Studying the consequences of immediate loss of gene function in the intestine: APC

A.R. Clarke1
Cardiff School of Biosciences, Cardiff University, Cardiff CF10 3US, Wales, U.K.

Abstract
The use of mouse models to study neoplasia is proving particularly powerful in dissecting the mechanisms underlying disease initiation and progression. However, the majority of these models have been somewhat limited in studying the very early effects of loss of gene function, as tumour initiation relies upon either constitutive loss of gene function or spontaneous somatic loss of function. We have therefore adopted a strategy of using an inducible Cre-lox-based system to analyse the effects of loss of gene function, the use of which is reviewed here for the intestinal tumour suppressor APC (adenomatous polyposis coli). Using this approach, we have conditionally and synchronously inactivated APC in virtually all the epithelial cells of the adult murine small intestine. After 5 days following induction of Cre-mediated recombination, mice show grossly altered crypt/villus architecture. Deficiency in APC perturbs migration, alters the normal programme of differentiation and results in increased proliferation and apoptosis. Microarray analysis reveals the transcriptome to be significantly altered; reflecting both gross phenotypic changes and changes in transcriptional activation. These findings demonstrate that APC is indeed the critical determinant of cell fate in the intestinal epithelium, explaining its role as the cellular ‘gatekeeper’ in preventing neoplasia.

The APC (adenomatous polyposis coli) gene encodes the APC tumour suppressor protein, germline mutation of which characterizes FAP (familial adenomatous polyposis), an autosomal syndrome that is characterized by multiple colorectal lesions [1]. APC is also mutated in the majority of sporadic colorectal cancers, and inactivation of APC is recognized as a key early genetic change in colorectal tumorigenesis. APC has been implicated in a wide range of cellular functions, including adhesion, migration, apoptosis and cell cycle control, although the relative contribution made by these activities to tumour suppression is somewhat unclear. Many of these activities have in turn been attributed to a role for APC in Wnt signalling [2–4]. Thus loss of APC in adenomas from both FAP patients and Apcm14 mice leads to elevated levels of β-catenin, as it is no longer targeted for degradation. The absence of APC is therefore considered to lead to activation of TCF (T-cell factor)/LEF (lymphoid enhancer factor)-dependent transcription. Targets of Wnt signalling include the oncogenes c-myc and Cyclin D1, providing an attractive hypothesis that loss of APC would lead to dysregulated proliferation [4].

Given the numerous diverse cellular activities ascribed to APC deficiency and the inherent difficulty in analysing systems characterized by multiple genetic change (such as adenomas), we have used an inducible Cre-lox system to investigate the immediate consequences of APC loss in otherwise normal murine epithelium and so specifically address the relevance of loss of APC function to the early stages of colorectal neoplasia [5].

To achieve this, mice were generated that carried an inducible Cre vector, pAhCre, which utilizes the rat CYP1A1 promoter to drive Cre expression. Cre activity could be induced in these mice following a single intraperitoneal injection of the inducing agent β-naphthoflavone [6,7]. This approach was then applied to mice homozygous for a loxP-flanked APC allele [8], such that activation of Cre would lead...
to conditional deletion of APC within the adult intestine. Histological analysis of the organs from the β-naphthoflavone-induced Cre\(^+\) Apc\(^{flox/flox}\) mice revealed a marked phenotypic change within the small intestine such that crypt-like cells occupied the majority of the crypt–villus axis. This ‘crypt-like’ area had expanded to contain in excess of 80 cells, in contrast with the normal 44 cells observed within wild-type crypts.

Loss of APC also perturbed the normal programme of differentiation in the intestine. Thus both the goblet and Paneth lineages were perturbed. Notably, the distribution of Paneth cells directly parallels that observed in another model of altered crypt development, namely EphB3\(^{−/−}\) mice [9]. Coincident staining for alkaline phosphatase and villin was used to define the extent of villus differentiation, which was confined to the remaining histologically normal areas in the Cre\(^+\) Apc\(^{flox/flox}\) mice.

Previous studies have characterized an amplification zone within the normal crypt [10]. To test whether this amplification zone was altered, mice were injected with BrdUrd (bromodeoxyuridine) and killed 2 h later. In control mice, cycling cells were confined to the expected zone, but APC-deficient crypts showed a marked increase in the proliferation index, with proliferation now occurring independently of position. BrdUrd incorporation can also be used to track cell movement within the crypt, by scoring cell position 24 h after exposure to BrdUrd. Using this approach, APC deficiency clearly blocks any cell movement within the crypt.

We also analysed the total number of BrdUrd-positive cells at 24 h. This number should increase arithmetically through each division, and, indeed, wild-type crypts show an approximate doubling of labelled cells from 2 to 24 h. No such increase was observed in the Cre\(^+\) Apc\(^{+/+}\) mice, implying that BrdUrd-positive cells either were failing to progress through mitosis or were being deleted. To investigate this further, we scored the mitotic index and found this not to be elevated in the mutant. This argues against a blockage within mitosis, implying that cells either fail to enter mitosis or are being deleted. Histological examination supports the latter notion, with greatly elevated levels of apoptosis observed in the APC mutant mice when scored either histologically or immunohistochemically with an anti-(caspase 3) antibody.

We also investigated levels of β-catenin within the mutant tissue by immunohistochemistry. This showed that, 5 days after the induction of recombination, β-catenin had relocalized to the nucleus in the mutant tissue. To test whether nuclear β-catenin was activating transcription of its known target genes, microarray analysis was performed using the Affymetrix platform. This revealed many hundreds of up-regulated genes [5], a proportion of which have been associated previously with Wnt signalling, such as c-myc, CD44 and EphB3 (e.g. [3,7]).

In summary, the Cre-lox-based system that we have used represents a very powerful analytical tool for any gene within the intestine. The principal benefit of this approach is the ability to inactivate genes within otherwise normal adult intestinal epithelium, and to do so at almost 100% penetrance. For APC, this has allowed us to characterize a critical determinant of cell fate in murine small intestinal epithelium [5]. By removing a single gene, we have produced many of the phenotypes that are associated with early colorectal lesions: failed differentiation, increased proliferation, aberrant migration and loss of euploidy. Furthermore, we show a large number of molecular changes centring on activation of β-catenin/TCF4 transcription. Therefore, from a very early stage in colorectal cancer, there are a number of molecules that can be used as markers for colorectal cancer and potentially as targets for chemoprevention. Given our improved understanding of the very early stages of colorectal cancer and the phenotype of APC-deficient cells, the challenge will now be to design therapies that use these properties to delete them selectively.

References

Received 30 March 2005