Intestinal cellular heterogeneity and disease development revealed by single-cell technology

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Abstract
The intestinal epithelium is responsible for food digestion and nutrient absorption and plays a critical role in hormone secretion, microorganism defense, and immune response. These functions depend on the integral single-layered intestinal epithelium, which shows diversified cell constitution and rapid self-renewal and presents powerful regeneration plasticity after injury. Derailment of homeostasis of the intestine epithelium leads to the development of diseases, most commonly including enteritis and colorectal cancer. Therefore, it is important to understand the cellular characterization of the intestinal epithelium at the molecular level and the mechanisms underlying its homeostatic maintenance. Single-cell technologies allow us to gain molecular insights at the single-cell level. In this review, we summarize the single-cell RNA sequencing applications to understand intestinal cell characteristics, spatiotemporal evolution, and intestinal disease development.

Keywords: scRNA-seq, Gut, Heterogeneity, Enteritis, Colorectal cancer

Background
The intestine plays an important role in nutrient digestion and absorption, hormone secretion, protection and immune regulation (Peterson and Artis, 2014; Sanger and Lee, 2008; Zorn and Wells, 2009). Also, the intestine is the main organ for microbiota residing that has been showing continuously emerging functions to influence other organs in the body (Tilg et al., 2020). These functions depend on the intestinal epithelium that constitutes the second-largest epithelial surface area of more than 30m² in the human body (Helander and Fandriks, 2014). The intestinal epithelial surface is expanded by millions of special structures, crypt-villus units in the small intestine or crypt units in the large intestine (Fig. 1A). The intestinal epithelium undergoes a fast turnover, which is driven by the intestinal stem cells (ISCs) located at the bottom of crypts (Fu et al., 2021). ISCs continuously self-renew and meantime generate transient-amplifying (TA) cells and progenitors, which then differentiate into mature functional cell types. Briefly, TA cells from ISCs give rise to secretory progenitors and enterocyte progenitors. Then the secretory progenitors further differentiate into goblet cells, enteroendocrine cells (EECs), Paneth cells, and perhaps tuft cells, while enterocyte progenitors differentiate into enterocytes (Fig. 1B). However, it has been reported that tuft cells may be from enterocytes but not secretory progenitors (Herring et al., 2018). During the differentiation progress, Paneth cells move down to the bottom of small intestinal crypts and are long-lived, whereas other mature lineages move up to keep the crypt or villus structures. These cell types cooperate with each other to form a multifunctional network. After the functional performance, these mature cells undergo cell death in a cycle of 4–5 days (Cheng and Leblond, 1974), and up to 10¹¹ epithelial cells are lost every day in the human intestine (Leblond and Walker, 1956). This dynamic renewal system provides the intestine epithelium the ability to endure continuous chemical stimulation and various damages.

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The dynamic balance of intestinal epithelium is maintained by the underneath niche cells and the microenvironment signaling (Zhu et al., 2021a). Most importantly, epidermal growth factor (EGF) and Wnt are abundant in the crypt to promote the proliferation of ISCs and TA cells, while bone morphogenetic protein (BMP) activity is higher at the villus, promotes cell differentiation and induces cell death (Fig. 1A) (Qi and Chen, 2015; Wang and Chen, 2018). These microenvironment signals are mainly from the niche cells, including stromal cells, fibroblast cells, immune-related cells, and others (Fig. 1A). For example, Forkhead box L1 (Foxl1) positive trophocytes can provide a source of Wnt-activation and BMP-inhibition signals for ISC self-renewal (Shoshkes-Carmel et al., 2018), and T helper cells can support ISC renewal through IL-10 (Biton et al., 2018). In short, the surrounding niche cells and signals are necessary for the epithelial function performance; the destruction of the microenvironment homeostasis may lead to intestinal disease development.

With the discovery of the specific ISC marker Lgr5 (Barker et al., 2007), we have gained a great
understanding of intestinal epithelium homeostasis in the last decade. A series of breakthroughs have been made in this field, such as intestinal organoid culture from single Lgr5+ cells (Clevers, 2016; Sato et al., 2009), stem cell contribution to intestinal tumorigenesis (Liado et al., 2015), and the repopulation of human colonic epithelium in vivo with potential therapeutic applications in the mouse model (Sugimoto et al., 2018). However, as in other scientific research fields, new findings beget new questions. For instance, the exact cell constitution and ratio of different cell types in small and large intestinal epithelia are unclear, the critical factors governing the fate trajectory of ISCs are not fully determined, and the cellular and molecular changes under intestinal pathological conditions are not well understood. In addition, cellular heterogeneity is an important theme for intestinal epithelium researches. For example, EECs can be divided into several subtypes (Gribble and Reimann, 2016), but the substantial crossover or the cellular heterogeneity is rarely revealed. Also, the mechanism of intestinal disease development is obscure, such as intestinal enteritis and colorectal cancer (CRC). These emerging questions require new advanced technologies to offer us a more comprehensive picture.

Although whole transcriptome analysis using bulk RNA-sequencing has yielded massive information on cell function and biological regulation, individual cells are the life identity and functional unit. Also, cellular heterogeneity is a general feature of biological tissues (Wen and Tang, 2016). To better understand the cellular composition and heterogeneity of primary tissues at the same time, single-cell PCR gene-expression analysis was developed about 10 years ago (Dalerba et al., 2011). Then, with the marked improvement of the single-cell RNA sequencing (scRNA-seq) technology during the past years (Grun et al., 2015; Magness et al., 2013), thousands of individual cells can be processed in a single experiment and more than 50,000 reads for each cell obtained (Chen et al., 2021b). Nowadays, scRNA-seq is widely used for routine transcriptional profiling at single-cell resolution (Zhao et al., 2021). Our cross-species analysis of the single-cell transcriptome of the ileum epithelium identified a new CA7+ cell type in pig, macaque, and human ileum and revealed the distinct expression pattern in enterocytes, EECs, and Paneth cells among different species (Li et al., 2022). In this review, we focus on an advanced understanding of the intestine biology gained from single-cell transcriptome analysis and provide an updated summary of the scRNA-seq database in the field.

**ScRNA-seq reveals cellular heterogeneity of the intestinal epithelium**

Multiple functions of the intestinal epithelium are based on complex cell constitution with each cell type performing specific functions while coordinating with each other (Liu and Chen, 2020). Therefore, the identification of cell types is critical for understanding their detailed functions. Before the scRNA-seq technology application, identification of different cellular signatures at the transcriptome level was usually achieved by bulk RNA sequencing after fluorescence-activated cell sorter (FACS) sorting by cell-type specific markers (Table 1). However, bulk RNA-seq cannot reveal the cellular heterogeneity among these cells. ScRNA-seq makes it possible to detect the subtypes and their function difference at the single-cell level of the intestinal epithelium and the surrounding niche cells. More information about ISCs can be found in a recent review (Fu et al., 2021).

**Table 1** Summary of the specific markers of intestinal epithelial cells

| Cell types                        | Marker genes                                      | Mouse intestine | Human intestine          |
|-----------------------------------|---------------------------------------------------|----------------|--------------------------|
| Enterocyte                        | Alpi, Fabp1, Sic26a3 (Apoa1 in SI *)               | ALPI, FABP1, SLC26A3 (APOA1 in SI *) |                         |
| Goblet cell                       | Muc2, Spdef, Cica1, Zg16, Spink4, Fcgbp           | MUC2, SPDEF, CLCA1, ZG16, SPINK4, FCGBP |                         |
| Enteroendocrine cell              | Neurog3, Neurod1, Chga, Chgb                      | NEUROG3, NEUROD1, CHGA, CHGB |                         |
| Paneth cell                       | LYZ1, Defa5, Defa6                                | LYZ, DEFAS, DEFA6 |                         |
| Tuft cell                         | Dclk1, Pou2f3, Trpm5, Il25                         | POU2F3, TRPM5, IL25 |                         |
| TA cell (Lgr5-negative)           | Mki67, Hmgb2, Top2a, Ube2c, Stmn1                 | MKI67, HMGB2, TOP2A, UBE2C, STMN1 |                         |
| Stem cell                         | Lgr5, Ascl2, Rgmb, Smoc2, (Olfm4 in SI *)         | LGR5, ASCL2, RGMB, SMOC2, (OLFM4 in SI *) |                         |
| BEST4 cell                        | Absent in mice                                    | BEST4, SPIB, CA7, OTOP2 | Need to be confirmed    |
| + 4 stem cell                     | Bmi1, Hopx, Tret, Lrig1, Mex3a                    |                 |                          |

* SI small intestine
Enterocytes

Enterocytes, a predominant cell type in the intestine, are responsible for food digestion and nutrient absorption, and these cells are derived from enterocyte progenitors (Pinto et al., 2003). Several transcript factors were found to determine the differentiation of TA cells to enterocyte progenitors (or immature enterocytes), such as Hes1, Cdx2, and Hnf4 (Clevers and Batlle, 2013). Based on scRNA-seq, more transcription factors (Sox4, Foxm1, Mxd3, and Batf2) were identified to associate with enterocyte maturation in mouse small intestine (Haber et al., 2017). Similarly, FABP1 and KRT19 were found to participate in enterocyte maturation in the human small intestine (Fujii et al., 2018). In addition, a new subtype of enterocytes was found in the human colon, BEST4+ enterocytes, which comprise about 1% of the human ileal epithelium (Huang et al., 2019; Parikh et al., 2019; Smillie et al., 2019) and are enriched with the genes responsible for pH sensing and electrolyte transportation, including GUCA2A, OTOP2, and CA7 (Brenna et al., 2015; Tu et al., 2018). These studies expand our knowledge about the functions of enterocytes, which are responsible for nutrient absorption and electrolyte balance.

Goblet cells

Goblet cells synthesize and secrete mucins to prevent pathogen invasion and stabilize the bacterial biofilm (Zhang and Wu, 2020). Goblet cells are more enriched and contribute to two mucous layers in the large intestine: the outer layer is relatively loose and contains symbiotic bacteria, and the inner layer is tight and impervious to bacteria, while there is only one antibacterial gradient in the small intestine (Paone and Cani, 2020). The active mucin secretion in the large intestine is consistent with more goblet cells there compared with the small intestine (Wang et al., 2020b). The destruction of mucous layers and goblet cell functions may lead to enteritis development (Birchenough et al., 2016). ScRNA-seq trajectory analysis of mCherry-Muc2 cells showed that goblet cells segregate into two separate trajectories: one is enriched with canonical markers (Clica1 and Fegbp), while the other one is typically associated with enterocytes (Dmbt1 and Gsdmc4) (Nystrom et al., 2021). This study also revealed a new subpopulation of goblet cells (intercrypt goblet cells, icGCs), which are especially located to the surface epithelium between crypts in the colon and contribute to a functional mucus barrier to protect the epithelium from microorganisms (Nystrom et al., 2021). Furthermore, scRNA-seq uncovered an immature goblet cell type, which is absent of Tbx10 expression, and was reduced during inflammation (Smillie et al., 2019). Another scRNA-seq study revealed that goblet cells could be divided into two main states: proliferation and differentiation. HES6 was found in the early stage of goblet cell differentiation, which can be used to mark the goblet cells that are not yet morphologically identifiable as goblet cells (Zhang et al., 2019a). Similarly, mitotic goblet cells were identified by the co-expression of Mki67, Lbe2c, Zg16, Tff3, and Clca1 in the human colon (Huang et al., 2019). Mature Tff1+ goblet cells are found only in the villus of the human ileum and in the top zone of crypts of the human colon and rectum, and they are highly enriched with the function of MHC antigen processing (Wang et al., 2020b). These results suggest that goblet cells show more heterogeneity along the differentiation trajectory.

Paneth cells

Paneth cells, which are interspersed between Lgr5+ ISC s at the crypt base in the small intestine, secrete antimicrobial molecules modulating host-microbe interactions and provide the factors promoting Lgr5+ ISC s (Clevers and Bevins, 2013; Zhang and Liu, 2016). However, whether there are functionally equivalent cells in the large intestine is still under debate. With the application of single-cell technology, we have gained more insights into it. Single-cell PCR gene expression analysis identified a subset of cKit+ goblet cells in the mouse colon, which might have the equivalent function of Paneth cells in supporting Lgr5+ stem cells (Rothenberg et al., 2012). Furthermore, scRNA-seq of the human embryo’s digestive tract and adult intestinal segments unraveled the Paneth-like cells in human fetal and adult large intestine, respectively (Gao et al., 2018; Wang et al., 2020b). Interestingly, another scRNA-seq analysis revealed that LYZ, a Paneth cell marker, was upregulated in lower-crypt goblet cells and might mark the “deep crypt secretory cells” that are required to maintain the colonic stem cell niche and to protect stem cells from bacterial damage during colitis (Parikh et al., 2019). Moreover, scRNA-seq also uncovered the marker genes of Paneth cells in human ascending, transverse, and descending colons (Burclaff et al., 2022). Noteworthy, the cells designated as Paneth cells or Paneth-like cells in previous reports were designed as BEST4+ cells based on the expression of LYZ, SPIB, BEST4, and CA7 (Burclaff et al., 2022; Wang et al., 2020b). Therefore, in addition to the canonical Paneth cells in the small intestine, the large intestine hosts cKit+/BEST4+/Paneth-like cells.

Enterendocrine cells

EECs can sense nutrients and secrete about 20 different kinds of hormones (Furness et al., 2013). They have been divided into several subtypes based on hormone secretion (Gribble and Reimann, 2016). The scRNA-seq analysis of mouse intestine has uncovered hormone
cross-expression among these subtypes. Haber and colleagues have re-defined the subtypes of mouse intestinal EECs into 12 sub-clusters and provided the hormones' secretion atlas (Fig. 2A) (Haber et al., 2017). However, 9 sub-clusters EECs were assigned in the human small and large intestine in a single-cell transcriptomic atlas (Beumer et al., 2020): motilin (M cells), gastrin (G cells), gip (K cells), and cholecystokinin (I cells) were enriched in the duodenum, while glucagon (L cells) enriched in the colon. These results reveal different enteroendocrine subtypes between human and mouse intestines, including gene expression, such as ASCL1 and MNX1 in the human but not in mouse EECs (Beumer et al., 2020). A hormone gradient along the crypt-to-villus axis was revealed by scRNA-seq. For instance, tachykinin 1 (Tac1) and glucagon (Gcg, induced by Glp1) are highly expressed in the crypt, while secretin (Sct) and neurotensin (Nts) are high in the villus (Fig. 2B) (Beumer et al., 2018). Moreover, a hormone gradient is also observed along the small intestine from the proximal to distal segment: cholecystokinin (Cck) and gastrin (Gast) are enriched in the proximal segment, while proglucagon (Gcg) and peptide YY (PYY) enriched in the distal segment (Fig. 2C) (Beumer et al., 2020). Moreover, 10 major EEC subtypes were identified in adult Drosophila midgut, and these subtypes produce approximately 14 different classes of hormone peptides with each subtype on average secreting approximately 2-5 classes (Guo et al., 2019), demonstrating the complexity of the endocrine system in the intestine.

Along with the exploration of enteroendocrine subtypes, new marker genes are also identified by scRNA-seq. Reg4 may be a novel marker for both Chga/Chgb\textsuperscript{high} and Chga/Chgb\textsuperscript{low} EECs (Grun et al., 2015) and Neurog3, Neurod1, and Sox4 as new markers for enteroendocrine precursors (Gehart et al., 2019). ScRNA-seq also revealed that the EECs marked by Bmi1 and Prox1 could serve as a reservoir to replenish homeostatic and injury-inducible ISCcs (Yan et al., 2017).

**Tuft cells**

Tuft cells, marked by Dclk1, Pou2f3, Trpm5, Il-25 (O’Leary et al., 2019), are rare solitary chemosensory...
cells in mucosal epithelia (Gerbe and Jay, 2016; Gerbe et al., 2012). These cells are involved in the initiation of immune type 2 responses (Gerbe and Jay, 2016) and produce a spectrum of biological effector molecules (II-33, II-25, prostaglandin D2, and the neurotransmitter acetylcholine) to mediate a cytokine-mediated cellular response (Gerbe and Jay, 2016; Schneider et al., 2019). Tuft cells closely interact with lymphoid cells (ILC2s) and recruit type 2 helper T cells (Th2 cells) to regulate immune activities in the intestine (Grencis, 2015; von Moltke et al., 2016). ScRNA-seq analysis has yielded new insights into tuft origin, differentiation progress, subtype, and functional subdivision.

Due to their secretion function, tuft cells were usually thought to originate from secretory progenitors that were regulated by Atoh1 (Gerbe et al., 2012). However, using scRNA-seq, Herring and colleagues reported that tuft cells in the small intestine appeared distinct from the secretory lineage goblet and Paneth cells while showing a common trajectory with enterocytes (Herring et al., 2018). This study suggests tuft cells may not originate from secretory lineage. ScRNA-seq revealed that Dclk1-positive tuft cells express high levels of Cox2 and Hopx in the mouse small intestine compared with the tuft cells in the colon (McKinley et al., 2017). ScRNA-seq studies also suggested that mature tuft cells may have two subtypes, one involved in the immune-related reaction and another related to neuronal development (Haber et al., 2017). These two subtypes present different enrichments in II-33, the pan immune marker CD45, and the Th2-related cytokines II-4 and II-13 (Haber et al., 2017). Interestingly, tuft cell number increases after Paneth cell ablation and may act as novel niche cells in Paneth cell-ablated crypts (van Es et al., 2019). Moreover, the single-cell transcriptomic analysis showed that tuft cells may play an important role in intestinal enteritis—the enrichment of PSMA6 (Proteasome 20S subunit alpha 6, associated with Crohn’s disease risk) in tuft cells may lead to impaired epithelial integrity and stress responses (Huang et al., 2019).

ScRNA-seq unveils spatiotemporal regulation

Along the crypt-villus axis, cells display multifarious states, and the same cell type may have specialized functions (Beumer et al., 2022; Moor et al., 2018). The functional diversity also exists from the proximal to the distal small and large intestine (Wang et al., 2020b). In other words, each cell could be precisely regulated, and the same type of cells may show cellular heterogeneity along with the spatiotemporal points. This spatiotemporal regulation makes the epithelium flexible to adapt to specialized functions and various environments such as nutrient constitution (Wang et al., 2020b), microbiota (Boulange et al., 2016), pathogens (Peterson and Artis, 2014), hypoxia (Zheng et al., 2015), chemical concentration changes (Williamson and Clifford, 2017) and chymopectinosis (Bhat et al., 2020). Spatial difference along the crypt-villus axis has been explored with bulk RNA sequencing (George et al., 2008; Mariadason et al., 2005), but the effect of cell heterogeneity and precise differentiation state cannot be taken into consideration. The studies based on scRNA-seq have provided insightful information on the spatiotemporal regulation of the intestinal epithelium.

Enterocytes exhibit significant functional differences along the crypt-villus axis according to scRNA-seq combined with RNA-seq of laser capture micro-dissected tissue (LCM-RNA-seq) (Moor et al., 2018). Amino acid and carbohydrate transporters were enriched in the middle of the villus, while the proteins involved in lipoprotein and chylomicron biosynthesis are mainly expressed at the top of the villus. Reg family members and other peptides involved in the microbiota-host interactions are highly expressed at the bottom of the villus. The spatially functional difference of enterocytes, such as the increased lipid uptake at the top of the villus and the Reg expression at the bottom of the villus, is confirmed by the analysis of scRNA-seq and Bmpr1a knockout mouse model, and the zonated gene expression is controlled by BMP signaling along the crypt-villus axis (Beumer et al., 2022). ScRNA-seq analysis also unraveled that enterocytes at the bottom and middle of the villus show more plasticity than the ones at the tip (Ayyaz et al., 2019).

Different intestinal segments exhibit distinct activities in nutrient absorption. ScRNA-seq analysis of the intestinal epithelium of the human ileum, colon, and rectum reveals the differences of signature genes and nutrient transporters in enterocytes among these three segments (Wang et al., 2020b). Although the genes related to protein digestion and absorption, mineral and organic substance transports are evenly expressed in all three segments, the genes participating in lipid metabolism and drug metabolic processes are highly expressed in the ileum, while the genes related to small molecule transport were enriched in the large intestine. The scRNA-seq analysis of the gene expression profiles of the human embryo digestive tract between 6 and 25 weeks of gestation showed that the genes involved in protein digestion and absorption were enriched in the small intestine compared with the stomach and large intestine (Gao et al., 2018). A large-scale single-cell spatiotemporal atlas of the human embryo digestive tract ranging from 8 to 22 post-conceptual weeks further revealed that the spatial distribution of absorptive genes is established in development prior to crypt formation (Fawkner-Corbett et al., 2021). Another spatial scRNA-seq of epithelial cells from
the human duodenum to descending colon also showed the segmental absorption difference—fatty acid, glucose, and cholesterol transporters were enriched in the small intestine, while sodium transporters were enriched in the colon (Burclaff et al., 2022).

Like enterocytes, EECs also display spatial distribution in mice and humans based on scRNA-seq. EECs show differential hormone secretion in the villus (Sct, PYY, Nts) and crypts (Tacl, Glp1, Trp1), while other hormones (Cck, Sst, Lapp, Tph1, Gip) show no significant difference along the crypt-villus axis in the mouse small intestine (Fig. 2B) (Beumer et al., 2018). Also, some hormones show segmental enrichment. The hunger-related hormones Ghrl and Gcg are enriched in the mouse duodenum, while PYY, an appetite reducer upon feeding, is found mainly in the mouse ileum (Fig. 2C) (Haber et al., 2017). In addition, some of the hormones are highly expressed in the human ileum (SCT, NTS, and CCK), while some are enriched in the human colon and rectum (PAM, NMB, and INSLS5) (Wang et al., 2020b). Gip-producing K cells are enriched in the proximal part of the mouse small intestine, while Glp1-producing L cells in the distal segment, Sst-producing D cells, and enterochromaffin cells are uniformly distributed (Beumer et al., 2018). Consistent with the mouse results, GAST-producing G cells and CCK-producing I cells are enriched in the proximal segment, whereas NTS-producing N cells and GHRL-producing X cells are in the human distal small intestine (Beumer et al., 2020).

Regional transcription factors may contribute to EEC diversity according to scRNA-seq of the Drosophila midgut. Class-specific Mirr and Ptx1 define Tk+ and AstC+ EEC types, respectively, while regional transcription factors contribute to segmental EEC identities. For instance, Drnn defines the ITP+ EEC type in the posterior midgut, and Esg defines the NPLP2+ EEC type in the middle midgut (Guo et al., 2019). A time-resolution EEC subtype differentiation tree is given to depict common and lineage-specific transcriptional regulators during fate trajectory (Williamson and Clifford, 2017). Using a bi-fluorescent reporter (Neurog3-Chrono) to precisely position single-cell transcriptome along a time axis in mouse organoids, scRNA-seq identified temporal transcriptional regulators during enteroendocrine differentiation: Neurog3, Dll1, C1qbp function in the early stage, Pax6, Insm1, Arx in the intermediate stage, and Isl1, Pax6, Elf4 in the late stage (Williamson and Clifford, 2017).

Like other organs, the intestine also undergoes structural and functional changes during organism aging (Pentimikko and Katajisto, 2020), for instance, disruption of the intestinal barrier (Parrish, 2017), increased risk of inflammatory conditions (Shemtov et al., 2022), the loss of the regenerative capacity of ISCs and epithelial renewal (Funk et al., 2020; Mihaylova et al., 2018; Nalapareddy et al., 2017). Like other organs, the intestine also undergoes structural and functional changes during organism aging (Pentimikko and Katajisto, 2020), for instance, disruption of the intestinal barrier (Parrish, 2017), increased risk of inflammatory conditions (Shemtov et al., 2022), the loss of the regenerative capacity of ISCs and epithelial
defining the disease-associated cell states and their possible interactions.

The altered immune response in enteritis development

The intestinal immune system undergoes dramatic alterations during IBD development and is perceived as a key for effective treatment (Gorreja et al., 2022). Several unique T cell subsets were found by scRNA-seq from two severe CD cases, including \( \text{NKp}30^{+} \gamma\delta T \) cells expressing RORγt and IL-26 (Jaeger et al., 2021). This study also revealed the increase of activated \( CD8^{+} \) T cells and the decrease of Treg cells in the inflamed regions (Fig. 3). Although the accumulation of \( CD8^{+} \) T cells was confirmed by another scRNA-seq analysis of immune cell populations in checkpoint inhibitor-induced colitis (CPI), the percentage of \( FOXP3^{+} \) Treg cells was found to be significantly elevated in CPI patients (Luoma et al., 2020), and the increase of special \( FOXP3^{+}/BATF^{+} \) Treg cells and \( IL1B/LYZ^{+} \) myeloid cells were confirmed by another study in inflamed UC patients (Devlin et al., 2021). Along with \( CD8^{+} \) T cells, \( CD4^{+} \) T cells were increased in CD patients, accompanied by an increased expression of the chemokines and cytokines, such as CXCL2, CXCL10, CXCL13, CCL11, and IL-6 (Elmentaite et al., 2020). Furthermore, the disease-specific patterns and metabolic changes were observed in \( CD4^{+} \) effector T cells according to the scRNA-seq analysis of 6 CD and 6 UC donors, including the increase of Toll-like receptor (TCR), Janus kinase (JAK), IL-17A, IL-22, TNF, et al. (Huang et al., 2021). Interestingly, \( CD39^{+} \) intraepithelial T cells are decreased in the pediatric IBD group, which may exacerbate colonic inflammation via platelet aggregation and 5-hydroxytryptamine (5-HT) release (Huang et al., 2019). These results confirmed the accumulation of activated \( CD8^{+} \) T cells during enteritis development, while the change of Treg cells was still unclear.

For other immune cell types, the expansion of \( IgG^{+} \) plasma cells, myeloid cells, and memory B cells were observed in the pediatric IBD group (Huang et al., 2019), and the increased \( IgG^{+} \) plasma cells and myeloid cells were also verified in CD patients (Elmentaite et al., 2020). Pathogenic expansion of \( IgG^{+} \) plasma cells and naïve B cells was also confirmed by scRNA-seq of human UC samples, which may be regulated by intestinal CXCL13-expressing follicular helper (TFH)-like T peripheral helper cells (Uzzan et al., 2022). Additionally, a shift from plasma cells to follicular cells and a decrease in the frequencies of \( IgA^{+} \) relative to \( IgG^{+} \) plasma cells were observed in the scRNA-seq survey of the 366,650 stromal cells from 18 UC patients and 12 healthy individuals (Smillie et al., 2019). Moreover, enrichment of mononuclear phagocytes was also observed in both

![Fig. 3](image-url) The alteration of cell types, signaling, and metabolism pathways in IBD. Some cell types are increased in IBD, such as microfold-like cells, memory B cells, \( IgG^{+} \) plasma cells, inflammatory fibroblasts, and \( CD8^{+} \) T cells, while \( SOX6^{+} \) mesenchymal cells, \( CD39^{+} \) intraepithelial T cells, \( WFDC2^{+} \) goblet cells, \( BEST4^{+} \) enterocytes, and \( VSTM2A^{+} \) stromal cell are decreased in IBD. Similarly, type I interferon signaling, Lox enzyme activity, TNF/IL-17 signaling, Kynurenien pathway, Butyrate/propanoate metabolism, \( \beta \)-oxidation, \( pH \)-sensing, epithelial integrity, et al. are increased in IBD, while \( \beta \)-oxidation, \( pH \)-sensing, epithelial integrity, et al. are decreased in IBD.
infliximab- and vedolizumab-treated non-responder samples from IBD patients by scRNA-seq analysis, which was validated in the dextran sulphate sodium (DSS)-induced colitis mouse model (Liu et al., 2019). Therefore, most immune cells are increased in enteritis, especially for IgG+ plasma cells and myeloid cells, but other cell types, such as naïve B cells and mononuclear phagocytes, need further investigation. These studies illustrate the detailed change in the intestinal immune system during enteritis development.

The association between environmental signaling and intestinal enteritis

Environment signaling also plays an important role in the pathogenesis of inflammation. Kinchen and colleagues surveyed the colonic mesenchymal atlas of health and IBD samples by scRNA-seq. They found that SOX6+ mesenchymal cells, which express TGF-β superfamily ligands BMP2 and BMP5, the non-canonical Wnt ligands WNT5A and WNT5B, and the Wnt antagonist FRZB, were decreased in inflamed UC colonic tissues (Kinchen et al., 2018). Similarly, PDGFRα, BMPs, WNT5A, SOX6, and matrix genes (ADAMDEC1 and GSN) in the crypt top fibroblasts were decreased in human IBD tissues and mouse DSS model (Elmentaite et al., 2020; Kinchen et al., 2018). Another study showed that MAP3K2-regulated intestinal stromal cells are the source of R-spondin 1 following intestinal injury, which can protect the intestine from DSS-induced colitis in mice (Wu et al., 2021). These results illustrate the importance of the microenvironment remodeling in intestinal enteritis.

A common therapeutic target for IBD is TNF, which is usually upregulated during enteritis development (Kinchen et al., 2018). Integration of scRNA-seq and proteomics revealed that activation of integrin signaling is associated with anti-TNF therapy resistance in IBD (Brubaker et al., 2020). Enrichments of IgG plasma cells, inflammatory mononuclear phagocytes, and activated T and stromal cells are also associated with resistance to anti-TNF therapy according to the scRNA-seq analysis of human ileal CD samples (Martin et al., 2019). ScRNA-seq of NOD2-driven CD in zebrafish suggests that gpi130 blockade could be used to complement anti-TNF therapy (Nayar et al., 2021). Therefore, upregulated TNF signaling, reduced activities of BMPs, and non-canonical Wnts may be associated with intestinal enteritis, which may guide therapy.

Epithelial barrier and metabolism changes during enteritis development

The intestinal epithelium establishes the fundamental barrier for microbiota defense, intestinal luminal stress and mucosal immunity, and dysfunction of the epithelial barrier contributes to enteritis development. ScRNA-seq analysis of human colonic epithelia in health and clinically inflamed and noninflamed UC mucosa identified a novel pH-sensing absorptive colonocyte population marked by BEST4/OTOP2 (Parikh et al., 2019), and its reduction was associated with IBD development (Fig. 3). A disease-associated cluster of goblet cells was also observed to highly express WFD C2, KLF2, LAMC2, LAMB3, PLEC, and F3, and these genes may play an important role in the integrity maintenance of the epithelial barrier (Parikh et al., 2019). In addition, other genes, which may contribute to pediatric IBD pathogenesis by causing impaired epithelial integrity and stress responses, were reported to be enriched in IBD epithelial cells, including ERN1, PSMA6, DVL1, CASP7, and PIEZO1 (Huang et al., 2019).

Niche cells also contribute to the integrity maintenance of the intestinal epithelium. A stromal subset marked by VSTM2A, SOX6, and AGT was functionally related to the epithelial basement membrane at the physiological condition, and its deregulation contributes to epithelial barrier breakdown during colitis (Fig. 3) (Kinchen et al., 2018). Similarly, inflammation-driven fibroblasts could regulate mucosal matrix remodeling and healing in mouse DSS-induced colitis by producing IL-11 and the metalloprotease Adamdec1 (Jasso et al., 2022). The enrichment of fibroblasts and endothelial cells that participate in matrix remodeling and type I interferon signaling was also revealed by the single-cell transcriptomic analysis from the children samples with undifferentiated colitis, CD, and UC (Huang et al., 2019).

Interestingly, enteritis-associated inflammation also leads to metabolic changes in epithelial cells, such as induction of the kynurenine pathway and arginine biosynthesis enzymes, increased metabolism of butyrate and propionate, impaired degradation of branched-chain amino acids, and downregulation of β-oxidation (Fig. 3) (Smillie et al., 2019). The blockade of Lox enzyme activity in Lox/LoxI1+ mesenchymal cells could attenuate DSS colitis and reduce circulating markers of oxidative stress by reducing hydrogen peroxide (Kinchen et al., 2018). In addition, other changes may be pathogenic, such as 5-HT over-secretion (Huang et al., 2019), integrin signaling activation (Brubaker et al., 2020), and redox imbalances (Kinchen et al., 2018). Although the survey at the single-cell level surely advances our understanding of IBD development, the causal relationship between the dysfunction of the epithelial barrier, metabolism changes, and intestinal enteritis needs experimental verification.

Mice as a model for human enteritis

DSS treatment or irradiation of the mouse intestine is often used as enteritis models (Zhu et al., 2021b).
Assessment of the difference and similarity between mouse and human enteritis is important for the proper application of mouse models. ScRNA-seq analysis of colon and colitis showed that embryonic-specific genes reappear in response to intestinal damage, which is conserved both in human IBD and mouse DSS-induced colitis, including MYO15B, SI00A11, and CDV3 (Fazilaty et al., 2021). ScRNA-seq combined with spatial transcriptomic analyses also defined a subset of human IBD-risk genes that occur in the mouse DSS-treated colitis, such as the genes related to immune cell recruitment (e.g., Itgal, Icam1, Itga4), activation (e.g., Cd6, Plec2, Ncf4, Il10ra), and antigen presentation (e.g., Tap1, Tap2, Psmb8) (Parigi et al., 2022).

However, different gene expression profiles were reported between human colitis and mouse model. For instance, Serpin1c (or PI3 in humans) was only increased in mice after intestinal damage (Fazilaty et al., 2021). ScRNA-seq analysis also revealed that a new skin-like epithelial population called squamous neo-epithelium marked by the expression of Sox2/Krt14/Krt7 could confer the resistance to colitis injury and rebuild the epithelial structure after colitis (Liu et al., 2022). This specialized population is only identified in the rectum of the DSS-treatment mouse model and need to be confirmed in human. These observations indicate that mouse colitis models should be cautiously used for human enteritis although some pathogenetic mechanisms are conserved.

**Single-cell technologies reveal novel insights into gut cancer development**

Deciphering tumor heterogeneity is a key to get a comprehensive understanding of tumor origination and to find better and more precise treatments. Our current knowledge of intra-tumoral heterogeneity is largely gained from the analysis of bulk tumor specimens, including bulk DNA/RNA sequencing. However, most bulk tumor specimens consist of a mixture of nonmalignant cells and diverse subpopulations of cancer cells. To gain more insight into intra-tumoral heterogeneity, single cancer cell clones have been generated (Roerink et al., 2018). But these clones still cannot cover all the cancer cells with distinct behavior. In this regard, single-cell technology exhibits its advantage in dissecting the multiple dimensions of intra-tumoral heterogeneity and their evolutionary relations (Francis et al., 2014; Navin et al., 2011). Combining single-cell DNA sequencing (scDNA-seq) and scRNA-seq, more details can be obtained at the single-cell resolution, such as tumor cell heterogeneity (Darmanis et al., 2017), epithelial-to-mesenchymal transition (EMT) (Pastushenko et al., 2018), cancer metastasis (Puram et al., 2017), and tumor microenvironment (Neal et al., 2017).

**Transcriptomic heterogeneity of CRC**

CRC development is influenced by numerous factors whose combinatory effects lead to various tumor subtypes and cellular heterogeneity (Dekker et al., 2019). ScRNA-seq has been used to profile various tumors and has revealed the cell constitution, tumor-initiating cells, and the microenvironment cells. For instance, it has been reported that stem-like cells accounted for 93% of tumor epithelial cells in CRC, but only 30% in normal mucosa epithelium, according to a new clustering method named reference component analysis (Li et al., 2017). This study also grouped colorectal tumors into subtypes with proliferating and differentiated states, which were previously assigned to a single cell type by bulk transcriptomics (Li et al., 2017; Wu et al., 2017). The distinct cell subpopulations resembling differentiation states of normal intestinal epithelial cells (ISCs-like, TA-like, and differentiated cells) were also revealed by other scRNA-seq analyses in CRC patients (Wu et al., 2017; Zowada et al., 2021). These ISCs-like and TA-like cells showed high levels of oxidative phosphorylation and mitochondrial membrane potential, which were linked to tumor-initiating activity (Zowada et al., 2021).

ScRNA-seq has also been employed to investigate the epithelial-mesenchymal interaction in CRC pathogenesis. Analysis of cancer stem cells in CRC in ApcMin/+ mouse adenoma at various time points during chemoradiotherapy suggests that tumor-initiating cells can shape the tumor environment into a landscape, which renders resistance to immunosuppression by forming an immune barrier against CD8+ T cells and promotes the proliferation of therapy-resistant cancer stem cells via Cox-2/PGE2 signaling (He et al., 2021). Moreover, cancer stem cell activity is also regulated by the niche BMP and WNT signaling secreted by the PDGFRAlow/CDBI+ stromal cells (Yum et al., 2021). The oncogene reporter mouse model combined with scRNA-seq analysis suggests that oncogene-expressing crypts can replace neighboring wild-type crypts over time, thereby leading to accelerated clonal drift and CRC development (Yum et al., 2021). In summary, these results implicate that the transformed epithelial cells can accelerate the tumorigenesis process themselves and cultivate the environment to facilitate tumor development.

**The role of the immune system in CRC therapeutic outcomes**

Apart from promoting tumor transformation of the intestinal epithelial cells, the immune cells also play an important role in CRC therapeutic outcomes and influence tumor metastases. ScRNA-seq transcriptomic profiling of T cells from peripheral blood, adjacent
normal and tumor tissues of CRC patients showed that the CXCL13+/BHLHE40+ subset of IFNG+ Th1-like T cells was preferentially enriched in microsatellite instability tumors (Zhang et al., 2019b). Treatment of a CD40-activating antibody could expand Th1-like and CD8+ memory T cells in colon cancer (Zhang et al., 2020a). Furthermore, the scRNA-seq analysis of 37,931 T cells from 16 CRC patients suggests the phenotypically and functionally distinguishable CD4+ and CD8+ effector T cell types are associated with clinical outcomes (Masuda et al., 2022). The GZMK+/KLRG1+ cytotoxic CD8+ T cells with a less dysfunctional phenotype were enriched in CRC patients with good outcomes. For CD4+ T-cell infiltrates, Helios+ Treg cells were associated with good outcomes, while Helios+/CD38+ peripherally-induced Treg cells were strongly associated with bad outcomes. These immune cells may be good targets for advanced treatments and the prognosis of CRC.

It has been reported that specific macrophage and conventional dendritic cell subsets may be key factors in the development of colorectal adenoma and liver metastases, according to the scRNA-seq of 18 treatment-naive CRC patients (Zhang et al., 2020a). ScRNA-seq and spatial transcriptomic analysis further revealed that immune-suppressive cells, especially MRC1+/CCL18+ M2-like macrophages, were enriched in live metastases of CRC (Wu et al., 2022). Single-cell transcriptomic profiling of lung metastases from CRC patients uncovered that a special subtype of B cells (ERBIN+) was involved in cancer metastases, and targeting Erbin as well as the combinatorial block of B cells and PD1 could suppress lung metastasis of CRC in mice (Shen et al., 2021). Therefore, special T cells may be associated with CRC development, and macrophages may play an important role in CRC metastases. These scRNA-seq results present a more comprehensive understanding of the immune roles in tumor development and metastasis and provide new insights to improve immune therapies.

**Single-cell DNA-seq confirms tumor heterogeneity**

In addition to scRNA-seq, scDNA-seq has also exhibited its power in the understanding of cellular heterogeneity of CRC (Jiang et al., 2021; Navin et al., 2011). An early study of 63 single tumor cells identified the SLC12A5 gene with a high frequency of mutation at the single-cell level but exhibited low prevalence at the bulk population level (Yu et al., 2014). By far, single-cell whole-exome sequencing of CRC has discovered many important gene mutations. One unique sub-clonal mutation of CSMD1 was detected exclusively in the adenomatous polyps by scDNA-seq, but not by bulk sequencing (Wu et al., 2017). A similar scDNA-seq analysis also revealed mutations and somatic copy number alterations hidden in bulk sequencing (Liu et al., 2017). ScDNA-seq also revealed that EpCAM/CD44 colonic cancer stem cells are critical for cancer development, metastasis, and drug resistance (Du et al., 2008; Went et al., 2004). Single-cell whole-genome sequencing of EpCAM(high)/CD44+ cancer stem cells and EpCAM(high)/CD44− differentiated cancer cells in colon cancer indicated that those cells from the same patient had a similar somatic copy number variation pattern. However, the somatic copy number variation was found to be different in certain patients and might confer different growth advantages at later stages (Liu et al., 2018). Moreover, scDNA-seq of the primary tumors and liver metastases of CRC unraveled the extensive intratumoral heterogeneity at the primary and metastatic tumor sites, as well as the late dissemination of metastases from early progenitor clones (Leung et al., 2017).

Multiple techniques were employed to simultaneously assess somatic copy number variation, DNA methylation, and transcriptome of 1,900 single cells from 10 CRC patients at the single-cell level (Bian et al., 2018). The results uncovered that the genome-wide DNA demethylation patterns of cancer cells were consistent in all 10 patients, while the DNA demethylation degrees were correlated with the density of the heterochromatin-associated H3K9me3 of normal tissues (Bian et al., 2018). Also, combined analyses of scDNA-seq and scRNA-seq on tumor niche cells from 21 colorectal patients showed that somatic copy number alterations were prevalent in immune cells, fibroblasts, and endothelial cells in tumors compared with adjacent tissues (Zhou et al., 2020). Furthermore, five genes (BGN, RCN3, TAGLN, MYL9, and TPM2) were identified as fibroblast-specific biomarkers for the poor prognosis of CRC. ScDNA-seq and scRNA-seq were also applied to the organoids, which were derived from single normal intestinal cells, subjected to RNAi-mediated downregulation of the tumor suppressor gene APC, and then transplanted to mice to investigate how intra-tumoral heterogeneity is generated. The results showed that the emergence of new transcriptional subpopulations was the key for the adaptation of cancer cells to drastic microenvironmental changes (Ono et al., 2021). All these studies clearly demonstrate that the single-cell studies provide novel insights into cancer initiation, metastases, heterogeneity, and relation to the microenvironment, and would advance disease diagnosis and treatment.

**Conclusions and Perspectives**

With the development of single-cell technology, we have gained a more comprehensive understanding on the intestinal cellular constitution, characterizations, and...
| Organism       | Sample type                  | Sample source                     | Cell type                                                                 | Library preparation method           | Cell number | Data resource | Reference                                      |
|---------------|------------------------------|-----------------------------------|----------------------------------------------------------------------------|--------------------------------------|-------------|---------------|------------------------------------------------|
| *Mus musculus*| Jejunum                      | Labeled stem cells                | Short-term and long-term label-retaining stem cells                          | Fluidigm                             | 598         | GSE134479     | (Li et al., 2016)                              |
| *Mus musculus*| Jejunum                      | Wildtype mice                     | Epithelial, mesenchymal, and immune cells                                   | MARS-seq                             | 399         | GSE130113     | (Bao et al., 2019)                             |
| *Mus musculus*| Jejunum                      | Stem cells                        | Prox1-GFP+, Bmi1-GFP+, Lgr5-eGFP+, and Lgr5-eGFP−cells                      | 10X Chromium                         | 3,521       | GSE99457      | (Sato et al., 2017)                            |
| *Mus musculus*| Ileum                        | Wildtype mice                     | Crypt cells                                                                 | 10X Chromium                         | 19,245      | GSE92332      | (Yan et al., 2017)                             |
| *Mus musculus*| Ileum                        | Massive small bowel resection     | Epithelial cells                                                            | 10X Chromium                         | 7,216       | GSE130113     | (Seiler et al., 2019)                          |
| *Mus musculus*| Small intestine              | Wildtype mice                     | Crypt cells                                                                 | 10X Chromium                         | 13,247      | GSE92865      | (Bottcher et al., 2021)                        |
| *Mus musculus*| Small intestine              | Labeled epithelial cells          | Lgr5-GFP− cells                                                             | 10X Chromium                         | 288         | Not described | (Glass et al., 2017)                           |
| *Mus musculus*| Small intestine              | Labeled epithelial cells          | Intestinal preproglucagon-expressing cells                                  | 10X Chromium                         | 192         | Not described | Not described                                 |
| *Mus musculus*| Small intestine              | Wildtype mice                     | Epithelial cells                                                            | 10X Chromium                         | 27,687      | GSE147319     | (Tetteh et al., 2016)                          |
| *Mus musculus*| Small intestine              | Lgr5-eGFP+ intestinal stem cells  | Intestinal stem cells                                                       | 10X Chromium                         | 6,644       | GSE123516     | (Wang et al., 2020)                            |
| *Mus musculus*| Small intestine              | Wildtype and irradiated mice      | Epithelial cells                                                            | 10X Chromium                         | 12,653      | GSE131630     | (Ayuso et al., 2019)                           |
| *Mus musculus*| Small intestine              | Lgr5-GFP− intestinal stem cells   | High β7 intestinal stromal cells and high β7+endothelial cells              | 10X Chromium                         | 7,906       | GSE124488     | (Haug et al., 2017)                            |
| *Mus musculus*| Small intestine              | Wildtype and high-fat diet mice   | Epithelial cells                                                            | 10X Chromium                         | 11,332² for WT, 10,763 | GSE1100070 | (Chen et al., 2019)                            |
| *Mus musculus*| Small intestine              | Wildtype and Fox2/Yγ− mice        | Epithelial cells                                                            | 10X Chromium                         | 60,000      | GSE152325     | (Bottcher et al., 2021)                        |
| Organism     | Sample type          | Sample source                                                                 | Cell type                                      | Library preparation method | Cell number | Data resource   | Reference                           |
|--------------|----------------------|-------------------------------------------------------------------------------|-----------------------------------------------|---------------------------|-------------|----------------|-------------------------------------|
| *Mus musculus* | Small intestine | Wildtype crypts in Red2-KrasG12D and Red2-PK3CAH1047R mice | Epithelial, mesenchymal and immune cells | 10X Chromium             | 21,183      | E-MTAB-8656  | (Yum et al., 2021)                  |
| *Mus musculus* | Small intestine | Wildtype mice and Casp3/7<sup>LEC</sup> mice and Casp3/7<sup>Wt</sup> littermates | Epithelial cells and immune cells | MARS-seq                  | Not described | GSE178586  | (Zinina et al., 2022)               |
| *Mus musculus* | Small intestine and colon | Casp3/7ΔIEC mice and Casp3/7FL/FL littermates | Epithelial cells and immune cells | 10X Chromium             | 7,584       | GSE183885  | (Ghazavi et al., 2022)              |
| *Mus musculus* | Distal small intestine and colon | Muc2-mCherry mice | Goblet cells | 10X Chromium             | 6123        | GSE144436  | (Nystrom et al., 2021)              |
| *Mus musculus* | Colon | Gli1<sup>+</sup> stromal cells | Regenerating crypt cells and Ascl2-deficient colonic stem cells | 10X Chromium             | 4,464       | GSE113043  | (Degirmenci et al., 2018)           |
| *Mus musculus* | Colon | LGR5<sup>+</sup> lineage tracing and lethal injury (irradiation) mice | Regenerating crypt cells and Ascl2-deficient colonic stem cells | 10X Chromium             | 3,254       | GSE130822  | (Murata et al., 2020)               |
| *Mus musculus* | Colon | Wildtype mice and epithelial cells | Regenerating crypt cells and Ascl2-deficient colonic stem cells | 10X Chromium             | 6,358       | GSE130681  | (McCarthy et al., 2020c)            |
| *Mus musculus* | Colon | NeuroD1-Cre x Rosa26-EYFP labeled mice | EECs | 10X Chromium             | 1,560        | Not described | (Billing et al., 2019)              |
| *Mus musculus* | Colon | Wildtype mice | Epithelial, endothelial and stromal cells | 10X Chromium             | 7,395       | GSE151257  | (Brugger et al., 2020)              |
| *Mus musculus* | Colon | Wildtype mice | T cells | 10X Chromium             | 7,012        | GSE160055  | (Kiner et al., 2021)                |
| *Mus musculus* | Colon | Wildtype mice and DSS-exposed 3 days | Epithelial and mesenchymal cells | 10X Chromium             | 11,495      | GSE156245  | (Wu et al., 2021)                   |
| *Mus musculus* | Colon | MC38 xenografts upon SHP099 treatment | Cancer cells, stromal cells, and immune cells | 10X Chromium             | 7,934(SHP099), 7,881(PBS) | GSE164908  | (Gao et al., 2022)                  |
| *Mus musculus* | Colon | Wildtype and DSS treated mice | Stromal cells | 10X Chromium             | 34,197      | GSE172261  | (Jasso et al., 2022)                |
| *Mus musculus* | Distal colon | Wildtype and DSS treated mice | Epithelial, stromal and immune cells | 10X Chromium             | > 35,000     | GSE168033  | (Liu et al., 2022)                  |
| *Mus musculus* | Colorectal cancer | Primary tumor and lung metastases | Immune cells | 10X Chromium             | 12,588 B220<sup>+</sup> cells, 3,748 CD38<sup>+</sup> cells, 1,588 CD79a<sup>+</sup> cells | Not described | (Shen et al., 2021)                 |
| *Mus musculus* | Small intestine, colon | Wildtype mice | Endothelial cells | 10X Chromium             | > 32,000     | E-MTAB-8077  | (Kalucka et al., 2020)              |
## Table 2 (continued)

| Organism    | Sample type                                                      | Cell type                                                      | Library preparation method | Cell number | Data resource                      | Reference |
|-------------|------------------------------------------------------------------|----------------------------------------------------------------|----------------------------|-------------|------------------------------------|-----------|
| Mus musculus| Intestine pLysDTR mice (DT-treated for 6 consecutive days)       | Paneth cells                                                   | CEL-seq                    | 288         | Supplementary data-set 301         | (van Es et al., 2019) |
| Mus musculus| Intestine Wildtype mice                                          | Intestinal stromal cells                                        | Drop-seq                   | 4,359       | GSE116514                          | (Ken et al., 2020) |
| Mus musculus| Intestine Endoderm                                               | Intestinal endodermal cells                                    | 10X Chromium               | 112,177     | GSE123046                          | (Novotny et al., 2019) |
| Mus musculus| Intestine                                                       | Intestinal endodermal cells                                    | 10X Chromium               | 217         | GSE87038                           | (Dong et al., 2018) |
| Mus musculus| Intestine                                                       | Endoderm                                                        | 10X Chromium               | 2,329       | GSE145866                          | (Sheng et al., 2020) |
| Mus musculus| Intestine                                                       | Endoderm                                                        | 10X Chromium Single Cell Kit | 200         | GSE133038                          | (Piccard et al., 2019) |
| Mus musculus| Intestine                                                       | Endoderm                                                        | 10X Chromium Single Cell Kit | 1 million   | https://community.10xgenomics.com/samples/single-cell-10X-Chromium |          |
| Mus musculus| Intestine                                                       | Endoderm                                                        | 10X Chromium Single Cell Kit | 217         | GSE119988                          | (Beumer et al., 2019) |
| Mus musculus| Intestine                                                       | Endoderm                                                        | 10X Chromium               | 238         | GSE62770                           | (Gurt et al., 2019) |
| Mus musculus| Intestine                                                       | Endoderm                                                        | 10X Chromium Single Cell Kit | 1 million   | https://community.10xgenomics.com/samples/single-cell-10X-Chromium | (Benner et al., 2019) |
| Mus musculus| Intestine                                                       | Endoderm                                                        | 10X Chromium Single Cell Kit | 23,421      | GSE115956                          | (Serres et al., 2019) |
| Mus musculus| Intestine                                                       | Endoderm                                                        | 10X Chromium Single Cell Kit | 200         | https://figshare.com/articles/dataset/Sing-Cell-10X-Chromium | (Cho et al., 2021) |
| Mus musculus| Intestine                                                       | Endoderm                                                        | 10X Chromium Single Cell Kit | >1 million  | GSE156438                          | (Takagapada et al., 2021) |
| Mus musculus| Intestine                                                       | Endoderm                                                        | 10X Chromium Single Cell Kit | 217         | https://community.10xgenomics.com/samples/single-cell-10X-Chromium | (Beumer et al., 2022) |
| Organism          | Sample type                                      | Sample source                                                                 | Cell type                                                                 | Library preparation method                      | Cell number | Data resource      | Reference                           |
|-------------------|--------------------------------------------------|-------------------------------------------------------------------------------|---------------------------------------------------------------------------|-----------------------------------------------|-------------|--------------------|-------------------------------------|
| Mus musculus      | Transgene-inducible intestinal organoids          | Induced organoids for KRAS, BRAF and CTNNB1                                  | Epithelial cells                                                          | BD<sup>TM</sup> Precise WTA Single Cell Kit   | 160,000     | GSE115242          | (Brandt et al., 2019)              |
| Mus musculus      | Small intestine and organoids                    | Wildtype mice and organoids                                                   | Epithelial cells                                                          | 10X Chromium                                  | 10,180      | GSE100274          | (Mead et al., 2018)                |
| Mus musculus      | Intestinal adenoma of Apc<sup>Min</sup>/mice at various time points | Colorectal cancer                                                             | Epithelial and mesenchymal cells                                         | 10X Chromium                                  | 79,801      | GSE136256          | (Zowada et al., 2021)              |
| Homo sapiens      | Ileum                                            | CD tissues                                                                    | Lamina propria cells                                                      | 10X Chromium                                  | 82,417      | GSE134809          | (Martin et al., 2019)              |
| Homo sapiens      | Ileum                                            | Health and CD tissues                                                         | Immune cells                                                               | 10X Chromium                                  | 16,731      | GSE157477          | (Jaeger et al., 2021)              |
| Homo sapiens      | Esophagus, stomach, duodenum                     | Healthy tissues                                                               | Epithelium; immune cells                                                  | SORT-seq                                      | Not described | GSE157694/EGAS00001004695    | (Busslinger et al., 2021)          |
| Homo sapiens      | Small intestine                                  | Donor- and recipient-derived cells after transplantation                      | T cells                                                                    | 10X Chromium                                  | 974 for scRNA-seq, 196 for smart-seq         | GSE162687          | (FitzPatrick et al., 2021)         |
| Homo sapiens      | Small intestine                                  | Healthy tissues                                                               | Epithelial cells                                                          | 10X Chromium                                  | 12,590      | GSE185224          | (Burclaff et al., 2022)            |
| Homo sapiens      | Ileum and ileum-derived organoids                | Healthy and viral infected tissues                                            | Epithelial cells                                                          | 10X Chromium                                  | 25,482      | GSE171620          | (Triana et al., 2021)              |
| Homo sapiens      | Colon                                            | Healthy and IBD tissues                                                       | Epithelial and mesenchymal cells                                         | Smart-seq2                                    | 11,175      | GSE116222          | (Parikh et al., 2019)              |
| Homo sapiens      | Colon                                            | Healthy and UC tissues                                                        | Epithelial and mesenchymal cells & immune cells                           | 10X Chromium                                  | 366,650     | SCP259             | (Smilie et al., 2019)              |
| Homo sapiens      | Colon                                            | Colorectal cancer                                                             | Cancer cells, stromal cells and immune cells                              | 10X Chromium                                  | 27,927      | GSE188711          | (Guo et al., 2022)                 |
| Homo sapiens      | Colon                                            | Colorectal cancer                                                             | Immune cells                                                               | 10X Chromium                                  | 178,630     | OEP001756          | (Wu et al., 2022)                  |
| Homo sapiens      | Colon                                            | Tumors and adjacent tissues                                                   | T cells                                                                   | 10X Chromium                                  | 37,931      | EMTAB-9455         | (Masuda et al., 2022)              |
| Homo sapiens      | Colon                                            | Healthy and UC tissues                                                        | Immune cells, stromal cells                                               | 10X Chromium                                  | 29,046      | GSE182270          | (Uzzan et al., 2022)               |
| Homo sapiens      | Colon                                            | Healthy tissues                                                               | Macrophages                                                               | 10X Chromium                                  | 63,970      | EGA-D00001007765/ EGA-S00001005377 | (Domanska et al., 2022)            |
| Homo sapiens      | Colon and rectum                                 | Familial adenomatous polyposis                                               | Environmental cells (endothelial cells, fibroblasts, macrophages, mast cells, T cells and B cells) and epithelial cells | STRT-Seq(modified)                            | 8,757       | Not described       | (Li et al., 2020)                  |
| Organism        | Sample type    | Sample source                                                                 | Cell type                                      | Library preparation method | Cell number | Data resource          | Reference                  |
|-----------------|----------------|-------------------------------------------------------------------------------|-----------------------------------------------|-----------------------------|-------------|------------------------|----------------------------|
| Homo sapiens    | Large intestine| Children with undifferentiated colitis, Crohn’s disease, and ulcerative colitis | Epithelium, mesenchyme and immune cells       | 10X Chromium                | 73,165      | GSE121380              | (Huang et al., 2019)       |
| Homo sapiens    | Large intestine| Colorectal cancer                                                              | Immune and non-immune cells                   | 10X Chromium and Smart-seq2 | 43,817 10,468 (Smart-seq2) | GSE146771              | (Zhang et al., 2020a)      |
| Homo sapiens    | Large intestine| Healthy; melanoma with Checkpoint inhibitor-induced colitis; melanoma without Checkpoint inhibitor-induced colitis | Immune cells                                  | 10X Chromium                | 51,652      | GSE144469              | (Luoma et al., 2020)       |
| Homo sapiens    | Large intestine| Healthy, CD and UC tissues                                                     | CD45+ immune cell                              | 10X Chromium                | 63,314      | PRJCA003980            | (Huang et al., 2021)       |
| Homo sapiens    | Large intestine| Healthy and UC tissues                                                         | Immune cells                                   | 10X Chromium                | 20,678 (16,678 Health) | GSE162335              | (Devlin et al., 2021)      |
| Homo sapiens    | Ileum, colon, rectum | Precancerous tissues                                                           | Epithelial cells                               | 10X Chromium                | 14,537      | GSE125970              | (Wang et al., 2020b)       |
| Homo sapiens    | Small intestine, large intestine | Fetal digestive tissues                                                       | Epithelial and mesenchymal cells              | STRT-seq                    | 5,227       | GSE103239              | (Gao et al., 2018)         |
| Homo sapiens    | Embryonic gastrointestinal tract | Healthy tissues                                                                | Epithelial cells                               | Not described               | 5,290       | Supplementary Table 1 and 2 | (Tan et al., 2019)         |
| Homo sapiens    | Human embryonic intestinal tract | Healthy tissues                                                                | Epithelial, mesenchymal and immune cells      | 10X Chromium                | 24,783      | E-MTAB-9489            | (Holloway et al., 2021)    |
| Homo sapiens    | Human embryonic and adult intestinal tract | Healthy tissues                                                                | Epithelial, mesenchymal and immune cells      | 10X Chromium                | 428,000     | E-MTAB-9543, E-MTAB-9536, E-MTAB-9532, E-MTAB-10386 | (Elmentaite et al., 2021) |
| Homo sapiens    | Small intestinal organoids | Crohn’s disease patient-derived tissues                                        | Epithelial cells                               | C1                          | 1,037       | Not described          | (Suzuki et al., 2018)      |
| Homo sapiens    | Human pluripotent stem cell-derived intestinal organoids | Organoids                                                                      | Epithelial, mesenchyme, endothelium, and neurons | 10X Chromium                | 13,289      | E-MTAB-9228            | (Holloway et al., 2020)    |
| Homo sapiens    | Intestine and organoids | Intestinal tissues and organoids                                               | EEC                                           | 10X Chromium                | 15,283      | GSE146799              | (Beumer et al., 2020)      |
| Homo sapiens    | Cell lines       | Colorectal cancer                                                               | SW480 cells                                   | 10X Chromium & SORT-seq     | 192         | Not described          | (Yi et al., 2020)          |
| Homo sapiens    | Cell lines       | Control and SFU-treated colon cancer                                            | SFU-treated RKO, HCT116, SW480                | Drop-seq                    | 10,421      | GSE149224              | (Park et al., 2020)        |

Table 2 (continued)
| Organism                        | Sample type                           | Sample source                          | Cell type                                    | Library preparation method | Cell number | Data resource          | Reference                        |
|--------------------------------|---------------------------------------|----------------------------------------|----------------------------------------------|----------------------------|-------------|------------------------|----------------------------------|
| Homo sapiens                   | Large intestine and cell lines         | Healthy tissues and colorectal cancer  | Epithelial cells                           | C1                         | 2,221       | GSE81861               | (Wu et al., 2017)                |
| Homo sapiens                   | Primary and metastasis tumor          | Colorectal cancer                      | Spheroids, Tumors, PDOs, PDXs, PDOs         | 10X Chromium and iCELL8    | 26,170      | EGA500001004064        | (Zowada et al., 2021)            |
| Homo sapiens                   | Tumors and adjacent normal tissues    | Colorectal cancer                      | T cells                                     | Smart-seq2                 | 8,530       | EGA500001002791 / GSE108989 | (Zhang et al., 2018)             |
| Homo sapiens                   | Embryonic, fetal, childhood/adolescence ileum | Healthy and pediatric Crohn's disease tissues | Epithelial, mesenchymal and immune cells   | 10X Chromium               | 74,106      | E-MTAB-8901            | (Elmentaite et al, 2020)         |
| Homo sapiens                   | Human embryonic intestinal tract      | Healthy tissues                        | Epithelial, mesenchymal and immune cells   | 10X Chromium               | 76,592      | GSE158328 / GSE158702  | (Fawkner-Corbett et al., 2021)   |
| Mus musculus; Homo sapiens     | Intestine and organoids               | Colonic mesenchyme; fibroblast-crypt cultures; Ptger4-knockout crypt epithelial cells | Epithelial cells and mesenchymal cells    | Drop-seq                   | 5,371       | GSE142431              | (Roulis et al., 2020)            |
| Mus musculus; Homo sapiens     | Colon                                 | Healthy, UC and DSS treated tissues    | Epithelial and mesenchymal cells           | 10X Chromium               | 11,549      | GSE114374              | (Kinchen et al., 2018)           |
| Mus musculus; Homo sapiens     | Human embryonic intestinal tract and mouse colon and colitis | Healthy and DSS treated tissues | Epithelial, mesenchymal and immune cells | 10X Chromium               | 22,579      | GSE151257 / GSE154007  | (Fazilaty et al., 2021)          |
| Mus musculus; Homo sapiens     | Intestinal organoids                  | Organoids                              | Epithelial cells                           | DisCo                      | 945         | GSE148093              | (Bues et al., 2022)              |
| Rhesus macaques                | Large intestine                       | Healthy and during acute graft-versus-host disease tissues | T cells                                   | 10X Chromium               | 21,490      | GSE142483              | (Tkachev et al, 2021)            |
| Zebrafish                      | Whole intestine                       | Healthy and DSS treated tissues        | Epithelial, mesenchymal and immune cells   | 10X Chromium               | 30,069      | https://github.com/Cho-lab-Sina/Scripts_Nayar_et_al | (Nayar et al., 2021)             |
| Swine                          | Ileum                                 | Healthy tissues                        | Epithelial cells                           | 10X Chromium               | 40,186      | GSE162287              | (Meng et al, 2021)               |
| Drosophila melanogaster        | Midgut                                | Healthy tissues                        | EECs                                        | 10X Chromium               | 4,661       | GSE132274              | (Guo et al, 2019)                |
heterogeneity (Table 2). Single-cell sequencing has also yielded new insights into the pathogenesis of enteritis and intestinal cancer. Although a significant advance has been made, many questions remain. Single-cell transcriptomic analysis has unraveled new subtypes of intestinal tuft cells, enterocytes, and EECs, but the heterogeneity of other cell types and the landscape of the intestinal immune system need further investigation. Epithelial progenitors have been divided into absorptive and secretory subtypes (Clevers and Batlle, 2013), but no clear subpopulations are reported. It may be due to a vague definition of progenitors and unclear differences between progenitors and TA cells. Attention has also been paid to the differences between $Lgr5^{\text{hi}}$ and $Lgr5^{\text{lo}}$ subpopulations (Baulies et al., 2020), but the heterogeneity among intestinal stem cells is still unclear. Even the dispute over whether there are quiescent stem cells or +4 stem cells is not settled. Microfold cells are indicated to be essential for antigens transport to the lymphoid cells underneath (Mabbott et al., 2013), but the atlas of microfold cells is rarely known because of their low cell number in the intestinal epithelium. The spatial heterogeneity of enterocytes and EECs has been explored along the crypt-villus axis and in different intestinal segments (Beumer et al., 2018; Wang et al., 2020b). More comprehensive investigations on spatial cell heterogeneity, pathophysiological significance, and the underlying mechanisms are needed. Also, the spatiotemporal transcriptional atlas of EECs has been reported. Similar studies should be applied to other epithelial cell types and niche cells during intestine development and regeneration, which would help address the question concerning what regulates the formation of the special structures. For instance, Hedgehog signaling has been reported to be critical for villus formation (Rao-Bhattia et al., 2020).

Nowadays, we can perform single-cell sequencing with millions of cells (Qin et al., 2020), but the sequencing depth in each cell is limited in the massive scRNA-seq. On another side, some sequencing technologies, such as Smart-seq2 (Picelli et al., 2013), shows a great sequencing depth, but it can only apply to small cell number due to the cost. If the depth sequencing can be combined with massive single-cell numbers cost-effectively, more information will be gained (Lai et al., 2020). Furthermore, other single-cell technologies are under development, such as single-cell protein mass spectrum, single-cell sequencing of whole epigenomes, and combinatorial DNA/RNA/epigenetic sequencing in one cell (Bian et al., 2018; Hutchins, 2021), the low-input but high-resolution analysis scRNA-seq methods (Bues et al., 2022). If the breakthroughs of these technologies on the scale, sensitivity, and cost-efficiency can be made, biomedical research will enter the single-cell era.

**Abbreviations**

*S-HT: 5-Hydroxytryptamine; BMP: Bone morphogenetic protein; CCK: Cholecystokinin; CD: Crohn’s disease; Chga: Chromogranin-A; CPI: Checkpoint inhibitor-induced colitis; CRC: Colorectal cancer; DISCO: Digital microfluidic isolation of single cells for-omics; DSS: Dextran sulphate sodium; EECs: Enteroendocrine cells; EGF: Epidermal growth factor; EMT: Epithelial-to-mesenchymal transition; FACS: Fluorescence activated cell sorter; Fox1: Forkhead box L1; GAST: Gastrin; GCG: Glucagon; GHRL: Ghrelin; GIP: Glucose-dependent insulinotropic polypeptide; HIST: Histamine; IBD: Inflammatory bowel disease; iCGCs: Intercrypt goblet cells; ISC: Intestinal stem cells; JAK: Janus kinase; LCM- RNA-seq: RNA-sequencing of laser capture micro-dissected tissue; MARS-seq: Massively parallel single-cell RNA-sequencing; MLN: Mollitis; NTS: Neurotensin; PSMA6: Proteasome 20S subunit alpha 6; PYY: Peptide YY; scDNA-seq: Single-cell DNA sequencing; scRNA-seq: Single-cell RNA sequencing; SCT: Secretin; SORT-seq: FACS sorting followed by single-cell RNA-sequencing; SST: Somatostatin; STRT-seq: Single-cell tagged reverse transcription sequencing; TA cells: Transient-amplifying cells; TAC1: Tachykinin 1; TCR: Toll-like receptor, Th2 cells: Type 2 helper T cells; TNF: Tumor necrosis factor, UC: Ulcerative colitis.

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**Authors’ contributions**

YW and YGC wrote the manuscript; WS integrated the reported single-cell data. SY andYL helped with the manuscript writing. The author(s) read and approved the final manuscript.

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