this further, we modelled the likelihood 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-enhanceosome, for intestinal transformation following loss of the tumour suppressor *APC*. While these genes were required for 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 tumour initiation in the proximal small intestine and blocked tumour growth in the colon. Furthermore, *Bcl9/9l* deletion completely abrogated β-catenin-driven intestinal and hepatocellular transformation. We speculate these results support the *just right* hypothesis of Wnt–driven tumour formation. Importantly, loss of *BCL9/9l* is particularly effective at blocking colonic tumourigenesis and suppressing the impact of mutations that most resemble those found in human cancer (Gay *et al.*, Nat. Comms. 2019; 10: 723).

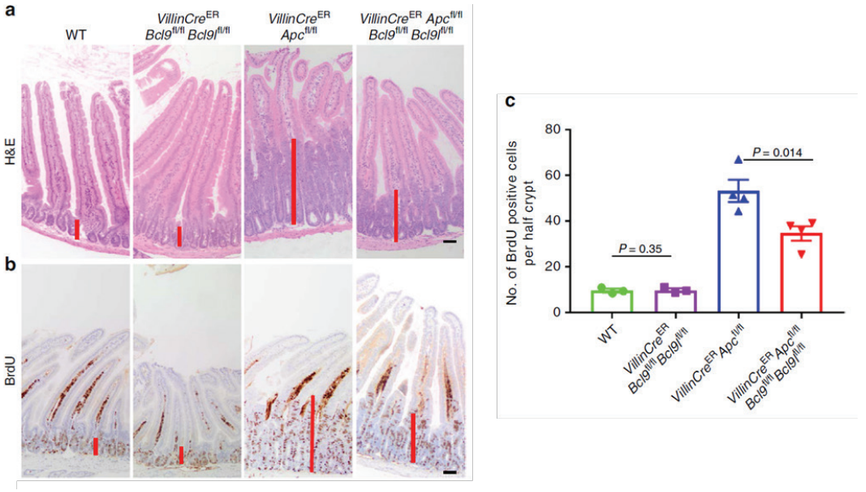

Figure 1: *Bcl9/9l* are required for intestinal transformation driven by *Apc* deficiency.
A) Representative images of haematoxylin/eosin stained wild-type (WT), *VillinCre^ER Bcl9/9l^fl/fl*, *VillinCre^ER Apc^fl/fl* and *VillinCre^ER Apc^fl/fl Bcl9/9l^fl/fl* intestinal tissue sampled at 4 days post-Cre-induction.
B) Mice were injected with BrdU prior to sampling, with subsequent immunohistochemical staining of BrdU incorporation highlighting proliferative cells in these tissue specimens as described in (A). Red bars indicate the proliferative crypt. Scale bar: 50 µm.
C) Quantification of cellular proliferation indicated by BrdU incorporation in small intestinal tissues from tissue specimens as described in (A).

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 oncogene *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 Cordero's group (University of Glasgow), we identified the Ral GTPases as critical regulators of Wnt signalling and maintenance of the intestinal stem (ISC) and enteroblast 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 Frizzled-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 dependency upon secreted Wnt ligands, as in the context of APC deficiency, Ral GTPase are no longer required for efficient pathway activation (Johansson *et al.*, *Cell Stem Cell*, 2019; 24: 592–607.e7). As a number of the Ral GEF (guanine nucleotide exchange factor) molecules which control Ral GTPase activity are in turn regulated by the RAS GTPase molecules, our ongoing research in this area focuses upon potential cross-talk between the RAS and Wnt signalling pathways.

Publications listed on page 91

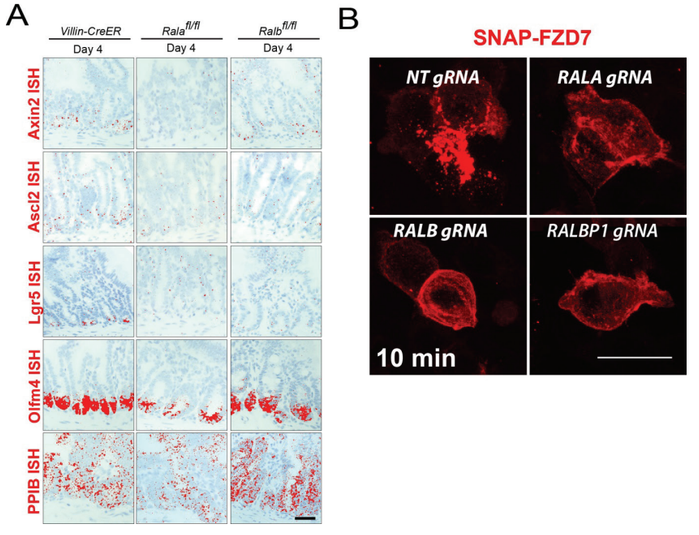

Figure 2: Ral GTPases are required for efficient activation of Wnt signalling.
A) Representative images of *VillinCre^ER*, *VillinCre^ER Rala^fl/fl* and *VillinCre^ER Ralb^fl/fl* intestinal tissue sampled at 4 days post-Cre-induction, stained by RNA in situ hybridisation (RNAscope) for the intestinal stem cell markers *Olfm4* or *Ascl2*, Wnt target genes *Lgr5* or *Axin2*, or the positive control *Ppib*, with red dots representing individual mRNA molecules. Scale bar: 50 µm.
B) Subcellular localisation of the Wnt-receptor Frizzled-7 visualised through confocal imaging of SNAP-tag labelled receptors following stimulation of HEK293T cells with serum for 10 minutes. Internalisation is assessed in populations of cells where *Rala*, *Ralb* or their common effector gene *Ralbp1* have been disrupted using CRISPR-Cas9 gene editing technologies and compared to control (NT gRNA) cells.