INTESTINAL TRACT

# Cellular diversity in the colon: another brick in the wall

*Isabelle Cleynen and Debby Laukens*

Single-cell RNA profiling of colonic epithelial crypts from healthy volunteers and patients with ulcerative colitis adds pH-regulating colonocytes and goblet cells expressing a major determinant of barrier maintenance to the current arsenal of uncovered colonic epithelial cell types.

*Refers to* Parikh, K. et al. Colonic epithelial cell diversity in health and inflammatory bowel disease. *Nature* **567**, 49–55 (2019).

The intestinal epithelial monolayer exhibits a diversity of functions: it is a physical barrier separating the luminal content from the underlying immune tissue, has absorptive and metabolic tasks, controls bacterial growth and actively contributes to immune responses and immunotolerance. Such a plethora of functions is reflected in the diversity of cell types that constitute the intestinal epithelial barrier[1]. In addition, this layer of cells self-renews every 4–7 days, during which the differentiation of cells from the stem cell niche along the crypt axis requires orchestrated molecular mechanisms[1,2]. Any deviation that might occur during this process can form a breach in the intestinal epithelium leading to pathology. Indeed, a rapidly growing number of intestinal and non-intestinal disorders have been linked with a dysfunctional intestinal barrier, including infectious disease, inflammatory bowel disease (IBD), coeliac disease, and type 1 diabetes mellitus. This paradigm has led to the concept of the 'leaky gut', supposedly a primary cause leading to uncontrolled systemic exposure of luminal antigens. However, many of these studies are associative by nature. Causality is often difficult to study[2], especially because specific tools or markers to evaluate gut barrier function are lacking. Nevertheless, the classic view of barrier dysfunction is that of a disruption of the junctional complexes between intestinal epithelial cells (IECs) in a (low-grade) inflammatory environment. Convincing experimental evidence for a leaky gut has been generated in IBD, in which intestinal barrier function is compromised by structural damage caused by cell death and tissue remodelling, or by changes in the signalling pathways regulating barrier function[1,3]. In addition, a surprisingly high number of IBD genetic susceptibility genes encoding proteins with key functions in gut barrier homeostasis have been identified[4].

Despite the increasing focus on the leaky gut, our understanding of barrier function has stagnated. Generally, we consider the intestinal epithelium to be composed of undifferentiated stem cells, absorptive enterocytes, different types of enteroendocrine cells, mucus-producing goblet cells, and Paneth cells secreting antimicrobial peptides. Less-well-studied specialized cells include tuft cells that express taste and other receptors to sense pathogens[5], and microfold cells guiding transport of luminal antigens to the lamina propria[6,7]. Since the introduction of high-throughput bacterial genome sequencing and the concept of dysbiosis of the gut microbiota, an intriguing question is how IECs contribute to bacterial homeostasis. Aside from controlling the composition of the mucus layer[8], it is unclear whether and how the enterocytes in the bacteria-loaded colon contribute to the maintenance of bacterial composition. Such studies are hampered by technical issues in isolating and culturing specific IECs, and have mostly relied on immunostaining of predefined markers. However, microarray or RNA-sequencing studies of full-thickness mucosal tissue have helped improve our knowledge. For example, a study by Vancamelbeke et al. found many intestinal epithelial barrier genes to be dysregulated in IBD, with an over-representation of mucus-layer and barrier-regulating genes[9].

Technical advances in single-cell RNA sequencing (scRNA-seq) have enabled the identification and quantification of cells based on their intrinsic transcriptome. Parikh et al. used this approach in a landmark study aimed at profiling colonic epithelial cell type composition in health and disease[10]. Their data showed the existence of a differentiation hierarchy characterized by gene expression gradients following the spatial segregation along the crypt axis, consistent with their origin from a common stem cell at the bottom of the crypt. Most interestingly, this study identified a novel absorptive cell type regulating pH balance. These cells, characterized by a high expression of the proton channel *OTOP2* and the chloride channel *BEST4*, conduct protons into their cytosol upon acidification of the extracellular space. Based on evidence from deep RNA profiling and proteomics, these cells are probably protected against subsequent intracellular acidification by the high expression of anti-apoptotic *BAG1*, and *GUCA2B* or uroguanylin, a peptide agonist of guanylate cyclase 2C regulating electrolyte and water transport.

Compared with crypts from healthy individuals, crypt-top colonocytes and goblet cells in inflamed crypts from immunotherapy-naive patients with ulcerative colitis contain the highest number of differentially expressed genes. Universal responses across cell populations converged to inflammatory pathways, whereas cell-type specific responses were, for example, linked with downregulation of metabolic processes and upregulation of genes related to reactive oxygen species and microbial killing in colonocytes (FIG. 1). Notably, IBD-associated risk genes (for example, *NOS2* and *JAK2*) were expressed differently in diverse epithelial cell types (FIG. 1), suggesting that the small genetic defects typical for these susceptibility loci manifest in distinct cell types, and thus contribute at different levels to failure of re-establishing barrier function. In crypts isolated from uninvolved areas of intestine from patients with ulcerative colitis, >50% of differentially expressed genes were also dysregulated in inflamed areas, suggestive of a subclinical pathology reflecting a dominance of regenerative pathways over damage pathways, or an early protective mechanism in anticipation of damage. The changes specific to uninvolved mucosa were linked with unfolded protein responses, the establishment of cell polarity, metabolism and apoptosis.

Focusing on goblet cells, five clusters were derived, one of which was specifically

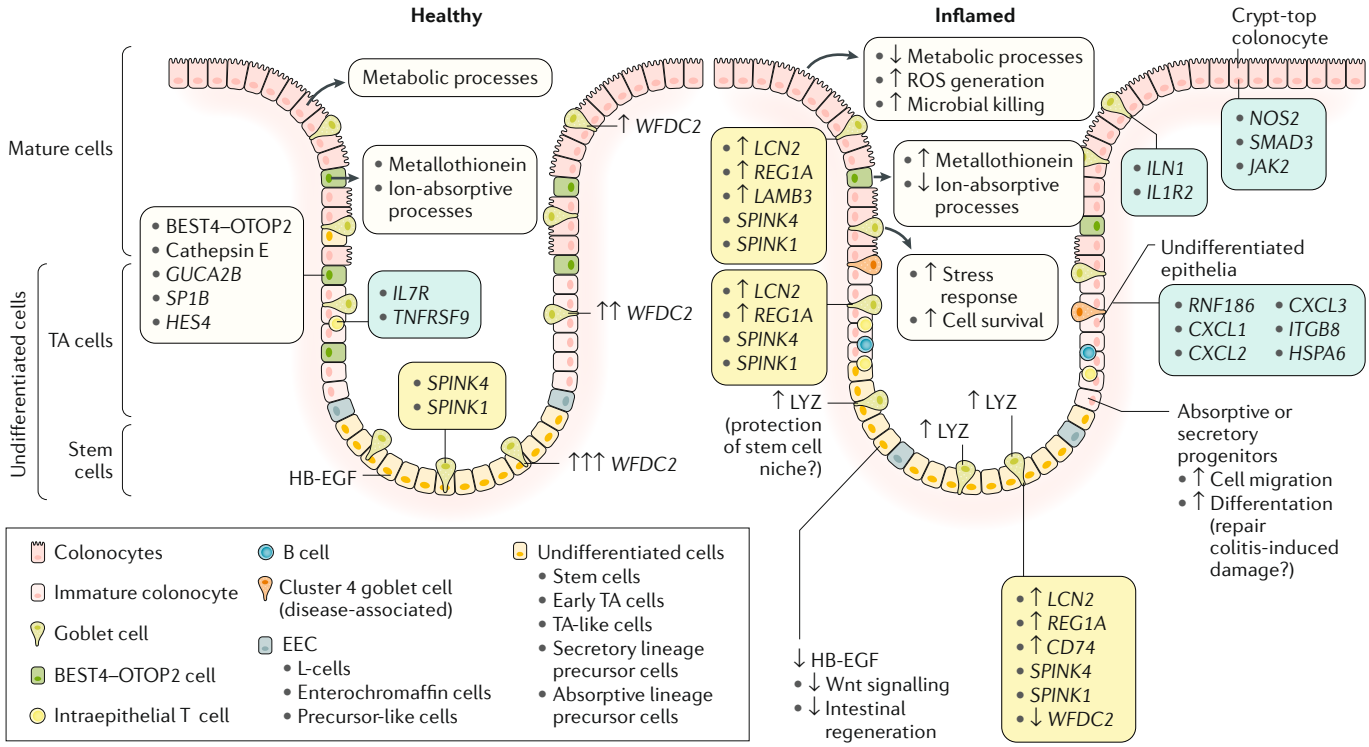


Fig. 1 | **Overview of colonic crypt structure in health and disease.** Parikh et al.[10] found: a differentiation hierarchy that ascends through the crypt (left panel); a novel absorptive cell type regulating pH balance (BEST4–OTOP2 cells) decreased in inflammation; and a pathway in goblet cells focused around *WFDC2* and linked with maintenance of barrier function. *WFDC2* shows an expression gradient in goblet cells, and is downregulated in inflammation. Changes in cellular composition, as well as cell-type specific responses were seen in disease (right panel): a balance between restoration of tissue integrity and response to aberrant tissue homeostasis. Expression of IBD disease susceptibility genes mapped to specific cell clusters are in blue boxes; spatial goblet cell gene expression is marked in yellow. EEC, enteroendrocrine cell; HB-EGF, heparin-binding EGF-like growth factor; LYZ, lysozyme; ROS, reactive oxygen species. TA cells, transit amplifying cells.

associated with ulcerative colitis and the expression of genes essential for integrity and barrier homeostasis (FIG. 1). In inflamed ulcerative colitis crypts, both spatial and crypt-wide differences in gene and protein expression in goblet cells were seen: some genes were induced throughout the crypts (*LCN2*, *REG1A*) others only at the crypt top (*LAMB3*) or bottom (*CD74*), and yet others normally limited to the crypt bottom persisted in crypt-top goblet cells (*SPINK4*, *SPINK1*). The authors focused on a gene belonging to the WFDC domain family of protease inhibitors (*WFDC2*), and saw that its expression was negatively correlated with ulcerative colitis disease severity. Using human organoid cultures, they showed that IFNγ reduced *WFDC2* expression, and speculated that the downregulation seen in ulcerative colitis might be related to the cytokine milieu. Further functional studies showed that WFDC2 is an antibacterial protein secreted from goblet cells that is important in maintaining the sterility of the inner mucus layer in the colon, and thus a major contributor to colonic barrier function.

Although therapeutic strategies to restore a leaky gut have mainly focused on the expansion of stem cells and restoration of junctional complexes, the identification of WFDC2 as a barrier-controlling protein could be exploited therapeutically to improve barrier function. Also, the maintenance of colonic pH by *BEST4–OTOP2* cells probably contributes to optimal microbial growth and represents a novel key component involved in host–microorganism interaction. In that regard, uroguanylin mimetics might be able to restore the typical dysbiosis observed in these patients. To prevent alterations in cell type composition, it will be important to understand the factors driving the generation of different cell types in the gut.

This study illustrates that single-cell profiling facilitates the characterization of cells, pathways and genes associated with human disease, and hence provides unprecedented opportunities for personalized medicine. However, with current prices, and technical and analytical challenges, scRNA-seq will probably remain difficult to implement in clinical practice. Nonetheless, this study opens up new opportunities to define a set of markers to evaluate the cellular composition of intestinal epithelia in health and disease.

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**Competing interests**
The authors declare no competing interests.