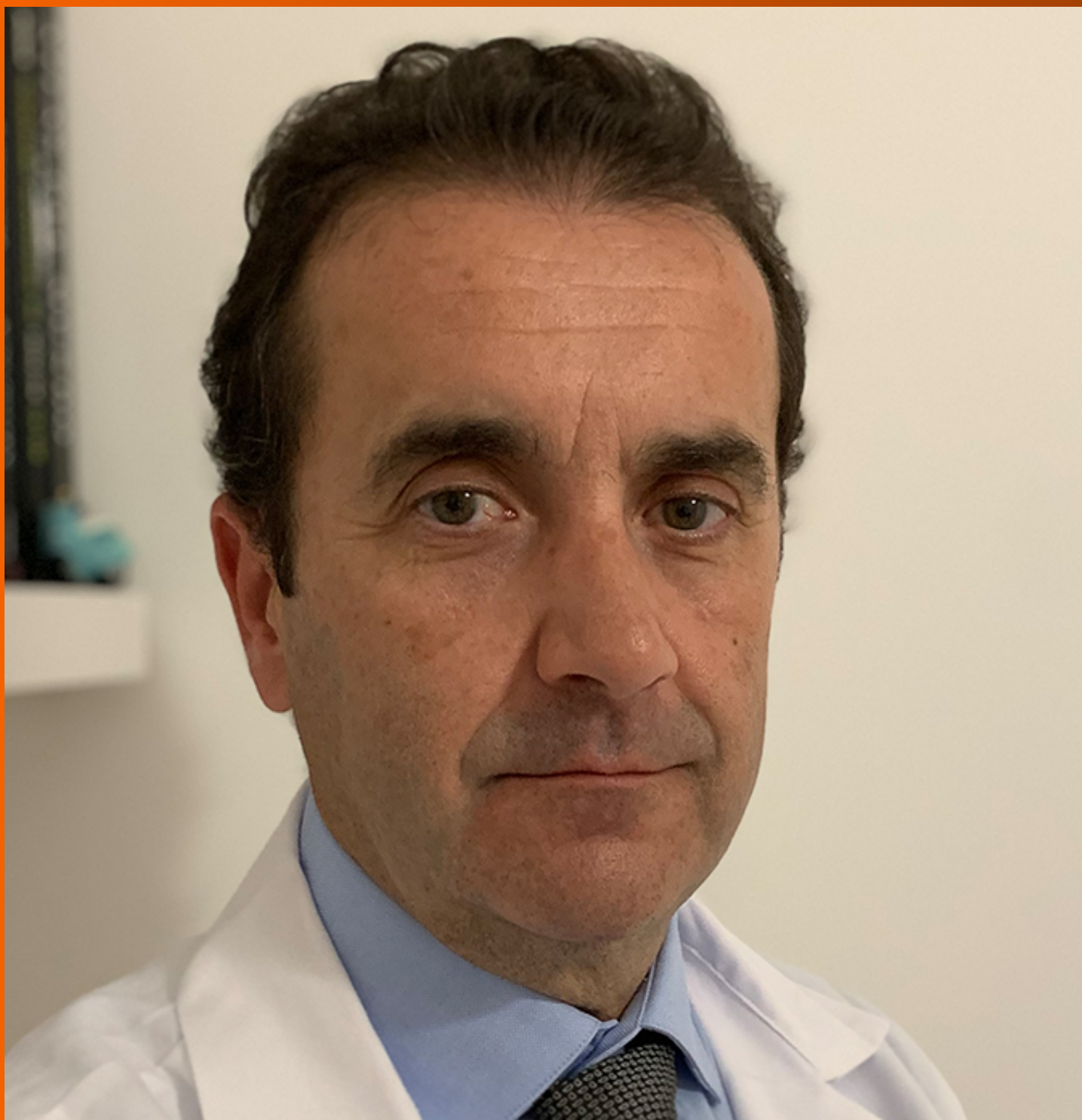


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## Application of single-cell omics in inflammatory bowel disease

Hengqi Betty Zheng

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**Hengqi Betty Zheng**, Department of Pediatrics, Seattle Children's Hospital, University of Washington, Seattle, WA 98105, United States

**Corresponding author:** Hengqi Betty Zheng, MD, Assistant Professor, Department of Pediatrics, Seattle Children's Hospital, University of Washington, 4800 Sandpoint Way NE, Seattle, WA 98105, United States. [betty.zheng@gmail.com](mailto:betty.zheng@gmail.com)

### Abstract

Over the past decade, the advent of single cell RNA-sequencing has revolutionized the approach in cellular transcriptomics research. The current technology offers an unbiased platform to understand how genotype correlates to phenotype. Single-cell omics applications in gastrointestinal (GI) research namely inflammatory bowel disease (IBD) has become popular in the last few years with multiple publications as single-cell omics techniques can be applied directly to the target organ, the GI tract at the tissue level. Through examination of mucosal tissue and peripheral blood in IBD, the recent boom in single cell research has identified a myriad of key immune players from enterocytes to tissue resident memory T cells, and explored functional heterogeneity within cellular subsets previously unreported. As we begin to unravel the complex mucosal immune system in states of health and disease like IBD, the power of exploration through single-cell omics can change our approach to translational research. As novel techniques evolve through multiplexing single-cell omics and spatial transcriptomics come to the forefront, we can begin to fully comprehend the disease IBD and better design targets of treatment. In addition, hopefully these techniques can ultimately begin to identify biomarkers of therapeutic response and answer clinically relevant questions in how to tailor individual therapy to patients through personalized medicine.

**Key Words:** Single-cell omics; Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Single cell RNA-sequencing; Precision medicine

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**Core Tip:** Single-cell techniques and omics have taken off in the last few years and the ability to detect individual cellular transcript details has revolutionized the world of research. In the field of gastroenterology in just the last five years, several single-cell techniques have been applied to inflammatory bowel disease research with the identification of novel cellular immune players in the pathogenesis of both ulcerative colitis and Crohn's disease.

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## INTRODUCTION

The gastrointestinal (GI) tract houses a complex network of immune cells, cellular signaling, and intestinal flora. By balancing host tolerance and microbial defense response, the mucosal immune system of the GI tract performs constant surveillance and sampling of antigens and microbes to induce either tolerance or mounts a vigorous immune response to pathogens[1]. Intestinal inflammation and disease occur when this delicate balance breaks down leading to over activation of mucosal immunity and aberrant response to host antigen and commensal organisms[2]. Microscopic disruption of the epithelial barrier, over activation of proinflammatory cells, and the lack of regulatory mechanisms are some pathways that present in clinical chronic GI disorders and distinguishes mucosal immunity from systemic immunity.

The ability to detect pathogenic pathophysiology is rooted in the organ, the GI tract, and may be missed through studying the systemic immune system. In order to understand the target organ and mucosal immune system, technical advances have been made in the last few years giving us the ability to interrogate down to the microscopic single cell level, thus revolutionizing GI research. Since its inception in 2009, single cell RNA-sequencing (scRNA-seq) has enhanced our ability to comprehensively map and resolve cell types, cellular subsets, and cells states present in both healthy GI tissue and diseased states[3]. The novelty of single-cell technologies *vs* previous technologies such as bulk-sequencing is the ability to detect rare subsets of cells that may be the aberrant drivers of disease[4]. The homogeneity of bulk-sequencing lacks the capacity to decipher cellular heterogeneity and loses dimmer signals in rare subsets that may be important in disease pathogenesis[4,5]. The inherent advantage of single-cell techniques has led to its continued popularity in research. Here, we aim to review the single-cell technologies that have emerged over the last decade and its published applications to GI disease, namely in inflammatory bowel disease (IBD).

IBD which includes both ulcerative colitis (UC) and Crohn's disease (CD) is a chronic complex autoimmune condition characterized by inflammation of the GI tract. The pathogenesis of IBD is thought to develop from an inappropriate immune response towards self-antigens and commensal microbiota in a genetically susceptible host. The advent of scRNA-seq has led to a boom in the number of publications with the application of single cell techniques in IBD research [6,7]. As a disclaimer, this is by no means a comprehensive review of all single-cell studies or single-cell techniques available as the technology has massively expanded in the last few years and will continue to grow in popularity in years to come.

## OVERVIEW OF AVAILABLE SINGLE-CELL TECHNOLOGY

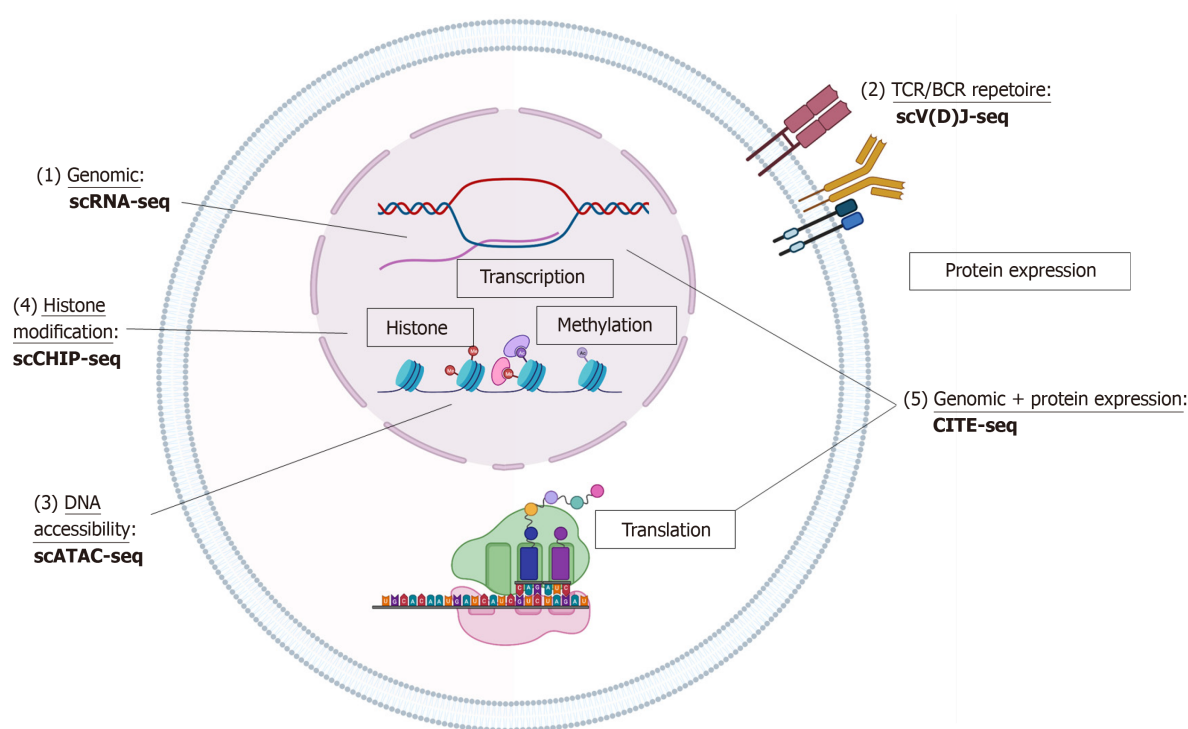
### scRNA-seq

With the commercialization of products and increasing user compatibility, scRNA-seq can be done using a variety of platforms and through multiple approaches (10 × Genomics, Fluidigm, BD Rhapsody, SmartSeq, *etc.*) whether with multiple well plates, microfluidics, or drop-seq[8,9]. Single-cell data sets can also be created from the 3' end or 5' end of mRNA. The basis behind the technology is partitioning individual cells with one uniquely barcoded mRNA-capture medium which has unique molecular code tag for each cell and its contained transcripts. The cell is then lysed and the mRNA is captured by these coded tags and each cell's mRNA is reverse transcribed into uniquely barcoded cDNA. cDNA libraries are amplified, indexed and sequenced using next-generation sequencing platforms (NextGen). Using the unique molecular code tags, the cDNA can be linked to the cell of origin and abundance of transcripts can be deciphered using the number of copies of the certain cDNA (Figure 1).

With large sets of data, bioinformatics analysis can be challenging. To perform this analysis, FASTQ files are generated from binary base call output from sequencing using software programs that then align the reads from the FASTQ files to a human reference genome/transcriptome using STAR[10]. Gene-cell matrices with principle component analysis can then be used to quantify transcripts of interest using standard workflows to give traditional visualization of single-cell datasets in the form of uniform manifold approximation and projection algorithms or T-distributed stochastic neighbor embedding plots (Figure 1).

In some cases, studies are interested not only in the current state of the cell but what the possible future of how the cell may develop ("pseudotime"). Software programs including Monocle[11] and Censu[12] offer toolkits of computational bioinformatic programs specifically designed for the analysis of scRNA-Seq data to look at the temporal resolution of





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**Figure 1 Single-cell technologies.** scRNA-seq: Single cell RNA-sequencing; TCR: T cell receptor; BCR: B cell receptor; scCHIP: Single-cell chromatin immunoprecipitation; scATAC: Single-cell assay for transposase-accessible chromatin; CITE: Cellular indexing of transcriptomes and epitopes. Created in biorender.com.

transcriptome dynamics using unsupervised algorithms on scRNA-Seq data collected at multiple time points. These programs can be used to recover single-cell gene expression kinetics from a wide array of cellular processes, including differentiation, proliferation and oncogenic transformation to identify branching patterns in the cells. The algorithm finds the longest path through the minimum spanning tree, one that corresponds to the longest sequence of transcriptionally similar cells and produces a ‘trajectory’ of an individual cell’s progress through differentiation that is expressed in units of pseudotime (Figure 1).

### T cell receptor/B cell receptor: scV(D)J-seq

T cell receptor (TCR) and B cell receptor (BCR) drive a range of antigen specific adaptive immune responses to pathogens with large highly diverse repertoires to allow for recognition of antigens[9]. The advent of 5’ single-cell sequencing allows researchers to better understand lymphocyte diversity and antigen specificity or essentially the clonality of T and B cells present in disease states. The technique of TCR/BCR is similar to scRNA-seq but utilizes a specialized switch oligo nucleotides and poly dT tail primers[9]. Untemplated C (cytosine) nucleotides to the 3’ end will pair with the end of switch oligo and reverse transcription occurs to capture the TCR/BCR sequences[9]. cDNA libraries of TCR/BCR are created and sequenced per usual protocol (Figure 1).

### Single-cell assay for transposase-accessible chromatin

The modification in gene expression through epigenetics can also be studied down to a single-cell level. Through epigenetics, although the DNA sequence is unaltered, expression patterns can be affected by DNA methylation or chromatin structure. DNA chromatic accessibility is maintained by regulatory elements such as transcription factors, DNA methylation, and histone modification where DNA is wound around into nucleosomes. Single-cell assay for transposase-accessible chromatin-seq (scATAC-seq) takes advantage of the Tn5 transposase which is a bacterial enzyme primed to find and cut open DNA positions[13]. Through scATAC-seq, cells are lysed and nuclei are harvested and then undergo transposition where open DNA fragments are “cut and tagged” with adaptors[13]. Single nuclei run through a similar process as the scRNA-seq mechanism to create barcoded cDNA and sequenced and mapped to reference genome and accessible chromatic regions. These peak calling reads can then be linked to areas such as promoters and enhancers. The distribution of reads across the whole genome, functional analysis the genes associated with the peaks, and peak distribution on functional gene elements can also be done with further analysis[13] (Figure 1).

### Single-cell chromatin immunoprecipitation-seq

Single-cell epigenetics in the measurement of transcription factor binding and histone modification can also be studied through single-cell chromatin immunoprecipitation-seq. Generally, cells are encapsulated and lysed within the droplets and chromatin is fragmented. DNA barcodes and chromatin fragments are merged *via* the microfluidics device[14]. DNA

barcodes are ligated to chromatin fragments and droplets are then immunoprecipitated with antibody with a carrier chromatin and library construction[14] (Figure 1).

### Single-cell cellular indexing of transcriptomes and epitopes

Cell cellular indexing of transcriptomes and epitopes-seq (CITE)-seq combines traditional protein marker detection (such as flow cytometry) with scRNA-seq to provide phenotypic information such as cell-surface protein expression with transcript information[15]. Antibodies are conjugated to oligonucleotides with antibody specific barcodes using streptavidin-biotin interaction and cells are processed per scRNA-seq technique[15]. The cells are then lysed and the Oligo-dT primers capture the oligonucleotides and mRNA to create cDNA[15]. cDNA is then processed into libraries and sequenced (Figure 1).

## SINGLE-CELL STUDIES IN IBD

### UC

Kinchen *et al*[16] published a study describing the activation of intestinal mesenchymal cells subpopulations in adult human UC and dextran-sodium sulfate colitis murine models. Through scRNA-seq, the authors identified SOX6, CD142, and WNT expressing colonic crypt mesenchymal cells consisting of fibroblasts subsets that when dysregulated can lead to impaired epithelial function and inflammation driving the UC state[16]. The human colonic epithelial layer in the UC state is again studied by Parikh *et al*[17] with further identification of various progenitor cells, colonocytes, and goblets cell also implicated in states of inflammation. The authors go on to describe a new subset of absorptive BEST4+ colonocytes cells expressing proton channel OTO2 and uroguanylin implicated in sensing intraluminal pH and dysregulated in UC. Goblet cell expression of antiprotease molecule WFDC2 was found to be vital in bacterial defense and tight junction barrier function[17]. The study utilized inflamed human UC tissue, adjacent human UC noninflamed tissue, and healthy colonic tissue, and interestingly, the transcripts upregulated in inflamed tissue was also found to be upregulated to a lesser degree in noninflamed UC tissue implying that scRNA-seq is able to detect disease activity prior to tissue level damage[17]. Similarly, Smillie *et al*[18] described the changing landscape of inflammation in human adult UC with inflamed and noninflamed tissues. In addition, the authors describe the potential cellular players in anti-tumor necrosis factor (TNF) resistance in oncostatin M expressing monocytes and fibroblasts, and expanded on an intracellular circuitry of diseased state involving inflammatory fibroblasts, inflammatory monocytes, microfold-like cells, and CD8/interleukin (IL)17 T cells[18]. Uzzan *et al*[19] provided the first expanded adaptive immunity studies of the application of scRNA-seq in mucosal and circulating B cell and plasma cell subsets in human UC. The authors found that B cell response was dysregulated in UC through peripheral gut-homing plasmablasts that correlated with disease activity and expansion of naïve B cells and immunoglobulin (Ig)G+ plasma cells along with auto-reactive plasma cell from inflamed UC tissue[19].

Boland *et al*[20] integrated scRNA-seq with scTCR-seq and scBCR-seq to describe cellular states and clonal relationships of mucosal and peripheral adaptive immune cells in human adult UC. The authors describe an increase in IgG 1+ plasma cells and increased colonic ZEB2 transcription factor regulatory T cells in colonic tissue and gamma delta T cell subset enrichment in the peripheral blood. Of note, the authors also saw a skew in heterogeneity of CD8+ tissue-resident memory T cells in inflamed UC tissue indicating potential pathogenic role of these tissue-resident memory T cells[20]. In the same year 2020, Corridoni *et al*[21] utilized scRNA-seq, scTCR-seq, and CITE-seq to examine the repertoire and cellular subsets of tissue-resident memory CD8+ T cells in human adult UC. The study describes the heterogeneity of CD8+ T cells as both destructive expanded effector type that leads to a disease state with TNF-alpha production and post-effector type that seem to act in a more regulatory fashion through IL-26 production[21]. These studies exemplify the power and unbiased nature of single-cell technologies to understand pathogenic signatures and signify the complexity of human disease states.

### CD

Martin *et al*[22] published one of the first CD scRNA-seq data sets linking anti-TNF therapy resistance with a cellular module they entitled GIMATS which stands for IgG plasma cells, inflammatory mononuclear phagocytes, activated T cells, and stromal cells. Through utilizing resected inflamed terminal ileum from adult human CD patients who did not respond to anti-TNF therapy along with uninflamed samples, the authors identified this unique cellular signature to potentially eventually develop biomarkers in prediction of therapy response[22]. Jaeger *et al*[23] further looked at the T cell composition of the terminal ileum from adult human patients with CD and resected terminal ileums, distinguishing between lamina propria and epithelial layers. Within the epithelial layer, intraepithelial lymphocytes from inflamed tissue included specific NKp30+ gamma delta T cells that expressed ROR gamma which produced IL-26 with an increased in active T helper 17 (Th17) cells[23]. The lamina propria layer also found a Th17 signature with increased CD8+ cells implicating the Th17 pathway in CD[23]. Yokoi *et al*[24] further classified T cell subsets in CD to identify CD4+ tissue-resident memory T cells that were increased in CD that expressed CD161, CCR5, and CD103 using scRNA-seq and CyTOF. Rosati *et al*[25] used both bulk TCR repertoire and scRNA-seq on peripheral blood in CD samples to identify a subpopulation of unconventional Crohn-associated invariant T (CAIT) cells with a distinctly unique TCR. These peripheral CAIT cells seem to show a gene expression similar to cells of the innate immune system and mucosal associated invariant T cells and NKT cells and seem to be present in peripheral blood in CD rather than UC[25]. Maddipatla *et al*[26] describes a pediatric CD scRNA-seq data set from treatment naïve pediatric patients, established CD patients in remission, and refractory Crohn's. Patients in remission showed elevated apolipoprotein and goblet cell trefoil factor though not in refractory Crohn's[26]. Cellular subsets of enterocyte, goblet cells and BEST4+ enterocytes, microfold and

tuft cells see, to undergo changes from treatment naïve to established CD[26].

### Both UC and CD

Huang *et al*[27] describes a pediatric cohort of patients with UC, Crohn's colitis, and undefined pediatric IBD using scRNA-seq, scTCR-seq, and scBCR-seq. The authors describe a common pathway of impaired cyclic AMP-response signaling in all three pediatric cohorts along with infiltration of PDE4B-expressing and TNF-expressing macrophages within the mucosal samples. The authors also describe a decreased abundance of CD39 expressing intraepithelial T cells along with platelet aggregation and release of 5-hydroxytryptamine at the mucosal level[27]. Furthermore, they demonstrated the ability to improve clinical symptoms of some of the pediatric patients with colitis and IBD by using a drug (phosphodiesterase inhibitor dipyridamole) that targeted the pathways identified in a pilot study[27].

Mitsialis *et al*[28] also reports a study with scRNA-seq to confirm their mass cytometry (CyTOF) findings in patients with UC and CD. Within both the CD and UC cohorts, the authors found an expansion of HLA-DR+CD38+ T cells, CXCR+ plasmablasts, and IL1B+ macrophages and monocytes[28]. Expansion of IL17A+CD161+ effector memory T cells and IL17A+ T regulatory cells along with HLA-DR+CD56+ granulocytes was found within the UC cohort. Within CD, IL1B+HLA-DR+CD38+ T cells, ILB+TNF+IFNG+ naïve B cells, and IL1B+ dendritic cells, and IL1B+ plasmacytoid dendritic cells were expanded in the mucosal samples. Expanded IL1B+ T regulatory cells, IL-B+ dendritic cells, IL1B+ plasmacytoid dendritic cells, and IL1B+ monocytes were found in the peripheral blood of patients with CD but not UC [28].

## FUTURE DIRECTIONS FOR SINGLE-CELL RESEARCH IN IBD

The future of single-cell technologies within gastroenterology research is already here in the ability to perform single-cell multi-omics and spatial transcriptomic on the target organ (GI tract) and peripheral blood. The ability to multiplex single-cell technologies is in development and the ability to use them on clinical samples will lead to even further growth in the field of IBD. The ability to combine transcriptomics (scRNA-seq), epitopes, (protein expression), and chromatin accessibility (scATAC-seq) from single cells was described by Swanson *et al*[29] on peripheral blood using transcriptomics, epitopes, accessibility-seq. These multimodal single-cell assays may provide a novel way to uncover and link gene expression, gene regulation, and phenotypic gene expression within a single cell and begin to fully cover genotype with phenotype in specific diseases such as IBD.

Another exciting technique that is more readily available now is spatial transcriptomics where architectural information is preserved with intact tissue[30,31]. The position of cellular location and interaction continues to be present and cell to cell interactions can be further interrogated[30,31]. Cell and tissue function can be probed to understand potential pathogenic cells and culprits of inflammation rather than bystander immune cells may be a detailed way to understand IBD. At the same time, as the GI tract is an immune active organ, spatial transcriptomics may be a way to detect the invasive pathogenic variants without losing more delicate cells in the tissue dissociation process.

## SINGLE-CELL OMICS IN OTHER GI DISEASES

Given the limited scope of this minireview, we have described these technologies only in the field of IBD and did not elaborate into its use in other GI disorders such as GI cancers and other GI diseases such as allergies of the GI tract. Spatial transcriptomics and various single-cell techniques have been successfully applied to colorectal cancers using the GI cancer tissue and resections allowing researchers to better understand the tumor microenvironment to derive better chemotherapeutic targets[32-36]. Allergic disease of the GI tract such as eosinophilic esophagitis is also under interrogation using single-cell technologies[37]. The ability to study tissue level molecular changes across multiple disease is unparalleled using single-cell technologies.

## LIMITATIONS

The limitation to single-cell technologies is most importantly cost of reagents and cost of sequencing[38]. The ability to integrate multiple single-cell data across multiple platforms and techniques is also a challenge as there are many commercial products out in the market[38].

## CONCLUSION

Single-cell techniques and omics have taken off in the last few years and the ability to detect individual cellular transcript details has revolutionized the world of research. In the field of gastroenterology in just the last five years, several single-cell techniques have been applied to IBD research with the identification of novel cellular immune players in the pathogenesis of both UC and CD. As we continue to develop further immunological techniques, we may begin to detect signals of treatment response in IBD and tailor therapies to immune signatures present in disease state.



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**Country/Territory of origin:** United States

**ORCID number:** Hengqi Betty Zheng 0000-0003-0575-6475.

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