

# Colorectal Cancer and Wnt Signalling

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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 that is mutated in approximately 80 percent of sporadic cancers. 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.

## Wnt signalling drives intestinal regeneration and tumourigenesis via FAK

We have previously shown that transformation of the intestine requires the activation of Wnt signalling. However, little is known about the physiological role of this pathway during intestinal regeneration. We have now shown that Wnt signalling mediates the normal homeostatic response following exposure to DNA damage or deletion of genes required for crypt cell survival. To further identify critical pathways downstream of Wnt signalling, we have performed microarray analysis on regenerating epithelia and compared this to intestinal enterocytes lacking *Apc*. We have found that the integrin signalling pathway is deregulated in both scenarios and that genetic deletion of the critical mediator of integrin signalling, FAK completely blocks intestinal regeneration. Mechanistically, this appears to be due to the inability of FAK-deficient intestine to upregulate AKT/PKB during intestinal regeneration. Moreover, FAK-deficient intestinal epithelium is completely resistant to tumourigenesis caused by *Apc* loss, arguing that FAK could be an important chemopreventive target in colorectal cancer (Ashton *et al.*, *Dev. Cell* 2010; 19: 259).

## Cyclin D2 drives proliferation following *Apc* loss

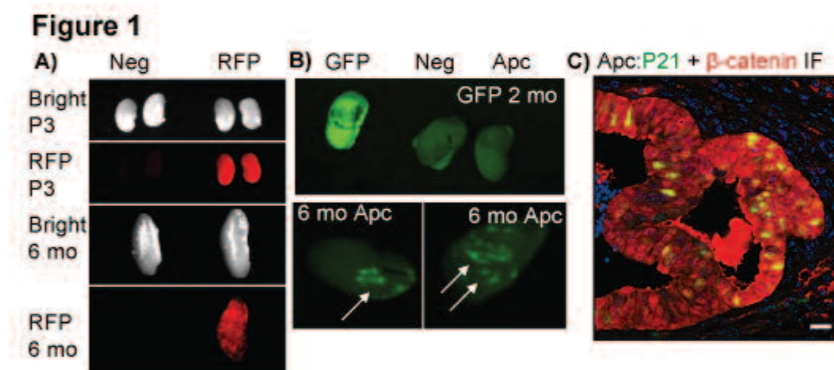
We have previously shown that c-Myc is the key effector downstream of *Apc* loss *in vivo*. Since it may be difficult to pharmacologically inhibit transcription factors such as c-Myc, investigating more druggable targets of the Wnt/c-Myc

pathway within the intestine may reveal potential therapeutic targets for colorectal cancer. Recent work in our laboratory has shown that the cyclin D2/cyclin-dependent kinase 4/6 (CDK4/6) complex promotes hyperproliferation in *Apc*-deficient intestinal tissue and *Apc*<sup>Min/+</sup> adenomas. We showed that the hyperproliferative phenotype associated with *Apc* loss *in vivo* was partially dependent on the expression of cyclin D2. Most importantly, tumour growth and development in *Apc*<sup>Min/+</sup> mice was strongly perturbed in those lacking cyclin D2. Furthermore, pharmacological inhibition of CDK4/6 suppressed the proliferation of adenomatous cells (Fig. 1). This suggests inhibition of this complex may be an effective chemopreventive strategy in colorectal cancer (Cole *et al.*, *Cancer Res.* 2010, published online 24 Aug).

## Combined *Apc* and *p21* loss provokes renal carcinoma

Senescence has been implicated as an important mechanism of tumour suppression in a number of human malignancies, including colorectal cancer. However, we still have a relatively poor understanding of how the underlying mutations that occur in cancer cause senescence and its relevance *in vivo*. Although *Apc* is the key colorectal cancer tumour suppressor gene, it is rarely mutated in other cancers. We have examined the capacity of *Apc* loss to induce senescence in the intestinal epithelium, where it is a *bona fide* tumour suppressor gene, and in the renal epithelium, where it is not closely

**Figure 1**  
A+B) Kidneys imaged on OVI100 microscope. A) Mice at P3 and 6 months imaged for RFP. Note kidneys from *AhCre* mice without the *Rosa26* tdRFP reporter (labelled Neg) show little RFP positivity, whilst *AhCre Rosa26* tdRFP mice (labelled RFP) on figure show marked RFP positivity. B) Mice imaged at 2 months (2 mo), top panel and 6 months (6 mo), bottom panels for GFP. Top panel (left kidney), note strong positivity for GFP in *AhCre+ ZIEG+ Apc+/+* (labelled GFP). Middle kidney is from *AhCre- ZIEG+ Apcfl/fl* (labelled Neg) shows only autofluorescence. Right kidney shows no detectable GFP positivity in *AhCre+ ZIEG+ Apcfl/fl* (labelled Apc) kidney at 2 month. Bottom panel shows at 6 months a small number of positive foci within *AhCre+ ZIEG+ Apcfl/fl* kidneys, suggesting that *Apc* deficient cells are being deleted. C) Co-immunofluorescence (IF) for *b-catenin* (red) and *p21* (green) showing high expression of both within premalignant lesions from *AhCre+ Apcfl/fl* mice.



associated with human cancer. Within the renal epithelium, loss of *Apc* function led to an induction of senescence, however bypassing senescence through combined *Apc* and *p21* or *Ink4A* gene deletion rapidly initiated renal carcinoma. Within the intestinal epithelium, loss of *Apc* did not induce senescence. Moreover, combined *Apc* and *p21* or *Ink4A* loss had no impact upon tumourigenesis. Taken together, these results show that *Apc* loss *in vivo* invokes a senescence programme in a context-dependent fashion (Cole *et al.*, *EMBO Mol. Med.* 2010; 2: 472). This implies escape from senescence is not a crucial pathway in colorectal cancers that are initiated by *Apc* loss. It also suggests that senescence following *Apc* loss may act as a barrier to renal carcinogenesis, potentially explaining why renal carcinoma is not observed in FAP (Familial Adenomatous Polyposis) patients who are germline heterozygous for *Apc*.

## *Pten* and *p53* suppress adenoma development and progression

*Pten* and *p53* have been shown to act as tumour suppressors in a range of tissue types. To study *Pten* and *p53* function in the intestine, we have used a conditional transgenic strategy to delete *Pten* or *p53* from the murine intestinal epithelium. We have shown that neither *Pten* nor *p53* affect the normal architecture or homeostasis of the epithelium. However, loss of *Pten* or *p53* in the context of *Apc* deficiency greatly accelerates intestinal tumourigenesis, leading to rapid development of adenocarcinomas. We conclude, therefore, that *Pten* and *p53* are redundant in otherwise normal intestinal epithelium but in the context of activated Wnt signalling, function to suppress progression to adenocarcinoma.

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**Figure 2**  
Treatment with the selective CDK4/6 inhibitor PD0332991 strongly suppresses proliferation in established adenomas from *Apc*<sup>Min/+</sup> mice. A) Box plot showing no change in intestinal enterocyte proliferation when *Apc*<sup>Min/+</sup> mice are treated 150mg/kg daily for 5 days with PD0332991 (Mann-Whitney,  $p=0.76$ ,  $n=3$ ). B) Box plot showing a significant reduction in proliferation in tumours arising from these *Apc*<sup>Min/+</sup> mice treated with PD0332991 when compared to vehicle treated (Mann-Whitney  $t$  test,  $p=0.04$ ,  $n=3$  mice, 5 adenomas per mouse). C) Immunohistochemistry for BrdU showing lower levels of BrdU incorporation within adenomas from *Apc*<sup>Min/+</sup> mice (black arrows) when compared to the surrounding normal tissues (red arrows). Scale bar represents 20 $\mu$ m.

