



Mini-symposium: Gastrointestinal pathology

# An update on the pathophysiology of the intestinal crypt

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**Summary** The epithelial cells of the intestinal crypts enable the gut to perform its physiological functions. The intestinal stem cell is responsible for maintaining the epithelial cell population of the crypts. Little is known of the stem cells in terms of their identity, numbers and locations within the crypts. The accumulation of genetic mutations within stem cells and the subsequent spread of these mutated clones of cells leads to the growth of adenomas. This article will focus on the maintenance of the cell population of the intestinal crypts by the intestinal stem cell, and will consider the evidence relating to stem cell identity, location and function, the molecular regulation of stem cell function and the development and growth of epithelial tumours within the gastrointestinal tract.

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## Introduction

The intestine is responsible for the digestion and absorption of ingested food and the excretion of numerous substances from the body. It also forms the largest endocrine organ in the body. These diverse roles are facilitated by the architecture of the intestinal crypts and villi, and the population of cells residing therein. The intestinal stem cell has the role of maintaining the epithelial cell population of the crypts. Surgical specimens demonstrate the morphological manifestations of damage to the intestinal stem cell, and a knowledge of the

molecular mechanisms involved provides the pathologist with a better understanding of the underlying process being examined. Much, however, remains unknown about the intestinal stem cells, such as their number and location, as well as their fundamental properties. Genetic and molecular changes in intestinal stem cells are thought to lead to tumorigenesis.

Recent work suggests that stem cells from extraintestinal organs may have the capacity to colonize the bowel and replenish the damaged bowel epithelium, raising possibilities for the therapeutic recovery of damaged intestinal mucosa following insults such as radiotherapy. This phenomenon is termed *stem cell plasticity*.

Inflammation of the bowel seen in conditions such as Crohn's disease leads to the formation of a cell lineage with unique properties, termed the

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ulcer-associated cell lineage (UACL), apparently directly from intestinal stem cells.

This article will review the structure and function of the crypts in the small and large intestines, and will focus on the central role of the intestinal stem cell in homeostasis and in pathological states such as inflammation and neoplasia.

## Basic physiology of the small and large bowel crypts

The major role of the small intestine is in the processing and absorption of ingested nutrients. Ingested food is exposed to pancreatic enzymes that act to create smaller molecules for further digestion. Pancreatic lipase and bile act intraluminally to generate free fatty acids and monoglycerides, which pass into the surface enterocytes and are then released into the portal venous system for systemic absorption. The brush border created by numerous apical microvilli on small intestinal enterocytes bears additional enzymes involved in nutrient breakdown and processing, the smaller molecules thus generated being absorbed across the epithelial layer, from where they pass into the portal venous system for systemic absorption.

The villus architecture increases the surface area over which this process can occur. The colon is involved in the terminal phases of digestion and, in doing so, forms and stores faeces through the further recovery of water and electrolytes, and the addition of mucus and bacteria. Four main cell types constitute the epithelial cell population of the intestinal crypts:

1. *Columnar cells* (colonocytes in the colon and enterocytes in the small bowel) are involved in the absorption of nutrients. They are the most abundant cell type in the crypt epithelium. They bear apical microvilli lined by a brush border which contains enzymes that function in the terminal digestive processes.
2. *Goblet cells* are involved in mucus secretion, which acts to trap and expel microorganisms and enables the creation of a moist viscid environment within the lumen of the small bowel.
3. *Neuroendocrine cells* contain dense-core neurosecretory granules that are involved in paracrine or endocrine hormone secretion.
4. *Paneth cells* have a role in keeping the crypt sterile. They bear apical secretory granules whose contents include lysozyme, defensins, cryptidins and tumour necrosis factor  $\alpha$ .<sup>1</sup>

Other cells found in the crypts, which are much fewer in number compared with those cell types described above, are the caveolated cells<sup>2</sup> and M (membranous) cells<sup>3</sup> of the Peyer's patches, which function in the transport of luminal antigens into extracellular spaces to expose them to cells of the immune system.

## The basic architecture of the intestinal crypts

Each epithelial cell type within the crypt is thought to originate from a stem cell. There is constant renewal and upwards migration of the epithelial cell population of the crypts, which occurs in a manner analogous to that of an escalator.

Towards the base of the crypt are thought to be located the stem cells. The stem cells usually divide asymmetrically, with formation of an identical daughter stem cell and committed progenitor cells. Progenitor cells retain the ability to divide until they terminally differentiate, thus forming a transit amplifying population that has a limited proliferative potential. After a small number of divisions, these cells decycle and undergo differentiation. The terminally differentiated epithelial cells then migrate towards the top of the crypt, from where they are lost to the bowel lumen by apoptosis, shedding or phagocytosis.<sup>4</sup> There is constant replacement of mature cells by newer cells migrating up this so-formed crypt-villus axis. The lifespan of a terminally differentiated intestinal epithelial cell is approximately 2–7 days. This constant renewal of the mature epithelial cells is thought to be a protective mechanism whereby any tumorigenic mutations in these mature cells are lost.

The lamina propria forms an important support function for the intestinal crypt. Within the lamina propria are located a type of myofibroblast called the intestinal subepithelial myofibroblast. These are subjacent to the epithelium. They form a fenestrated sheath, innervated by the enteric nervous system and connected in a cellular syncytium via gap and adherens junctions.<sup>5</sup> These cells are functionally diverse, with roles in mucosal protection, water and electrolyte transport. They secrete growth factors such as hepatocyte growth factor, keratinocyte growth factor and transforming growth factor (TGF)  $\beta$ 2, which are essential for epithelial cell differentiation and proliferation, representing an example of epithelial–mesenchymal cross-talk.<sup>6</sup>

## The intestinal stem cell

The functional definition of a stem cell is a primitive, undifferentiated cell that has the capacity for both self-renewal and the production of daughter cells that are committed to the formation of every adult cell lineage within their tissue of origin.<sup>7</sup> Stem cells can regulate the rate of cell production within tissues in order to maintain homeostasis, for example upregulating daughter cell production in response to increased demand.

The stem cells are maintained in a specialized *niche*, which provides an optimal microenvironment for stem cell function. The niche is formed by the supporting mesenchymal cells, which regulate stem cell behaviour through the paracrine secretion of growth factors and cytokines.<sup>7</sup> The niche is functionally defined through a cessation of stem cell potential when stem cells are removed from the niche; in effect, the stem cells are committed to differentiation when removed from the stem cell niche environment.<sup>8</sup>

### Location of stem cells

In the small intestine, the stem cells are located in the crypt base just superior to the Paneth cells. The colon is thought to contain basally located stem cells, except in the ascending colon, where the stem cells are thought to be located towards the middle of the crypt.<sup>9</sup>

Experimental evidence demonstrating the location of the stem cells in the small intestine (called the *stem cell zone hypothesis*) came from microscopic morphometry and autoradiography labelling studies. These demonstrated that stem cells were located at positions 1–4 in the small intestinal crypt base. Here they are induced to proliferate but not differentiate until they reach position 5.<sup>10</sup> These experiments additionally suggested that Paneth cells are also thought to appear initially at position 5 and then migrate down into the crypt base.<sup>11</sup>

### The number of intestinal stem cells within crypts

There is a debate over whether a single stem cell or multiple stem cells give rise to the epithelial cells of the crypt. Support for the existence of a single stem cell comes from observation of the existence of a monoclonal crypt following irradiation damage; in effect, a single surviving stem cell can regenerate an entire crypt.<sup>12</sup>

Stem cell number may vary with different sites in the bowel and the crypt cycle.<sup>13</sup> Indeed, some

workers<sup>14,15</sup> suggest the existence of 4–6 stem cells at the bottom of the crypt at cell positions 4–5, just superior to the Paneth cells in the small intestine. Others have proposed the existence of 16 or more stem cells within a single intestinal crypt.<sup>16</sup> Overall, it is thought that a single or very small number of stem cells gives rise to the epithelial population of a single crypt.<sup>9</sup>

With age, the mitochondrial DNA of the intestinal crypt acquires mutations enough to produce a biochemical deficit in cytochrome c oxidase activity, which can be detected using immunohistochemical techniques after staining for cytochrome c oxidase activity, mutations resulting in a lack of cytochrome c oxidase staining. Some crypts are uniformly negative, suggesting that the mutation occurred in a single stem cell that clonally expanded to form the entire epithelial cell population of the crypt. Other crypts show strands of negativity extending from the crypt base to the top of the crypt occupying only partial regions of the crypt. This suggests that one of multiple stem cells within the crypt had acquired a mutation, the defect being passed onto its progeny and accounting for the strand-like staining pattern.<sup>17</sup>

### Are the crypts monoclonal or polyclonal structures?

The unitarian hypothesis supports the idea that a single gastrointestinal stem cell is multipotent and has the capacity to produce the entire repertoire of adult cell types in the intestinal crypts.<sup>18</sup> This would give rise to a monoclonal crypt population. In man, the intestinal crypts appear to be monoclonal and the villi appear to be polyclonal structures.

Studies in man have utilized natural mutations and polymorphisms to analyse the clonality of the intestinal crypts. The clonal origin of the small intestinal epithelium was elegantly demonstrated by Novelli et al.<sup>19</sup> using tissue from an XO/XY patient with familial adenomatous polyposis (FAP). From the uniform expression within a single crypt of a single genotype (either expression of or lack of expression of a Y chromosome), intestinal crypts were demonstrated to be monoclonal structures. The villus epithelium contained a mixture of XO and XY cells, demonstrating the polyclonal nature of the villus.

O-Acetyl transferase (OAT) is an enzyme responsible for the O-acetylation of sialic acid in goblet cell mucin. Non-O-acetylated mucus stains positively with mild periodic acid Schiff (mPAS)

reagent. Approximately 9% of the Caucasian population have the homozygous mutation (OAT-/-), resulting in mPAS staining of goblet cells carrying the mutation, thus allowing their identification. Of the total population, 42% are heterozygous (OAT+/-) and do not show positive staining with mPAS unless further mutation causes loss of the remaining allele. Second hits in the heterozygote increase with age, and this results in randomly located positive mPAS-stained crypts with all intracryptal goblet cells affected from base to lumen.<sup>20</sup> Following irradiation, the crypts initially showed a partial staining pattern that became more uniform with time. The time for partially transformed crypts to become clonal after radiation damage was about a year (termed the *crypt stabilization time*).<sup>21</sup>

Monoclonal origins of crypts were also demonstrated using heterozygous polymorphism in the gene encoding glucose 6-phosphate dehydrogenase (G6PD) to demonstrate patch size.<sup>22</sup> Lyonization in females results in mosaicism for the expression of X-linked genes such as G6PD. Groups of cells sharing the same X-inactivation expression pattern are termed a *patch*. Sardinian women heterozygous for the G6PD gene demonstrated large monophenotypic patches of G6PD-positive or G6PD-negative crypts. Patches may be formed from the progeny of one cell or several cells that share the same X-inactivation pattern. Patches of up to 450 crypts in size with distinct patch boundaries and no evidence of mixed crypts at the patch border were seen. This observation supports the view that human colonic crypts are clonal populations.

## Stem cell division and niche succession

Studies in the mouse small intestine have shown that intestinal stem cells commonly undergo asymmetrical division. By labelling DNA template strands with tritiated thymidine during development or tissue regeneration, and by labelling the newly synthesized daughter strands with bromodeoxyuridine, both strands can be visualized during cell division. Results showed that the original template DNA is retained within the stem cell, and the newly synthesized strands are passed onto the daughter cells that differentiate into adult intestinal cell lineages. Newly synthesized DNA is more prone to replication-induced mutation; therefore, by not retaining this newly synthesized DNA, the intestinal stem cell utilizes an inherent mechanism of genome protection.<sup>23</sup>

There are three possible outcomes of stem cell division:<sup>24</sup>

1. asymmetrical division producing one stem cell and one daughter cell per division ( $r$  divisions);
2. symmetrical division with self-replication in which two stem cells are produced ( $p$  divisions);
3. symmetrical division with stem cell loss in which both daughter cells go onto differentiate ( $q$  divisions).

The term 'niche succession' refers to the stochastic extinction of stem cell lines by self-replication ( $p$  divisions), resulting in the crypt containing a monoclonal cell population derived from this single cell. This model of niche succession is suggested as the mechanism that may allow stem cell mutations to reach clonal dominance.<sup>25</sup>

## Crypt fission

This is the means by which crypts spread within the human intestine. Crypts undergo basal bifurcation followed by longitudinal division, with the ultimate formation of two daughter crypts. This process is called the crypt cycle, which lasts 9–18 years in the normal human colon. Crypt fission is rarely observed in the normal mucosa. Physiologically, the process is important in the postnatal period<sup>26</sup> and for regeneration following irradiation.<sup>27</sup>

It is thought that a doubling in stem cell number is the factor prompting crypt fission.<sup>24</sup> This has implications for any genetic events that cause an increase in the replication rate (symmetrical  $p$  divisions) of the stem cell, as such mutations would promote the spread of a clone through crypt fission. There is a significant increase in the rate of crypt fission in the colorectal mucosa in a range of pre-neoplastic states.<sup>28,29</sup> The main mode of growth of colonic adenomas in FAP is through crypt fission.<sup>29</sup> The crypt fission index (the proportion of crypts in fission) is upregulated in adenomas and in non-adenomatous crypts in FAP, in which is seen unusual asymmetrical budding from the superficial and mid crypts.<sup>30,31</sup> Mutated clones can spread by crypt fission.

## Molecular regulation of intestinal stem cell function

The molecular signalling pathways within stem cells that control their proliferation, development and differentiation are under intensive investigation.

Defects in these pathways are implicated in the causation of intestinal neoplasia. The pathways involved include the Wnt, bone morphogenetic protein (BMP), Notch, TGF- $\beta$  and SMAD signalling pathways.

### The Wnt signalling pathway

This pathway is thought to play a role in the establishment of the stem cell compartment in the developing intestine. T-cell factor (Tcf) 4, a member of this pathway, is expressed in the developing intestine. Mice with a targeted deletion of Tcf4 lack proliferating cells in the small intestine and are presumed to lack a functional stem cell compartment. Therefore, Tcf4 transcription, activated by Wnt signalling from cells in the underlying mesenchyme, is vital for the establishment of a stem cell population in the developing small intestine.<sup>32,33</sup>

The Wnts are a family of secreted growth factors, which are evolutionarily conserved cysteine-rich glycoproteins that can signal in both a paracrine and an autocrine fashion.<sup>34</sup> They act on frizzled (Fz) receptors<sup>35</sup> and low-density lipoprotein-related protein.<sup>36</sup>

In the canonical Wnt pathway, the adenomatous polyposis coli (APC) protein forms a subcellular complex with glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ) and axin. This complex leads to the phosphorylation, ubiquitination and proteasomal degradation of  $\beta$ -catenin, thus maintaining low cytosolic and nuclear levels of  $\beta$ -catenin.

In the presence of Wnt signalling through Fz, there is activation of the cytoplasmic protein 'dishevelled'. This causes an inhibition of GSK3 $\beta$ , leading to raised cytosolic levels of  $\beta$ -catenin.<sup>37</sup>  $\beta$ -Catenin then translocates to the nucleus, where it interacts with the Tcf/lymphocyte enhancer factor (LEF) family of DNA-binding proteins, converting them from transcriptional repressors to activators, which results in the transcription of target genes that increase cellular proliferation. Some of these genes include those implicated in tumorigenesis in the gastrointestinal tract, such as *c-myc*, *c-jun* and *Fra-1*, and those for the urokinase-type plasminogen activator receptor, fibronectin, CD44 and matrilysin.<sup>38,39</sup> In the absence of Wnt signalling, the resultant low levels of nuclear  $\beta$ -catenin restore the transcriptional repressor function of Tcf.

As well as having a role in the control of cellular proliferation, the Wnt pathway allows positioning of Paneth cells within the crypt-villus axis and the expression of Paneth cell markers.<sup>40</sup>

### BMP pathway

BMP is a member of the TGF family of proteins. The pathway is involved in the regulation of intestinal development.

BMP proteins bind to their type II receptor, which recruits type I receptors (BMPRI1A or BMPRI1B). A signal is then transduced to the nucleus via SMAD transcription factors. The BMP signal is antagonized by Noggin (Nog), an extracellular protein that binds BMP and prevents its activity.<sup>41</sup> In humans and adult mice, the highest levels of BMP4 have been seen in differentiating and mature colonocytes.<sup>42</sup>

The BMP pathway exerts an inhibitory effect on  $\beta$ -catenin that appears to be mediated through the phosphate and tensin homologue deleted on chromosome 10 tumour suppressor gene product (PTEN); a dual protein and lipid phosphatase; this acts via phosphatidylinositol-3 kinase to inhibit the serine threonine kinase Akt, which normally promotes cell cycle progression, inhibits apoptosis<sup>43</sup> and enhances  $\beta$ -catenin activity.<sup>44</sup> BMP activity appears to be required to control the proliferation of intestinal stem cells, an effect mediated through a suppression of Wnt signalling. Work on conditionally inactivated BMPRI1A mice suggests that BMP signalling may have a role in preventing stem cell renewal by inhibiting  $\beta$ -catenin. Mutated BMPRI1A mice developed characteristic polyps containing increased numbers of colonic crypts and had a five-fold increase in the number of stem cells in comparison to wild-type mice.<sup>44</sup>

Inhibition of the BMP pathway results in a characteristic pattern of epithelial development with ectopic crypt development, perpendicular to the crypt-villus axis, with excess branching and budding of the epithelium along with dilated cysts, an inflammatory infiltrate and a high rate of dysplastic change.<sup>45</sup> The changes mimic those seen in juvenile polyposis in humans.

### TGF- $\beta$ and SMAD signalling pathway

The TGF- $\beta$  family are inhibitors of gastrointestinal epithelial cell proliferation. In the normal situation, TGF- $\beta$  forms a multimeric complex with the serine-threonine TGF- $\beta$  type I and type II receptors, causing the phosphorylation of Smad2 and Smad3 cytoplasmic proteins and their formation of a heteromeric complex with Smad4. This complex translocates to the nucleus, where it interacts with transcriptional activators and co-activators to generate TGF- $\beta$  target gene transcription.<sup>46,47</sup>

## Forkhead pathway

This pathway involves the action of the Fox genes, which are usually expressed by the mesenchymal cells of the gastrointestinal tract. Mice with a mutation in the gene encoding Forkhead homologue 6 or Fox1 have increased levels of heparan sulphate proteoglycans, which increases the efficiency of Wnt binding to the Fz receptors, resulting in overactivation of the Wnt/ $\beta$ -catenin pathway and a subsequent increase in target cell proliferation. Their gut histology shows branched and elongated glands in the stomach, elongated villi, hyperproliferative crypts and goblet cell hyperplasia secondary to increased epithelial cell proliferation.<sup>48</sup>

## The role of the intestinal stem cell in neoplasia

Vogelstein et al.<sup>49</sup> proposed that colorectal carcinogenesis proceeded from early dysplastic lesions to invasive carcinoma by a step-by-step accumulation of genetic mutations, named the *adenoma–carcinoma sequence*. The events involved early loss of tumour suppressor genes such as APC and later activating mutations in proto-oncogenes such as *K-ras*. Molecular and genetic events in the intestinal stem cell are implicated in tumorigenesis as the other cells within the intestinal crypt have a limited lifespan that is insufficient to allow the accumulation of genetic events for tumorigenesis.

The following is a summary of dysregulation in the most well-known molecular pathways affecting stem cell function that are implicated in colorectal tumorigenesis. There is considerable interaction between different signalling pathways affecting the stem cell.

## Dysregulation of the Wnt signalling pathway

A mutation in APC is thought to be one of the first if not the initiating mutation in colon carcinogenesis.<sup>50</sup> Mutated APC is found in 80% of sporadic colorectal carcinomas. All patients with FAP who have a germline mutation in their APC gene will develop colorectal carcinoma when they acquire a somatic mutation in their second copy of APC. In FAP, a second hit in the APC gene is sufficient to give rise to microadenoma development.<sup>51</sup> Mutation in APC results in the transcriptional activation of target genes as a consequence of increased nuclear  $\beta$ -catenin/Tcf transcriptional complexes in the nucleus.<sup>33,52</sup> The APC protein is a large multi-

domain protein with multiple functions, several of which may be involved in tumorigenesis. These include the following:

1. Loss of functional Wnt signalling leads to inappropriate proliferation and a lack of differentiation.
2. APC loss is thought to cause changes in cytoskeletal regulation that affects the microtubules and F-actin, leading to defects in directed cell migration. Cells may therefore accumulate in the toxic gut environment for longer and accumulate genetic mutations, resulting in malignant transformation. Supporting this is the finding that APC mutations found in colonic tumours lack most regions involved in direct interactions with the cytoskeleton.<sup>53</sup>
3. A loss of APC may result in an increased incidence of mitotic errors resulting from defects in the mitotic spindles. This may lead to aneuploidy and tumour progression.<sup>54</sup>

In 50% of colon cancers that express wild-type APC,  $\beta$ -catenin is mutated in a phosphorylation site in one of its regulatory domains and does not undergo proteasomal degradation by GSK3 $\beta$ .<sup>55</sup> Small adenomas showing a loss of  $\beta$ -catenin are less likely to progress to larger adenomas and invasive carcinomas, as are adenomas with mutations in the APC allele, indicating that the inhibition of additional function(s) of APC above its regulation of  $\beta$ -catenin levels via the Wnt signalling cascade is conducive to the malignant progression of colorectal adenomas.<sup>56</sup> Mutations in APC and  $\beta$ -catenin arise independently in colon cancer, and some colon cancers have wild-type copies of both the  $\beta$ -catenin and APC genes, indicating that other members of the Wnt pathway may also be important in neoplastic change in the colon.<sup>55</sup>

The Wnt receptors Fz are also differentially expressed in the normal and neoplastic colon, with no expression of Fz receptor-1 and -2 mRNA in normal colon and well-differentiated tumours, but both receptors strongly expressed in poorly differentiated tumours.<sup>57</sup> The results strongly suggest an involvement of Wnt2, Wnt5a and the Fz1/2 receptors in the progression of colon cancer. Wnt2 was absent in normal human colon but expressed in colon cancer.

Several downstream targets of Tcf/LEF transcription include molecules involved in cell cycling, such as C-myc and cyclin D1.<sup>58–62</sup> The initial morphological manifestation of loss of APC function is an aberrant crypt focus, which may progress to micro- and macroadenomas and ultimately to colorectal carcinomas.

## SMAD pathways in tumorigenesis

Smad2 and Smad4 are frequently inactivated in human cancers, confirming their function as tumour suppressor genes.<sup>63</sup> There is thought to be a reciprocal interaction between the Wnt and TGF- $\beta$  signalling pathways in the progression of intestinal carcinogenesis, wherein loss of heterozygosity of both pathways is required before malignant transformation can occur.<sup>64</sup>

Mice with heterozygous targeted mutations of the *Smad4* and *APC* genes develop adenomatous polyps in the small intestine and colon owing to loss of heterozygosity of the *APC* and *Smad4* wild-type alleles. These lesions progress to form adenocarcinomas with an increased malignant nature over those formed in mice with only a heterozygous mutation of the *APC* allele.

## The clonal origin of intestinal tumours

Mutational theories of tumour development suggest that tumours arise from a series of mutations occurring in one cell and its progeny.<sup>65,66</sup> Others have argued that tumours are not clonal in origin but require the interaction of multiple cells,<sup>67,68</sup> the outgrowth of a dominant clone during subsequent development accounting for the apparent monoclonality. Novelli et al.<sup>19</sup> showed that approximately 76% of adenomas (above monocryptal size) were polyclonal in origin. Analysis showed that a random collision of tumours could not account for levels of polyclonality as high as those observed. This could be explained by the adenomatous transformation of non-involved crypts under the influence of transformed crypts.<sup>29,31,69</sup>

## The growth of adenomas—top-down versus bottom-up growth

The cellular mechanisms of spontaneous adenoma formation from a mutated stem cell clone are explained by two competing hypotheses. Both hypotheses feature mutation in the intestinal stem cell as the initial factor promoting tumorigenesis. The hypotheses are the *top-down* and the *bottom-up* theories of adenoma development.

The top-down hypothesis is based on the theory that the mutated intestinal stem cell is located in the intracryptal zone between two crypt orifices and that, when it proliferates, the mutated clone spreads downwards and laterally displaces the normal crypt epithelium (Fig. 1). This theory is

based on work on early FAP adenomas, in which dysplastic cells exhibiting loss of heterozygosity for *APC* and  $\beta$ -catenin were found exclusively at the tops of crypts with the crypt bases appearing phenotypically normal. Loss of heterozygosity was demonstrated in the top half of the crypts, and most of them had a truncating mutation confined to the top half of the crypts. Those cases without loss of heterozygosity had a truncating mutation at the top of the crypt.<sup>54</sup> A variation on this hypothesis suggests the intracryptal zone is the area to which a mutated basally located stem cell migrates in order to clonally expand.

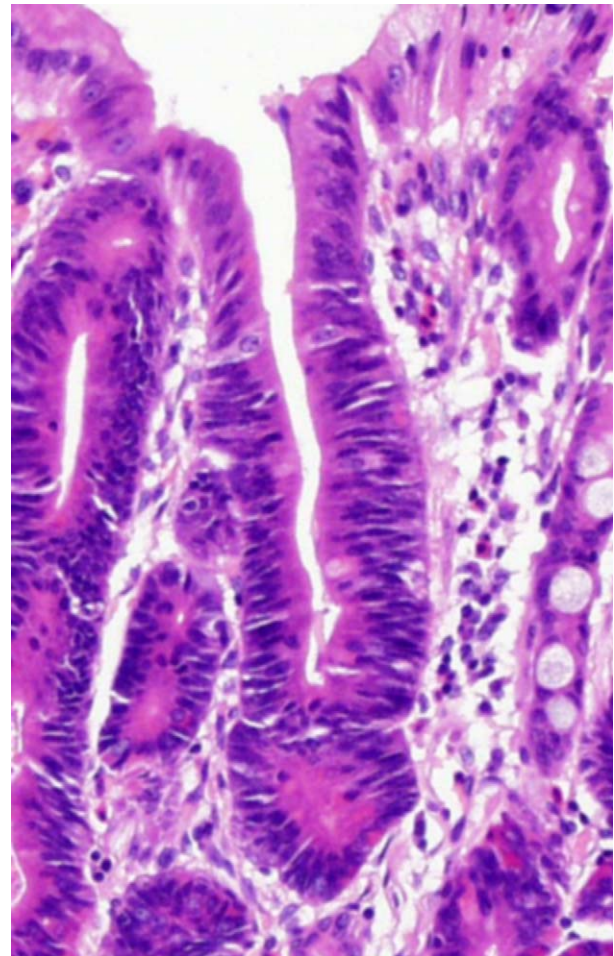
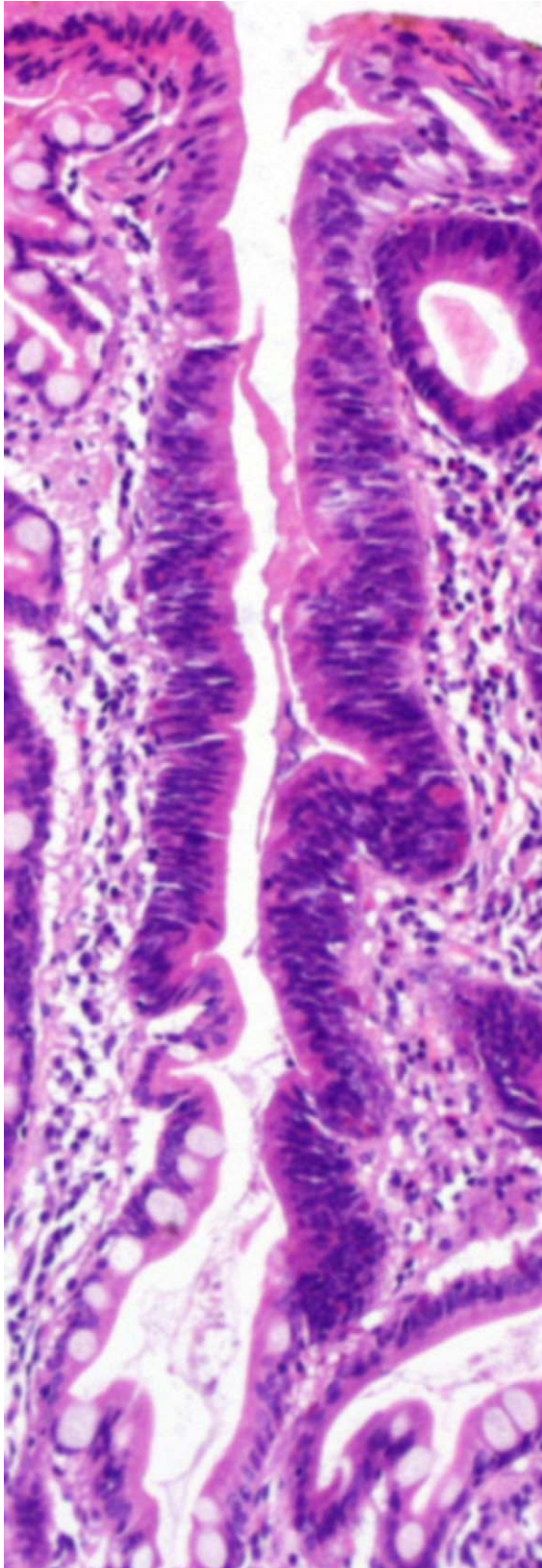
The competing hypothesis is the bottom-up hypothesis (Fig. 2), which states that an adenomatous clone of cells is produced by the stochastic expansion of a mutated stem cell in the base of a crypt. The mutated clone colonizes the entire crypt to form a monocryptal adenoma,<sup>19</sup> which is commonly seen in FAP and consists of a clonal population of cells. The mutated crypts then expand and replicate by crypt fission.

Evidence supporting the bottom-up theory came from immunohistochemical studies of tubular adenomas smaller than 3 mm showing a nuclear accumulation of  $\beta$ -catenin in dysplastic cells at the bases of adjacent crypts, as well as those crypts in the process of fission. There was a sharp cut-off between the dysplastic, adenomatous cells at the base of the crypt, which expressed nuclear  $\beta$ -catenin, and those at the top of the crypt, which did not.

Analyses of methylation histories of colorectal adenomas reveal a stem cell architecture that would be consistent with the bottom-up spread of adenomatous growth. Mitotic events are evenly distributed throughout the cells of an adenomatous crypt and not restricted to cells superficially located within the crypts, as would be expected with the top-down model of adenomatous development.<sup>70</sup>

In effect, both mechanisms may exist,<sup>71</sup> according to the size and stage of development of the adenoma. In larger adenomas, there was unequivocal evidence of surface cells growing down and replacing normal crypt epithelium, suggesting an overflow of dysplastic tissue or an adenomatous field effect on neighbouring crypts—suggesting that both the top-down and bottom-up processes may occur—with initial mutation of the stem cell within the niche at the crypt base resulting in bottom-up clonal expansion to form a monocryptal adenoma. Top-down spread into adjacent crypt territories is then a later secondary event in larger adenomas. The initial event in the genesis

of colorectal adenomas of both sporadic and FAP adenomas appears to be the monocryptal adenoma.<sup>31</sup>



**Figure 2** The bottom-up hypothesis. A small intestinal crypt showing dysplastic cells at the base of the crypt extending upwards. Towards the top of the crypt, the cells show less nuclear pseudostratification and appear to be maturing.

The following model has been proposed to account for adenoma development.<sup>7</sup> The initial event in the genesis of colorectal adenomas is the monocryptal adenoma, in which initial growth occurs via crypt fission, and spread into adjacent territories is a later secondary event. Crypt fission of adenomatous crypts appears to be the main mechanism of adenoma progression in FAP and in sporadic adenomas.<sup>29,72</sup> Analysis of colorectal adenomas has shown that the early expansion of these lesions occurs by crypt fission, with later

← **Figure 1** The top-down hypothesis. A small intestinal crypt showing dysplastic cells (nuclear enlargement and pseudostratification) extending from the top of the crypt downwards, with morphologically normal appearing enterocytes at the bottom of the crypt.

spread by the invasion of local crypt territories and 'top-down' growth.<sup>31</sup>

## The crypt response to inflammation

In the presence of chronic intestinal ulcers in the human gut, structures are induced that contain mucin-producing cells which differ from the indigenous cell lineages.<sup>73</sup> They have been shown to have new properties that include a definable life history during which they acquire different antigens, giving them a distinct phenotype, and synthesize large amounts of regulatory peptides. These cells appear to induce peptide gene expression in the local intestinal cells, and they also develop their own proliferative organization.<sup>74–76</sup> These cells are the differentiation progeny of intestinal stem cells but constitute a cell lineage in their own right—the Ulcer Associated Cell Lineage (UACL).

The UACL is commonly seen in the small intestine in association with Crohn's disease and duodenal ulcer disease, but is less common in the colon. Peptides produced by the UACL include lysozyme, trefoil peptides and epidermal growth factor. These peptides play a role in healing mucosal damage.

## Bone marrow stem cell plasticity in the gastrointestinal tract

Transplanted bone marrow stem cells show transdifferentiation to both epithelial and mesenchymal cell lineages in both human and mouse intestinal tract. Patients with a peripheral blood stem cell transplant showed epithelial cells of donor origin throughout the gastrointestinal mucosa.<sup>77</sup> In gastrointestinal biopsies from female patients with graft-versus-host disease after bone marrow transplant from a male donor, transplanted bone marrow stem cells were shown to contribute to a population of myofibroblast cells, the intestinal subepithelial myofibroblasts.<sup>78</sup> Transplanted bone marrow stem cells also transdifferentiate to form fibroblasts and smooth muscle cells in the lamina propria and mucosa, contributing to the multiple specialized adult gastrointestinal mesenchymal lineages in diseased tissue. Bone marrow-derived stem cells appear as single, random entities, so they may not contribute to the gastrointestinal stem cell compartment. Columns of bone marrow stem cells have, however, been observed, and it is possible that bone marrow may contribute to a myofibroblast stem cell.<sup>6</sup>

### Practice points

- The intestinal stem cell is central to the maintenance of normal crypt architecture in the small and large intestine.
- A large body of evidence exists that throws light on some aspects of stem cell function.
- There are numerous signalling pathways that involve the stem cell, and dysregulation of the molecules involved in these pathways may lead to tumorigenesis in the gut.
- Much remains to be learnt about the intestinal stem cell and its role in normal and pathological states.

### Research agenda

The following need to be investigated:

- Characterization of molecular markers of the stem cell.
- Characterization of the molecular interactions of the APC gene product.
- Further elucidation of the signalling pathways acting on the gastrointestinal stem cell.

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