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Review article

Decoding the mosaic of inflammatory bowel disease: Illuminating insights with single-cell RNA technology

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ABSTRACT

Inflammatory bowel diseases (IBD), comprising ulcerative colitis (UC) and Crohn's disease (CD), are complex chronic inflammatory intestinal conditions with a multifaceted pathology, influenced by immune dysregulation and genetic susceptibility. The challenges in understanding IBD mechanisms and implementing precision medicine include deciphering the contributions of individual immune and non-immune cell populations, pinpointing specific dysregulated genes and pathways, developing predictive models for treatment response, and advancing molecular technologies. Single-cell RNA sequencing (scRNA-seq) has emerged as a powerful tool to address these challenges, offering comprehensive transcriptome profiles of various cell types at the individual cell level in IBD patients, overcoming limitations of bulk RNA sequencing. Additionally, single-cell proteomics analysis, T-cell receptor repertoire analysis, and epigenetic profiling provide a comprehensive view of IBD pathogenesis and personalized therapy. This review summarizes significant advancements in single-cell sequencing technologies for enhancing our understanding of IBD, covering pathogenesis, diagnosis, treatment, and prognosis. Furthermore, we discuss the challenges that persist in the context of IBD research, including the need for longitudinal studies, integration of multiple single-cell and spatial transcriptomics technologies, and the potential of microbial single-cell RNA-seq to shed light on the role of the gut microbiome in IBD.

1. Introduction

Inflammatory bowel diseases (IBD), including ulcerative colitis (UC) and Crohn's disease (CD) [1], are chronic inflammatory conditions of the intestinal tract, driven by dysregulated immune responses and environmental triggers in genetically susceptible individuals [2]. The distinct differences between UC and CD contributes to the complexity of IBD, posing significant challenges. These include the roles of various immune and non-immune cell populations in disease development, pinpointing dysregulated genes and pathways, and deciphering treatment mechanisms [3,4]. Although effective in some patients, current therapies such as anti-TNF treatment fail to provide sustained remission for the majority of IBD patients [5]. Moreover, a serious complication in both UC and CD [6,7] known as fibrosis-induced structuring disease lacks targeted treatment options [8]. Addressing these clinical challenges can be possible by harnessing cutting-edge, high-throughput technologies capable of efficiently detecting multiple cell subtypes.

Traditional approaches for studying IBD, such as microscopic examination of tissues and tissue homogenization, provide valuable insights into disease pathology but have notable limitations. While microscopic examination offers qualitative information on inflammation severity, it lacks the specificity needed to understand the underlying cellular mechanisms of IBD. Similarly, tissue homogenization captures global changes in cytokine milieu and gene expression but fails to attribute findings to specific cell types [9], hindering the identification of key contributors to disease. Moreover, isolating cells from IBD tissues is challenging due to low yields and a lack of viable markers for specific cell subpopulations. These methods also struggle to explore dynamic cell interactions within the gut tissue, limiting our understanding of disease progression. To address these shortcomings, advanced techniques like

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single-cell RNA sequencing (scRNA-seq) offer unprecedented resolution and insights into IBD pathogenesis [10], providing a more comprehensive and deeper understanding of the disease [11].

With the advancement of scRNA-seq, there are various analytical tools and software developed for the in-depth analysis of cellular heterogeneity and gene expression at an unprecedented resolution. These tools facilitate a range of analytical processes such as data preprocessing, normalization, dimensionality reduction, clustering, and visualization, allowing researchers to uncover intricate details of cellular diversity and function. Commonly used tools such as Seurat [12], Scanpy [13], and Giotto [14] provide robust platforms for quality control, comprehensive analysis, and intuitive visualization of single-cell RNA sequencing data. To identify the most reliable and efficient methods in the rapidly evolving field of single-cell transcriptomics, benchmarking single-cell RNA-seq analysis tools is essential. There are existing benchmarking studies [15–17] that involve systematically comparing different tools and algorithms on various metrics such as accuracy, scalability, and computational efficiency. Key aspects evaluated include data preprocessing, normalization, cell clustering, and annotation. These evaluations help researchers select the best tools for their specific needs, ensuring robust and reproducible results in single-cell RNA-seq studies.

In this review, we will summarize recent advancements in the field of IBD in terms of pathogenesis, diagnosis, treatment, and prognosis through single-cell technologies. Furthermore, we will discuss the challenges that remain unaddressed by current single-cell sequencing technologies in the context of IBD.

2. Single-cell technology in IBD research

Single-cell RNA sequencing is a cutting-edge technology that enables the exploration of individual cells' genetic activity within tissues, offering unprecedented insights into disease mechanisms like Inflammatory Bowel Disease (IBD). The scRNA-seq workflow typically encompasses several key steps. Firstly, cells are isolated from tissue samples using methods like enzymatic digestion or microfluidic devices. Following isolation, single-cell capture is achieved using platforms such as the Chromium System by 10x Genomics [18], which encapsulates individual cells into nanoliter-sized droplets [18]. Within these droplets, cells undergo lysis, and their RNA is reverse-transcribed into complementary DNA (cDNA). Importantly, each cDNA molecule is tagged with a unique molecular barcode, facilitating the subsequent identification of transcripts originating from the same cell. Following reverse transcription and cDNA barcoding, PCR amplification is performed to amplify cDNA molecules within each droplet. Subsequently, libraries are constructed from the amplified cDNA, and sequencing adapters are added. Once libraries are prepared, high-throughput sequencing is conducted to obtain the transcriptomic profiles of individual cells. Finally, bioinformatics analysis is employed to process the vast amounts of sequencing data, including quality control [19], data normalization, dimensionality reduction [20], clustering [21], and cell type identification. This comprehensive approach enables the elucidation of cellular heterogeneity and gene expression patterns within IBD tissues [11,22–25] at single-cell resolution, facilitating the discovery of novel disease mechanisms and therapeutic targets (Fig. 1).

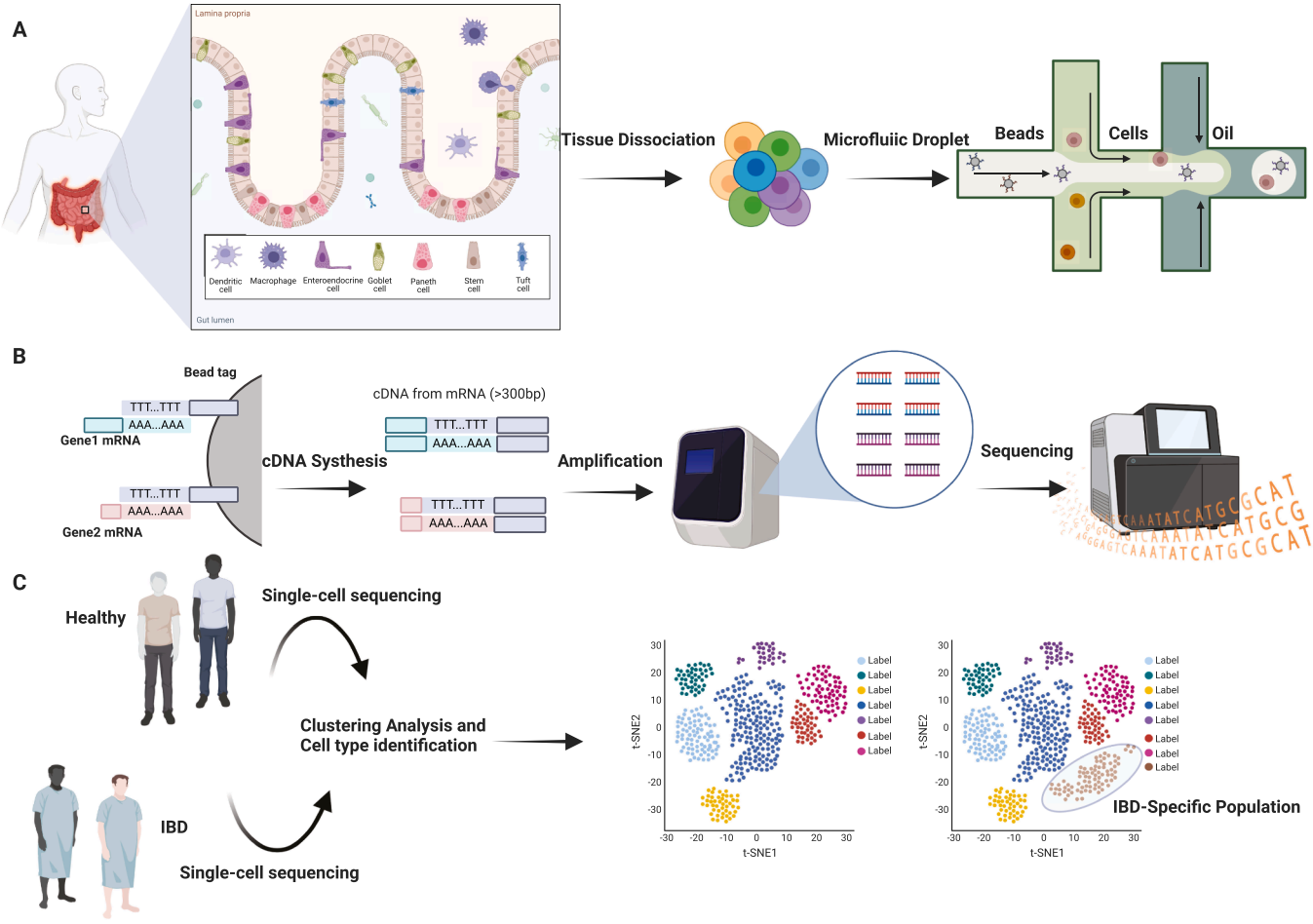

Fig. 1. Diagram showing the workflow of Single-Cell RNA sequencing. This diagram provides an overview of the workflow of scRNA-seq, including cell capture (A), reverse transcription and cDNA barcoding, PCR amplification and library construction (B), downstream data analysis (C), and phonograph-based visualization on the tSNE plot of T cells from IBD patients or healthy control (D), the graph D is quoted from Reference 31.

scRNA-seq enables the comprehensive transcriptomic profiling of various cell types at the individual cell level of IBD patient tissues, overcoming the limitations of conventional methods such as bulk RNA sequencing, which is unable to discern cellular heterogeneity. This traditional approach fails to detect subtle signals in rare subpopulations that may play pivotal roles in the context of the disease, although it can provide overall gene expression profiles in a tissue or cell population at a lower cost [10] (Table 1). Moreover, flow cytometry is a suitable alternative to determine lymphocyte subset populations and the detection of intracellular antigens [26], but this analysis relies solely on known protein markers and can result in the loss of important unspecified cell types or states. Furthermore, the simultaneous detection of multiple protein indicators is limited (Table 1). On the other hand, scRNA-seq has its limitations as well, including the loss of essential spatial localization information within native tissue from the isolation of individual cells during sample processing [27] (Table 2). Sample processing may also induce aberrant gene expression, which could subsequently lead to misinterpretations of specific cell subpopulations [28].

Mass cytometry/Cytometry by time of flight (CyTOF) has developed alongside single-cell RNA sequencing and emerged as an important tool for single-cell analysis of the proteome [29]. Mass cytometry is an innovative method that combines mass spectrometry and flow cytometry. It employs stable isotopes instead of traditional fluorophores as reporting agents [30,31], allowing for the simultaneous measurement of over 40 cellular markers and representing a significant improvement over conventional multi-parameter flow cytometry [32]. Despite being much more expensive than conventional flow cytometry, various algorithms have been developed for computational analysis, enabling the characterization of cellular proteomic heterogeneity at the single-cell level [33,34] (Table 2). Mass cytometry has also been used in extensive validation of most transcriptomic features from scRNA-seq in IBD, confirming that mass cytometry can capture key parameters of IBD pathogenic colonic stromal behavior associated with clinical disease activity [35–41].

Single-cell Cellular Indexing of Transcriptomes and Epitopes Sequencing (CITE-seq) is an innovative technology that has revolutionized the field of single-cell analysis by combining multiplexed protein marker detection with simultaneous unbiased single-cell RNA sequencing (scRNA-seq) [42]. This cutting-edge approach leverages the streptavidin-biotin interaction to conjugate antibodies to oligonucleotides, each carrying a unique antibody-specific barcode. These barcode-conjugated antibodies are then processed alongside scRNA-seq technology to capture cellular heterogeneity at an unprecedented resolution. During the workflow, oligo-dT primers are employed to capture both mRNA and antibody-derived oligonucleotides, facilitating the generation of complementary DNA (cDNA). The reverse transcription process indexes both mRNA and antibody-derived oligonucleotides using shared barcodes, enabling seamless integration of transcriptomic and proteomic information within individual cells. In practice, single-cell suspensions are first stained with antibodies following standard flow cytometry protocols. Subsequently, these antibody-stained cells are processed for scRNA-seq analysis [42]. Notably, unlike traditional flow cytometry and mass cytometry techniques, the detection of oligo-barcoded antibodies in CITE-seq is not constrained by signal overlap. With a 10-nucleotide sequence, a vast number of unique barcodes can be encoded, surpassing the diversity of human proteins. This capability facilitates large-scale immune phenotyping analysis, allowing the simultaneous interrogation of tens to hundreds of antibodies in a single experiment (Table 2). Moreover, CITE-seq offers the unique advantage of concurrently capturing mRNA and protein information within individual cells. Through the use of barcodes, the mRNA and protein detected by CITE-seq are precisely matched on a one-to-one basis. This alignment ensures that the expression profiles of both mRNA and protein can be accurately deciphered in each cell, providing comprehensive insights into cellular function and heterogeneity.

Single-cell assay for transposase-accessible chromatin-seq (scATAC-seq) is a comprehensive method for measuring open chromatin that utilizes prokaryotic Tn5 transposase to mark regulatory regions by inserting sequencing adapters into accessible regions of the genome [43] [44]. In scATAC-seq, individual cells are captured and analyzed using a programmable microfluidic platform (Fluidigm). After transposition and PCR on an integrated fluidic circuit (IFC), libraries are collected and PCR amplified with cell-identifying barcode primers. Single-cell libraries are then pooled together and sequenced on a high-throughput sequencing instrument [45]. Recently, Satpathy et al. introduced a droplet-based method for highly multiplexed single-cell chromatin accessibility profiling. The scATAC-seq libraries generated with this method exhibit a high quality, lower amplification bias compared to previous methods and do not require cell sorting or non-commercial reagents [46] (Table 2).

Single-cell TCR repertoire analysis. The recent advancements in single-cell isolation techniques have made it possible to capture paired TCRαβ chain information, initially introduced for bulk populations [47]. The earliest single-cell paired TCRαβ analysis methods relied on microdissection and microfluidic manipulation techniques to isolate individual T cells, followed by multiplex PCR and Sanger sequencing [48]. While these methods played a crucial role in the initial characterization of paired TCRαβ sequences, they were time-consuming and limited in efficiency and throughput. Currently, a common strategy for performing paired TCRαβ sequencing for single cells is through cell-based emulsion PCR methods [49,50]. In these methods, individual cells are captured in an emulsion containing TCR primers and RT-PCR

Table 1
Comparison between single-cell technology and traditional technology.

Technology	Main Applications	Advantage	Deficiency
scRNA-seq	Discerning cellular heterogeneity and dynamic changes.	Enabling revealing the characteristics of cell heterogeneity and rare cell populations, enabling studying the dynamic changes of cells in different stages of development or disease states.	High cost, complex data processing, and high technical requirements.
Bulk RNA-seq	Analysis of gene expression in the whole tissue or cell population.	Low cost, large amount of data, simple sample handling and data analysis.	It is not possible to distinguish differences in gene expression between different cell types in the sample, and only average expression levels can be obtained. Rare cell populations are difficult to detect.
Microscopic examination of tissues	Pathological diagnosis.	Intuitive tissue structure observation, cell and tissue localization, staining diversity, pathological diagnosis: providing diagnostic basis and assessment of disease severity.	Low throughput, difficult quantitative analysis, lack of specificity needed to understand underlying cellular mechanisms.
Tissue homogenization	Providing global gene expression and protein information, suitable for large-scale studies	High throughput, suitable for a variety of analytical techniques, and fast sample processing.	Lack of spatial and cell heterogeneity information.
Flow cytometry	Multiple protein markers were analyzed simultaneously to identify and sort different types of cells.	Multiparameter and quantitative analysis, high throughput: processing and analyzing large quantities of samples quickly.	The analysis relies on known protein markers and may miss unknown cell types or states. Loss of Spatial information.

Table 2
Summary of single-cell techniques.

Single-cell Technology	Main Applications	Advantage	Deficiency
scRNA-seq	Discerning cellular heterogeneity at the transcriptional level.	Enabling the comprehensive transcriptomic profiling of various cell types at the single cell level.	Lack of spatial transcriptomics information.
CyTOF	Single-cell analysis of the proteome.	Enabling simultaneously testing over 40 cellular markers and significantly improvement over conventional flow cytometry.	More expensive than conventional flow cytometry and high requirements are placed on data analysts.
CITE-seq	Simultaneous measurement of the mRNA and protein in a single-cell level.	Concurrently capturing mRNA and protein information within individual cells, and the information of mRNA and protein are precisely matched on a one-to-one basis. More proteins can be detected than CyTOF.	The cost of generating and analyzing CITE-seq data remains high Compared to scRNA-seq.
scATAC-seq	Comprehensive measurement for open chromatin in a single-cell level.	High quality, lower amplification bias compared to previous methods, and do not require cell sorting or non-commercial reagents.	Compared to other single-cell techniques, scATAC-seq has relatively higher cost, the data processing and analysis are more complex, requiring specialized bioinformatics skills and substantial computational resources.
scTCR-seq	Revealing the dynamic changes in the evolution and development process of disease-associated T cell clones contributes to understanding the pathogenesis and progression of the disease.	High efficiency and throughput than the earliest single-cell paired TCR sequence.	The coverage of full-length transcripts is lost due to fragmentation of cDNA fragments during library preparation, resulting in lower sensitivity compared to full-length strategies (detecting fewer genes).
Spatial RNA-seq	Determine the distribution of RNA in tissues.	High depth of coverage, detecting transcripts across multiple cells within a tissue sample.	Lacks of single-cell resolution as RNA signals.

reagents. After encapsulation and cell lysis, T cells undergo overlapping extension RT-PCR (OE-PCR), where primers target the C region and a set of V region primers, including a complementary sequence, allowing for the connection of TCRαβ transcripts within the emulsion [51]. These fused products contain two chains and can then be sequenced while preserving the natural pairing of TCR-α-β (Table 2).

Spatial RNA analysis encompasses two main techniques: spatial transcriptomics and spatial imaging. Spatial transcriptomics involves profiling RNA expression within tissue sections to obtain spatially resolved gene expression information. This technique captures RNA molecules directly from cells in an interested region within the tissue sections and sequences them, revealing gene expression patterns within defined spatial regions. Spatial transcriptomics offers high depth of coverage, detecting transcripts across multiple cells within a tissue sample. However, it typically lacks single-cell resolution as RNA signals are aggregated within these regions [52], potentially overlooking cellular heterogeneity and interactions.

Spatial imaging techniques, such as spatially resolved transcript amplicon sequencing (STARS), multiplexed fluorescence in situ hybridization (FISH), or Spatial Molecular Imager (SMI) [53] offer single-cell resolution by visualizing RNA molecules within individual cells. These methods often employ barcoding strategies to label individual cells and their RNA transcripts. Barcoding cells enables the assignment of RNA expression profiles to specific cells within tissue sections, providing detailed insights into the spatial distribution of gene expression at the single-cell level. However, the depth of coverage in spatial imaging techniques depends on the availability of probes, and they may struggle to detect low-abundance transcripts.

In spatial imaging, cells are often barcoded using various approaches. For instance, in STARS, cells are captured on a microarray chip, and each cell is assigned a unique spatial barcode. Similarly, in multiplexed FISH, cells are labeled with fluorophore-conjugated probes, each specific to a target RNA sequence. These barcoding methods enable researchers to map the spatial organization of gene expression in tissues, facilitating the study of complex biological systems and interactions within the tissues. Despite limitations, such as depth constraints, spatial imaging techniques play a crucial role in advancing the understanding of spatial gene expression dynamics (Table 2).

3. Distinctions between Ulcerative Colitis and Crohn's Disease at a single cell resolution

There is a collective effort to unravel the intricacies between the two subtypes of inflammatory bowel disease (IBD), Ulcerative Colitis (UC) and Crohn's Disease (CD). UC predominantly affects the colon and rectum, with inflammation typically starting in the rectum and extending continuously along the colon. Conversely, CD can impact any part of the digestive tract from the mouth to the anus, commonly involving the end of the small intestine (ileum) and the beginning of the colon. CD may also affect deeper layers of the bowel wall and manifest as patches of healthy tissue between inflamed areas. Despite sharing the IBD classification, UC and CD exhibit unique characteristics necessitating different management approaches. UC treatment often targets the colon specifically with medications like aminosalicylates (5-ASA) and rectal therapies such as suppositories or enemas. Surgery, notably colectomy, can be curative for UC. Conversely, CD treatment may involve medications targeting the entire gastrointestinal tract, including the small intestine, with biologic therapies like TNF-alpha inhibitors frequently used due to their ability to address systemic inflammation. Surgery for CD aims to alleviate complications like strictures, fistulas, or abscesses but does not offer a cure. While some medications overlap in UC and CD treatment, such as corticosteroids and immunomodulators, CD management often emphasizes biologic therapies due to the disease's systemic nature. Despite identifiable macroscopic differences, the clinical diagnosis between UC and CD remains challenging.

3.1. Distinct cytokine profiles between UC and CD

Two recent studies employed distinct but complementary techniques to dissect the cellular landscape of UC and CD (Fig. 2). Mitsialis and colleagues utilized CyTOF in conjunction with scRNA-seq to scrutinize immune cell populations in both intestinal mucosa and blood samples from patients with UC and CD [40]. They observed a discernible cytokine milieu, wherein IL-17A-producing cells were prominent in UC, while IL-1β-secreting cells were predominant in CD. Notably, in UC, the expanded regulatory T cells (Treg) emerged as a significant source of

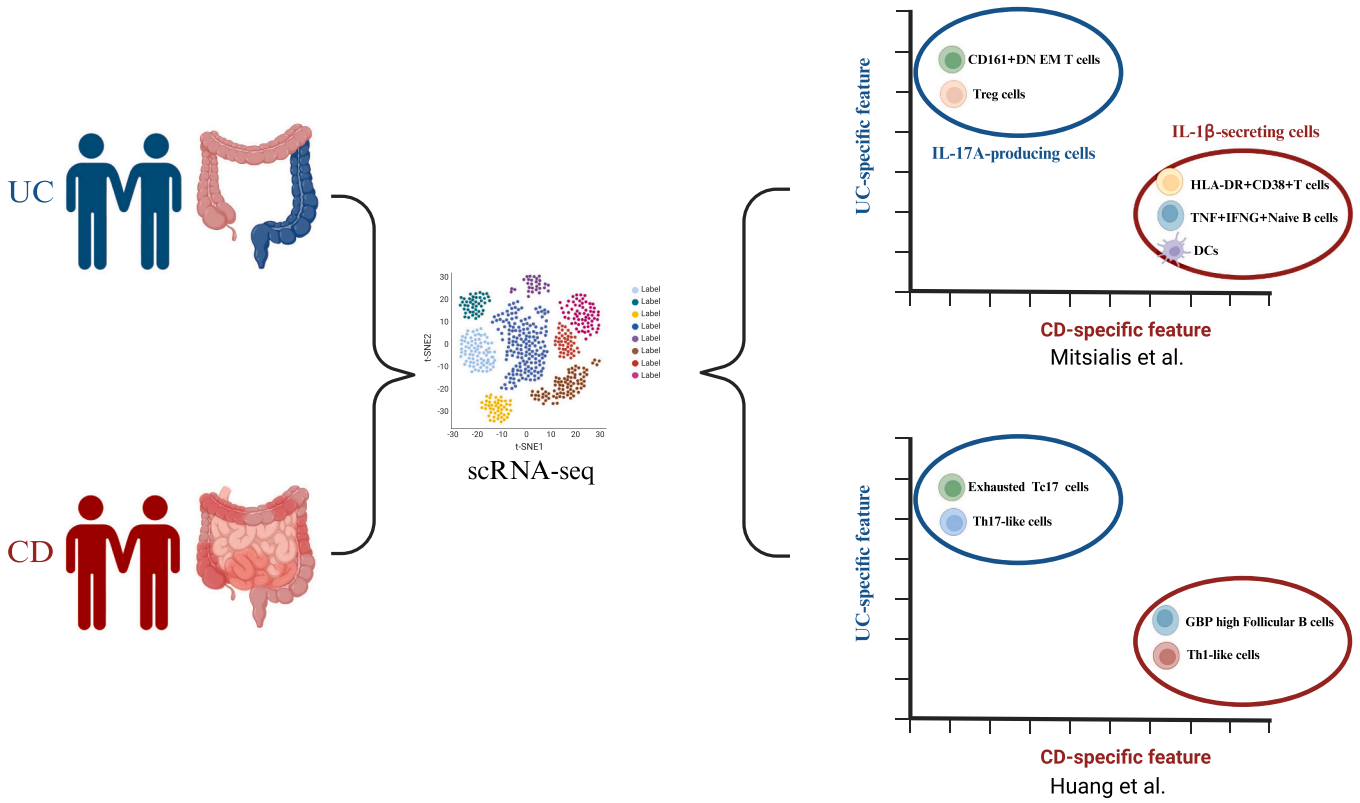

Fig. 2. : Distinctions between Ulcerative Colitis and Crohn's Disease at single cell resolution. Analysis using single-cell techniques reveals a distinct cytokine milieu, with IL-17A-producing cells being prominent in UC, while IL-1β-secreting cells predominate in CD. Abbreviations: Ulcerative Colitis, UC; Crohn's Disease, CD; Single-cell RNA sequencing, scRNA-seq; Regulatory T cells, Treg; CD4-CD8- effector memory T cells, DN EM T cells. Mitsialis et al. involved to Reference 34; Huang et al. involved to Reference 52.

IL-17A, hinting at a functional transformation of Treg cells during inflammation. Furthermore, a novel cell type capable of producing IL-17A was identified. These cells displayed a hybrid phenotype, sharing characteristics of both effector memory T cells and innate cells [54]. Given IL-17A's role as a chemoattractant for neutrophils, there was an elevation in granulocytes within the UC mucosa, accompanied by a concurrent decrease in peripheral levels, indicating a potential tissue-homing phenomenon. This observation aligns with prior findings suggesting a correlation between the infiltration of intestinal neutrophils and the severity of UC [55].

In contrast to UC, CD is distinguished by IL-1β signatures [40]. Various cell types have been identified as sources of this cytokine, including T cells, B cells, dendritic cells, and macrophages/monocytes, as previously reported by Mahida et al. [56]. While B cells have traditionally been viewed primarily as antibody-producing cells, their capacity for cytokine production should not be overlooked. Research indicates that B cells can amplify cytokine production from T cells in an antigen and MHC-independent manner [57]. This finding may explain the naive phenotype observed in cytokine-producing B cells isolated from individuals with CD [40].

While samples may have been collected from patients at varying disease stages and with different treatment histories, it is highly unlikely that these factors influenced the cytokine signature significantly. This assertion finds support in another study conducted among treatment-naïve individuals with IBD [58]. Specifically, in UC, clusters of Th17-like T cells were identified, whereas Crohn's disease patients, clusters of Th1-like T cells were revealed in the colon. The presence of Th1-like T cells mirrors the IL-1β signatures associated with CD. Our findings, corroborated by other studies, illustrate that the presence of IL-1β and IL-23 can trigger the transition of Th17 cells into Th1-like cells, marked by the production of Th1 signature cytokines including IFN-γ in addition to IL-17A.

Despite the efficacy of IL-17-blocking drugs like secukinumab and brodalumab in autoimmune diseases such as psoriasis [59–61], they have not yielded the expected results in moderate to severe CD, with some patients experiencing severe complications [62,63]. This inconsistency may be due to the controversial [64–68] or organ-specific roles of IL-17A [69,70], potentially explaining the variable efficacy of anti-IL-17A therapy. However, recent scRNA-seq investigations as mentioned here offer a new explanation by illuminating the distinct cytokine profiles associated with UC and CD, highlighting IL-17A predominance in UC and IL-1β and IFN-γ dominance in CD. Consequently, blocking IL-17A may offer therapeutic benefits in UC, although clinical trials are necessary to validate this hypothesis. Additionally, targeting IL-1β may show promise as a therapeutic approach for CD. These findings can also explain the therapeutic effects of Ustekinumab, a monoclonal antibody targeting both Th1 and Th17 cells, in both CD and UC [71,72].

3.2. Tissue-specific subtypes in Crohn's Disease suggested by scRNA analysis

Recent studies suggest the potential for further categorizing CD into distinct subtypes based on the involved tissues [73]. While histological analysis struggles to differentiate cell compositions between CD sites or between active and inactive disease states, single-cell RNA sequencing (scRNA-seq) has revealed that cellular responses to the inflammation in CD manifest in a cell-type-specific and tissue-specific manner. Firstly, tissues throughout the digestive tract harbor different cell types, and inflammation in CD has varying impacts on cell composition, particularly on epithelial and stromal cell subsets. Secondly, although tissue-specific differences in overall cell types and immune cell

transcriptional profiles are subtle [25], transcriptional changes are more pronounced across epithelial and stromal subsets [23]. Thirdly, inflammation-induced transcriptional alterations are largely site-specific and more pronounced in the colon compared to the terminal ileum. Significantly, within the colon, numerous metabolic pathways experience substantial downregulation, largely attributed to the suppression of the ketogenic pathway. Intriguingly, the ketogenic diet has shown diverse efficacy levels in treating CD, likely influenced by the tissue-specific distinctions inherent in CD [74,75]. This underscores the importance of delineating CD subtypes and customizing therapeutic approaches accordingly.

3.3. Unique features of pediatric IBD revealed by scRNA analysis

In the realm of pediatric IBD, it becomes evident that young patients frequently exhibit atypical characteristics that challenge conventional classifications of CD or UC [76]. A central enigma in this context lies in the potential evolution of pediatric-onset colitis into full-fledged IBD, along with the exploration of common pathogenic mechanisms shared among these distinct conditions. One recent study scrutinized the immune phenotypes within the colonic mucosa of a pediatric cohort affected by colitis, UC, and CD [77], through using scRNA-seq together with B-cell and T-cell receptor profiling. This study unveiled both shared and disease-specific pathogenic features among the three pediatric subcohorts. Notably, the study identified a common characteristic marked by impaired cyclic AMP (cAMP)-response signaling, which extended across all three pediatric IBD variants. Specifically, this suggests that platelet aggregation and high inflammation are important common pathological pathways supported by defects in the cAMP response pathway. The cAMP response pathway is extensively related to the acquisition of the regulatory function of T cells [78,79]. However, Zimmerman et al. demonstrated that administration of cAMP-elevating agents decreased infiltration of damage-causing leukocytes but inhibited epithelial repair and barrier maintenance [80]. While impaired cyclic AMP-response signaling reveals shared pathogenic mechanisms in pediatric IBD, controversy surrounds the use of cAMP-elevating agents, emphasizing the need for further research into their therapeutic implications.

4. Recently identified cell subsets contributing to IBD pathogenesis

4.1. Subsets of Intestinal Epithelial Cells (IEC)

Intestinal epithelial cells (IECs), lining the inner surface of the intestine, play pivotal roles in maintaining intestinal homeostasis, regulating mucosal immunity, and mediating host-microbiome interactions [81]. The functional diversity of IECs is underscored by their heterogeneity, encompassing absorptive enterocytes, secretory goblet cells, antimicrobial Paneth cells, and enteroendocrine cells. Intestinal stem cells (ISCs), situated at the base of intestinal crypts, continually renew the intestinal epithelium. Lgr5, a cell surface receptor protein, serves as a marker for Intestinal stem cells. However, recent advancements in scRNA-seq have revealed expanded epithelial cell heterogeneity (Table 3), with Lgr5 expression identified in several novel cell types, like Paneth cells [82]. The presence of the stem cell marker Lgr5 in Paneth cells raises intriguing possibilities regarding their role and origin during inflammation. One possibility is that these Lgr5-positive Paneth cells represent newly formed cells, where Lgr5 expression persists. This suggests rapid Paneth cell generation from stem cells as part of a regenerative response during inflammation. Alternatively, Paneth cells may re-express Lgr5 to acquire stem cell-like properties, possibly enhancing their self-renewal capacity or facilitating tissue repair processes, consistent with the previous finding that Paneth cell precursors can recall the stem-cell state upon intestinal injury [83]. Other potential explanations involve dynamic changes or plasticity within the intestinal epithelium, wherein Paneth cells transiently adopt stem cell-like properties under inflammatory conditions to meet tissue demands. Further research is imperative to elucidate the precise mechanisms underlying Lgr5 expression in Paneth cells and its functional implications during inflammation.

Intestinal stem cells reside at the base of intestinal crypts, where gradients of progenitor cells, colonocytes, and goblet cells have recently been identified [84]. Despite their delicate nature, these cells are protected from the extreme pH in the gut lumen through various mechanisms. Traditionally, goblet cells were considered the primary contributors to the protective mucus layer in the gut epithelium. Mucins secreted by goblet cells help maintain mucosal barrier integrity, indirectly influencing pH balance [85]. In contrast, a type of absorptive colonocytes has been newly recognized for their direct involvement in regulating pH balance within the gut [23]. These colonocytes likely achieve this by modulating ion transport and acid-base balance mechanisms in the intestinal lumen. The depletion of these cells has been associated with ulcerative colitis, potentially leading to the damage of stem cells and preventing the regeneration of epithelial cells and tissue repair.

Impaired function of stem cells has also been observed in Crohn's disease (CD), albeit through different mechanisms. In a comparative analysis of intestinal epithelial cells (IECs) between treatment-naïve adult patients with CD and those without inflammatory bowel disease (IBD) [86], Kanke et al. highlighted a shift in the microenvironment from supporting the classical $Lgr5^{+}$ stem cells located at the base of the

Table 3
Summary of subsets of intestinal epithelial cell.

IBD patient Population	Cell Population	Features	Main Discovery	References
Pediatric IBD	Colonic stem cells, absorptive cells, secretory progenitors	Colonic stem cells: *ASCL2*+LGR5 +*SMOC2* + , absorptive cells:FXYD3 +FABP1 +SLC26A2 + , secretory progenitors: HEXIM1 +METTL12 + .	Novel risk genes expressed in epithelial cells contribute to PIBD pathogenesis.	Huang et al. [70]
Healthy	Paneth cells	Lgr5 + .	Paneth cells may re-express Lgr5 to acquire stem cell-like features, thus enhancing their self-renewal capacity or facilitating tissue repair processes.	Grun et al.[75]
UC	Absorptive cells, goblet cells	Absorptive cells: BEST4 +OTOP2 + , goblet-cell -secreted antibacterial protein, WFDC2.	Critical for maintaining pH balance and mucosal homeostasis.	Parikh et al. [17]
UC	Enterocytes, microfold cells	Enterocytes: BEST4 + , microfold cells: CCL20 +CCL23 + .	BEST4 +enterocytes are enriched in genes related to pH sensing and electrolyte balance.	Smillie et al. [19]
CD	Colonocytes, goblet cells, SPIB+ cells	Colonocytes: CEACAM7 + , CA1 + , goblet cells: CLDN4 + , SPIB+ cells are similar to BEST4 +enterocytes.	A shift in the microenvironment from supporting the classical Lgr5 + stem cells located at the base of the crypt to favoring a unique subpopulation of mature colonocytes situated at the crypt apex.	Kanke et al. [79] Smillie et al.[19]
CD	Enterochromaffin (EC) cells	EC THP1 +CES+ and EC REG4 +NPW+ .	EC REG4 +NPW+ cells were negatively enriched in oxidative phosphorylation.	Kong et al.[66]

crypt to favoring a unique subpopulation of mature colonocytes situated at the crypt apex. This shift resulted in a significant alteration in the distribution of IECs in the early stages of the disease, potentially compromising the integrity of the gut epithelium and promoting the development of CD.

4.2. Subsets of Intestinal Mesenchymal Cells

Intestinal mesenchymal cells encompass a heterogeneous population of cells, including fibroblasts, myofibroblasts, pericytes, and smooth muscle cells, distributed within the intestinal mucosa and submucosa. These cells serve as crucial mediators of tissue homeostasis and repair, orchestrating interactions between epithelial cells, immune cells, and the extracellular matrix [87]. In IBD, Intestinal mesenchymal cells exhibit dysregulated activation, contributing to the perpetuation of inflammation and tissue damage through the secretion of pro-inflammatory cytokines, chemokines, and matrix metalloproteinases. Additionally, they play roles in processes such as fibrosis, angiogenesis, and epithelial barrier function, further influencing the disease course. Although studies have highlighted the involvement of IMCs in IBD pathogenesis, the specific subsets of IMCs involved, their functional heterogeneity, and their dynamic interactions with other cell types remain poorly understood. Additionally, the mechanisms driving IMC activation and dysregulation in IBD are not fully elucidated. Moreover, the spatiotemporal dynamics of IMC responses during IBD, including their contributions to tissue repair, fibrosis, and angiogenesis, are areas requiring further investigation.

In addition to the established cell types of intestinal mesenchymal cells, several subsets of fibroblasts expressing distinct transcriptional regulators have been identified by different laboratories (Table 4). Kinchen et al. identified a colonic crypt niche subset expressed in both human ulcerative colitis (UC) and dextran-sodium sulfate colitis mouse models, characterized by the unique expression of transcription factor SOX6 [88]. In the intestine, SOX6 is largely expressed within epithelial cells and facilitate their differentiation and maintenance through regulating the expression of genes involved in mucin production, tight junction formation, inflammatory responses, and tissue repair processes. SOX6 was reportedly expressed in the mesenchyme of the developing intestine, particularly in the mesenchymal cells surrounding the epithelial structures. Identification of the Sox6-expressing fibroblast subset from UC might reflect interactions of these cells with epithelial cells, hinting at a role in epithelial repair and renewal. Dysregulation of these fibroblasts can also lead to barrier dysfunction. Moreover, they observed the emergence of another subpopulation that was barely detectable in the healthy mesenchyme and may play a role in T cell recruitment and activation due to the high expression level of pro-inflammatory genes, including chemoattractant (*CCL19* and *CCL21*), T cell co-stimulatory ligand (TNFSF14/LIGHT), and fibroblastic reticular cell (FRC)-associated genes [89].

Jasso and colleagues identified a unique IL-11^{+} mucosal-associated fibroblast (MAF) subset, which is generated during chronic inflammatory responses and exhibits upregulated inflammatory genes, including C4B, CXCL5, and SAA3 [90]. It is noteworthy that IL-11 belongs to the IL-6 family and has previously been reported to be involved in inducing MAPK/ERK activation in fibroblasts through autocrine mechanisms, which drives the production of inflammatory chemokines and extracellular matrix (ECM)-degrading enzymes such as matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) for ECM deposition [91,92], contributing to tissue damage and fibrosis observed in IBD. A transgenic mouse model driving IL-11 expression in smooth muscle cells or fibroblasts has been shown to spontaneously develop colitis, as well as inflammation in other organs [93]. In humans, expansion of IL-11^{+} MAF cells has recently been described in patients with ulcerative colitis and Crohn's disease [25,94]. Overall, these findings suggest that IL-11 signaling in fibroblasts is a conserved molecular circuit that may be shared across various inflammatory fibrotic diseases, thus representing a potential therapeutic target for preventing fibrosis during IBD.

The emergence of previously unrecognized mesenchymal cell subsets may arise from the expansion of specific mesenchymal populations or, at least partially, from the transcriptional reprogramming of existing subsets in response to inflammatory regulators during IBD. Kong et al. compared transcriptomic data and identified gene clusters associated with transitions between two subtypes of myofibroblasts, which exhibit varying abundances in diseases and possess distinct collagen-producing characteristics. This process results in the generation of CD-associated myofibroblasts, which play a role in fibrotic complications. Such transition may present novel targets for preventing fibrosis during IBD [73].

Moreover, these studies have illustrated that the expansion of certain mesenchymal cell populations exhibits location-specific traits. For example, diverse stromal cell subsets were found to proliferate in the terminal ileum during inflammation, whereas their abundance decreased in the colon [73]. Additionally, previously described mucosal-associated fibroblasts were found to be enriched in the

Table 4
Summary of subsets of intestinal mesenchymal cells.

IBD patient Population	Cell Population	Features	Main Discovery	References
UC	Stromal cells	S1: COL14A1, COL15A, FBLN1, FBLN2, FBLN5, EFEMP1, FN1, S2: SOX6, WNT5A, S3: OGN, GSN, FBLN1, S4: fibroblastic reticular cell (FRC)-associated genes, lymphocyte trafficking cytokines (CCL19 and CCL21), T cell co-stimulatory TNF-superfamily ligand (TNFSF14/LIGHT),CD74, CD24.	Stromal remodeling in IBD is functionally distinct in a subset-specific manner.	Kinchen et al. [81]
Pediatric IBD	Fibroblasts	Myofibroblasts: MYH11, ACTA2, perivascular pericytes: RGS5, epithelial proximal fibroblasts: COL4A5, COL4A6, BMP2, WNT5A, laminal propria fibroblasts: ADAMDEC1, CCL8, DCN, Fibroblast-TACI: WNT2B.	Enrichment of genes participating in TNF, NF-κB signaling, and stress responses in the in the WNT2B^{hi} and the inflammatory fibroblasts subsets.	Huang et al. [70]
IBD, DSS-treated mice	Mucosal-associated fibroblasts, myofibroblasts, and interstitial fibroblasts	Mucosal-associated fibroblasts: IL-11, or Grem1, Myofibroblasts: Tagln, Myl9, and Acta2, Interstitial fibroblasts: Pi61, CD81.	IL-11 signaling in fibroblasts is a conserved molecular circuit that may be shared across various inflammatory fibrotic diseases.	Jasso et al. [83] Martin et al.[87]
UC	Inflammatory associated fibroblasts (IAFs)	OSMR+ .	IAFs may be implicated in the OSM-mediated anti-TNF resistance.	Smillie et al. [19]
CD	Myofibroblasts	HHIP+ NPNT+ and GREM1 + GREM2 + myofibroblasts.	Identification of gene clusters associated with transitions between two subtypes of myofibroblasts, which exhibit varying abundances in diseases and possess distinct collagen-producing characteristics.	Kong et al. [66]

inflamed colon but were absent in the terminal ileum [25,90]. This phenomenon may play a role in the differentiation between UC and CD and could also account for the unique stromal remodeling observed in individuals with IBD.

4.3. Subsets of Intestinal Immune Cells

Amidst the wealth of studies underscoring the involvement of T cells in the pathogenesis of inflammatory bowel disease (IBD) [95,96], recent research endeavors have shifted their focus toward intraepithelial lymphocytes (IELs) [97,98]. Due to their limited abundance, understanding the functions of IELs has posed a challenge, prompting investigations leveraging single-cell RNA sequencing. Compared to the healthy population, notable abnormalities in the composition of IBD-associated IEL T cells include: (a) an escalation in inflammatory Th17 cells; (b) a decline in Treg cells, potentially exacerbating inflammation; (c) a reduction in T_{FH} cells, which may elucidate the previously observed impaired mucosal IgA production in CD [99]; and (d) an overall decrease in $CD8^+$ T cells and γδ T cells (Table 5).

In recent years, an increasing body of research has shown that Th17 cells play a crucial role in the pathogenesis of IBD [100]. Under normal immune function, Th17 cells can play a protective role. They mediate the proliferation and maturation of lymphocytes, macrophages, and neutrophils. Th17 cells also resist extracellular microbial infections, protecting mucosal and epithelial tissues, and play important roles in immune responses to autoimmune diseases and mucosal infections [101]. However, in the case of immune dysregulation, the abnormal proliferation of Th17 cells can induce abnormal inflammatory immune responses, mediating the development of IBD. Jaeger et al. identified transcriptionally distinct subsets of Th17 cells originating from different compartments of the intestinal mucosa [102]. They observed that Th17 cells in the intestinal lamina propria appear to be in a more quiescent state compared to their counterparts in the IELs. Notably, these IEL inflammatory Th17 cells are distinguished by their expression of CD39. CD39, an ectonucleoside triphosphate diphosphohydrolase-1 (ENTPD1) enzyme, plays a pivotal role in regulating purinergic signaling and immune responses. While CD39 expression is typically associated with regulatory T cells (Tregs) rather than effector T cells, emerging evidence suggests heterogeneity within Th17 cell populations, with certain subsets expressing CD39, particularly in inflammatory contexts. However, whether $CD39^+$ Th17 cells represent a distinct subset with unique effector functions or acquire CD39 expression under specific inflammatory conditions remains an area of ongoing research. Interestingly, IEL $CD39^+$ Th17 cells may comprise two subsets with opposing functional characteristics. While one subset expresses cytokine genes IL17A and IL26 [66,69,70], known for their protective effects on the gut epithelium and antibacterial function [103], the other subset exhibit pathogenic features by expressing GZMB and CCL4, mediators of Th17 pathogenicity [104]. These findings align with our group's recent research in which we demonstrated that the inflammatory Th17 cells involved in autoimmune colitis are distinct from those activated during colonic infection, characterized by their different responses to pharmacological molecules [105]. The dual functionality of Th17 subsets poses challenges for therapeutic strategies in IBD and may explain the susceptibility to infections following treatment with anti-Th17 biologics. Further research is needed to fully elucidate the role of CD39 expression in Th17 cell biology and its implications in immune regulation and inflammatory diseases.

Significant alterations within mucosal B cell populations are another hallmark of the immune cell compartment changes associated with IBD.

Table 5
Summary of subsets of intestinal immune cells.

IBD patient Population	Cell Population	Features	Main Discovery	References
UC and CD	CD4 + effector cells, Follicular B cells.	UC: Th17-like cells, exhausted Tc17 cells, CD: Th1-like cells, GBP+ Follicular B cells.	Identification of a series of disease-specific immune cell types during the pathogenesis of CD and UC.	Huang et al. [51]
UC and CD	Treg, effector memory T cells, granulocytes, naïve B cells, dendritic cells (DCs), plasmacytoid DCs.	UC: IL17A+ CD161 + effector memory T cells, IL17A+ T-regulatory cells, HLA-DR+CD56 + granulocytes, CD: IL1B+HLA-DR+CD38 + T cells, IL1B+TNF+IFNG+ naïve B cells, IL1B+ dendritic cells (DCs), and IL1B+ plasmacytoid DCs.	Identification of immune cell populations specific to patients with CD and UC.	Mitsialis et al.[33]
Pediatric IBD	Macrophages, intraepithelial T cells (IET).	PDE4B- and TNF-expressing macrophages, CD39-expressing intraepithelial T cells.	CD39 + IET deficiency, platelet aggregation, and hyper-inflammation are substantial common pathogenetic marked by the defective cAMP response pathway.	Huang et al. [70]
CD	Th17 cells.	IEL CD39 + Th17 cells.	IEL CD39 + Th17 cells may comprise two subsets with opposing functional features.	Jaeger et al. [95]
CD	Intraepithelial lymphocytes (IELs).	ID3 + ENTPD1(CD39)+ IELs.	Reduction in the frequency of bona fide ITGAE(CD103)+ ENTPD1(CD39)+ IELs both in inflamed and non-inflamed tissue.	Kong et al. [66]
CD	CD4 +Tissue-resident memory T cells (Trm).	CD103 +CRR7-CD27-.	Identification of a unique CD4 + Trm subset contributing to CD pathogenesis.	Yokoi et al. [30]
UC	TFH-like T peripheral helper (TPH) cells, B cells, plasma cells (PCs).	TPH cells: CXCL13 + , B cells: CD19, MS4A1, BANK1 and HLA-DRA, and PCs: SDC1, XBP1, TNFRSF17, PRDM1, MZB1 and SEC11C.	Association with the pathogenic B cell response in ulcerative colitis.	Uzzan et al. [100]
CD	Macrophages.	HLADR+SIRPα+CD14 + macrophages have two subpopulations: CD64hiCD163-/dim and CD64hiCD163hi.	These two subpopulations showed distinct phenotypically, morphologically and functionally features.	Chapuy et al.[105]
UC	Mast cells.	MRGPRX2 + .	Identification of loss-of-function allele of MRGPRX2 exhibits a protective effect on UC and MRGPRX2 is a receptor primarily expressed by mast cell.	Chen et al. [108]
CD	GIMATS (IgG plasma cells, inflammatory mononuclear phagocytes, activated T cells, and stromal cells).	GIMATS: IgG-producing plasmablasts, inflammatory mononuclear phagocytes, and activated T and stromal cells.	The enrichment of this module before treatment was associated with resistance to anti-TNF therapy.	Martin et al. [87]
UC	Inflammatory monocytes and DC2s.	OSM+ .	Inflammatory Monocytes and Inflammatory associated fibroblasts (IAFs) are Associated with Resistance to Anti-TNF Therapy.	Smillie et al. [19]

Uzzan et al. conducted a comprehensive analysis of the compositional, clonotypic, and transcriptional profiles of intestinal mucosal and circulating B cells in patients with UC, revealing the expansion of naïve B cells and the presence of IgG^+ plasma cells. Additionally, they identified a subset of intestinal T_{FH}-like T peripheral helper (T_{PH}) cells, previously reported to drive pathological B cell responses in rheumatoid arthritis [106], which were associated with the pathogenic B cell response in UC. These cells may promote the expression of auto-reactive antibodies targeting integrin αvβ6 in the inflamed intestinal mucosa of UC patients [107].

Another recent focus has been on investigating events preceding the activation of adaptive immunity. This attention stems from the significant functional and genetic interconnections between dysregulated innate immune responses within the intestinal tract and the onset of IBD. For instance, in patients with CD or UC, a distinct subset of macrophages ($CD14^+HLA\text{-}DR^{dim}$) is notably enriched in the inflamed regions of the ileum and colon [108,109]. These macrophages express CD14, a surface marker commonly associated with monocytes and macrophages, while exhibiting reduced expression of HLA-DR (Human Leukocyte Antigen-DR), a major histocompatibility complex (MHC) class II molecule involved in antigen presentation. The diminished expression of HLA-DR indicates a less mature or less activated state compared to macrophages with higher HLA-DR expression levels. Although $CD14^+HLA\text{-}DR^{dim}$ macrophages are believed to exhibit immunoregulatory functions, their functions vary during inflammation. Conversely, $CD14^+HLA\text{-}DR^+$ macrophages have been found to be equally prevalent in both inflamed and non-inflamed mucosal areas [110,111]. Chapuy and colleagues provided detailed insights into two distinct colonic $CD14^+HLA\text{-}DR^+$ subsets based on their expression levels of CD63, with unique phenotypic and functional genes [112]. CD63 is typically associated with lysosomal membranes and is involved in intracellular trafficking and membrane fusion events. Notably, $CD63^{-/dim}$ macrophages, which exhibit reduced endocytic or phagocytic pathways, were found to produce IL-1β and IL-23, amplifying the pathogenic response of mucosal effector Th17/Th1 cells. These cells demonstrated a selective increase in the inflamed colon, and their presence correlated with disease severity.

Furthermore, mast cells have also garnered attention in the context of IBD. The existing literature presents varied conclusions regarding the abundance of mast cells in UC. While some studies report an increase in their numbers [113], others suggest a decrease [114]. However, a recent study utilizing scRNA-seq failed to identify significant differences in mast cell numbers between inflamed and non-inflamed UC tissues [115]. Interestingly, they discovered that a loss-of-function allele of MRGPRX2 (Mas-related G protein-coupled receptor X2) exhibits a protective effect on UC. MRGPRX2 is a receptor primarily expressed by mast cells, and its activation triggers the release of proinflammatory mediators. Therefore, blocking mast cell activation may represents a promising targeted therapeutic approach.

5. Clinic applications of single-cell techniques

5.1. Integration of scRNA analysis into diagnostic models

The diagnostic and therapeutic challenge of IBD arises from the significant variability in its clinical manifestations, however recent efforts have been made to incorporate scRNA-seq technology into the clinic. Dai et al. introduced a diagnostic model for Crohn's disease (CD) based on single-cell sequencing data, focusing on T cell-associated genes [116]. Their approach involved the integration of scRNA-seq data, the simulation of stage-specific alterations in T-cell development, and the establishment and validation of a comprehensive multigene diagnosis model. Furthermore, by analyzing how different cell types respond to treatment at the single-cell level, researchers can identify mechanisms of drug resistance or sensitivity. Maddipatla et al. employed scRNA-seq on colonoscopically obtained terminal ileal biopsies from non-IBD control subjects, treatment-naïve CD patients, and treated CD patients [117]. They observed notable changes in the cellular composition of the epithelial compartment in treatment-naïve CD patients, as compared to those with established CD. Specifically, increased apolipoprotein and goblet cell trefoil factor activity were detected in remission but not in refractory established CD patients. Additionally, Devlin et al. discovered that IL1B+ /LYZ+ myeloid cells were the most closely associated cell subtype within the inflammatory network and were linked to non-responsiveness to anti-integrin therapy in patients with ulcerative colitis (UC) [118]. IL1B+ /LYZ+ myeloid cells may thus serve as a biomarker for intestinal inflammation and non-responsiveness to anti-integrin blockade.

5.2. Prediction of therapy responses through scRNA analysis

Significant therapeutic advancements have been achieved through biological therapies in IBD. For instance, anti-TNF drugs such as infliximab, adalimumab, and certolizumab neutralize TNF-α activity, thereby reducing inflammation in the gastrointestinal tract. Tumor necrosis factor-alpha (TNF-α) is a pro-inflammatory cytokine that plays a key role in the pathogenesis of IBD through activating immune and mesenchymal cells, like fibroblast. Anti-TNF drugs can inhibit this activation, thereby reducing inflammation, fibroblast-mediated tissue remodeling, and fibrosis, facilitating the preservation of tissue integrity.

However, only 30–40 % of patients exhibit a sustained response to this treatment [5]. Several explanations for this phenomenon have been proposed using scRNA techniques. First, the variation in treatment response could be attributed to the unique nature of inflammation in different individuals, as reflected by the composition of cells involved in the inflammatory process. Martin and colleagues identified a specific cellular module known as GIMATS in a subset of ileal Crohn's Disease (CD) patients using scRNA-seq and CyTOF [94]. GIMATS comprises IgG plasma cells, inflammatory mononuclear phagocytes, activated T cells, and stromal cells, and its dynamics are governed by a distinctive cytokine-chemokine network dependent on mononuclear phagocytes (MNP), which may not depend on TNF-α signaling. The enrichment of this module before treatment was thus associated with resistance to anti-TNF therapy. Similarly, Maddipatla and colleagues utilized scRNA-seq on terminal ileal biopsies obtained via colonoscopy from CD patients [117]. Notably, they attribute the refractory to the treatment partially to the altered epithelial cell composition which result in the reduced apolipoprotein and goblet cell trefoil factor activity. These factors contribute to the maintenance of mucosal homeostasis, protection against mucosal injury, and promotion of mucosal repair and regeneration. A third investigation conducted by Brubaker and colleagues unveiled α1 integrin (ITGA1)-expressing T cells associated with resistance to anti-TNF treatment [119], since disrupting the α1 integrin subunit can enhance the response to anti-TNF treatment in immune cells. $ITGA1^+$ T cells might represent a subset of T cells with alternative inflammatory pathways that are not effectively targeted by anti-TNF therapy and selectively enriched in response to anti-TNF therapy as part of a compensatory mechanism to maintain inflammation.

An alternative explanation is some other signaling compensates the loss of TNF-α signals. In a study by Smillie and colleagues utilizing scRNA-seq, they elucidated the role of inflammatory monocytes and inflammation-associated fibroblasts (IAFs) in mediating resistance to anti-TNF therapy. These cells were found to express Oncostatin M (OSM) and OSM receptor (OSMR), respectively. Their interaction essentially mimics the TNF pathway and counteracts the effects of anti-TNF treatment [25].

Despite significant therapeutic advancements achieved through biological therapies in IBD, such as anti-TNF drugs, which target TNF-α activity to reduce inflammation and fibrosis, the variable response rates highlight the need for personalized treatment strategies. Findings from single-cell RNA sequencing studies shed light on the diverse cellular and molecular mechanisms underlying treatment resistance, emphasizing the importance of tailored approaches to address individual patient

needs.

6. Identifying genetic triggers of IBD by integrating GWAS and scRNA-seq

The development of inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), involves complex interactions between genetic and environmental factors. Several genetic factors have been implicated in the development of IBD, such as variations affecting genes NOD2, IL23R, and ATG16L1. GWAS provides valuable insights by identifying pathogenic single nucleotide polymorphisms (SNPs) associated with IBD across the genome. However, the cellular targets of these SNPs often remain elusive due to GWAS's limitation in pinpointing affected cell types [120–123]. In contrast, scRNA-seq offers a high-resolution view of gene expression patterns across diverse cell populations, allowing for the identification and characterization of distinct cell types based on their gene expression profiles. Integration begins by correlating the identified SNPs from GWAS with the gene expression profiles obtained from scRNA-seq data (Fig. 3). This data integration process facilitates the postulation that SNPs identified through GWAS may exert significant effects on specific cell types expressing genes associated with these SNPs. By examining the expression levels of genes linked to the identified SNPs across different cell types, inferences about which cell types are likely affected by the genetic variants can be made. Furthermore, advanced computational methods, such as colocalization analysis or expression quantitative trait loci (eQTL) mapping, can be applied to pinpoint specific cellular contexts where the genetic variants exert their effects.

Through the integration of cross-tissue GWAS and scRNA-seq, Smillie et al. revealed that 29 GWAS-implicated risk genes were enriched in specific lineages during IBD [25]. Beyond previously known associations such as NKX-2–3 in microvascular cells and HNF4A in enterocytes [124,125], they identified new associations, including intelectin1 (ITLN1), a lipid raft protein localized in the epithelial brush border [126], which showed overexpression in immature goblet cells. Furthermore, their findings suggested that multiple putative IBD risk genes belong to the same gene module, implying their involvement in critical disease pathways. For instance, JAK2 in Ulcerative Colitis-associated Microfold (M) cells, along with four other risk genes (PTGER4, CCL20, SH2B3, and AHR), may play a role in regulating the TNF signaling pathway in M-like cells. Similarly, Nie et al. collected 318 GWAS-implicated genes at risk loci which were enriched in specific immune cell subtypes, including Th17 cells, $CD8^{+}$Tc17 cells, and inflammatory monocytes [127]. Kanke et al. found that ATG16L2 was notably enriched in the $SPIB^{+}$ cluster, particularly in the $SPIB^{+}/LYZ^{+}$ subcluster [86]. Interestingly, Uellendahl et al. identified shared risk genes in both IBD and schizophrenia, including NR5A2, SATB2, and PPP3CA [128]. Notably, PPP3CA exhibited the highest expression in neurons, enteroendocrine, and Paneth-like cells of the ileum, colon, and rectum, suggesting a possible link to the gut-brain axis.

Moreover, the integration of cross-tissue GWAS and scRNA-seq facilitates the delineation of the pathogenic roles of susceptibility genes [2]. For instance, scRNA-seq analysis revealed a significant elevation of Gasdermin B (GSDMB) levels in inflamed tissues compared to non-inflamed and healthy tissues [129], particularly enriched in colonocytes with diminished expression in goblet cells. As a member of the gasdermin protein family involved in regulating inflammatory cell death processes like pyroptosis, which can lead to the swift rupture of epithelial cells, GSDMB may play a role in the resolution of inflammation and subsequent tissue repair during IBD. This observation suggests that naturally occurring mutations of GSDMB identified through GWAS may impede its ability to independently facilitate epithelial restitution and repair. By employing this integrated methodology, insights into the molecular pathways and cellular networks through which these genetic variants contribute to disease susceptibility or progression can be gleaned, thus advancing our comprehension of complex diseases and facilitate the development of targeted therapies.

7. Conclusions

In the last decade, the emergence of single-cell omics technologies has significantly advanced our comprehension of IBD. Most existing studies focus on investigating IBD specific pathological states using single-cell technologies. Nevertheless, several challenges such as the

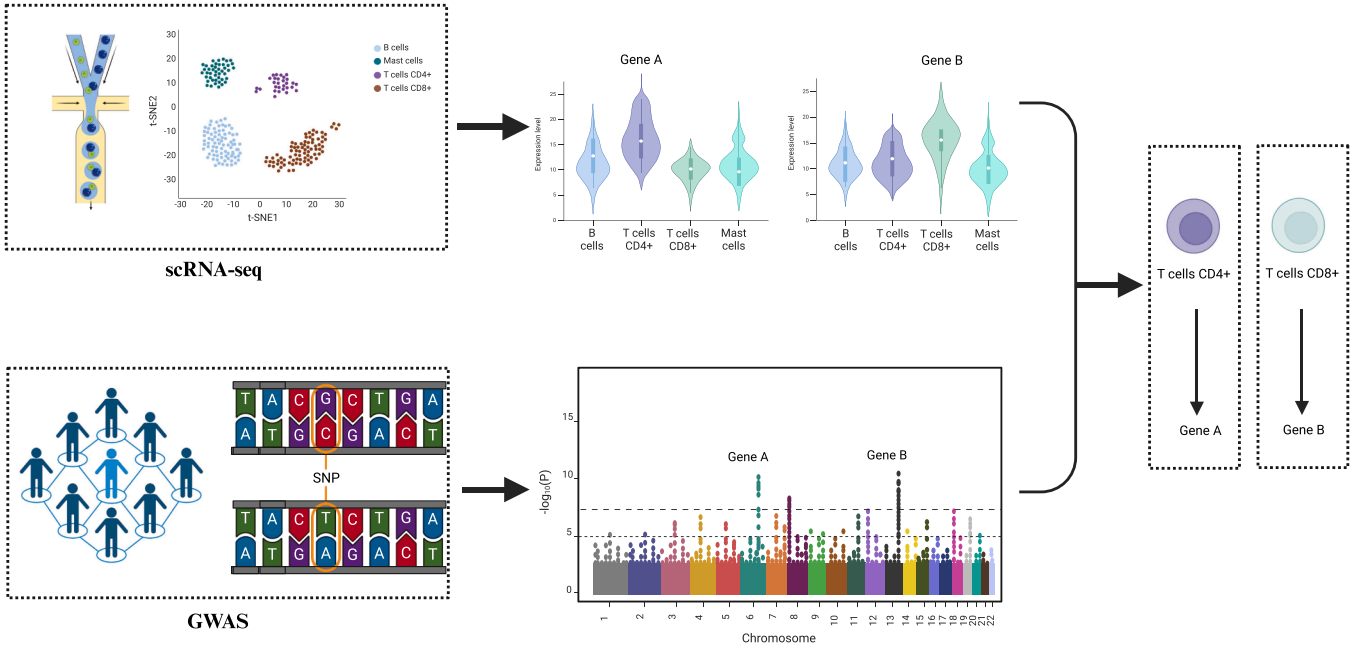

Fig. 3. : Identification of cell-type-specific IBD-risk genes by integrating GWAS and single cell RNA-seqencing (scRNA-seq). ScRNA-seq allow identification and characterization of distinct cell types based on their gene expression profiles. GWAS provides valuable insights by identifying pathogenic single nucleotide polymorphisms (SNPs) associated with IBD across the genome. Integration of GWAS and scRNA-seq allow revealing cell-type-specific IBD-risk genes. Abbreviations: Single-cell RNA sequencing, scRNA-seq; Genome-wide association studies, GWAS.

dynamic insights into IBD progression remain unexplored. Such challenges exist due to the nature of the samples used in most existing studies, which primarily consist of mucosa or peripheral blood samples from specific stages. This results in the lack of samples from various stages from the same subject. To comprehensively explore the dynamic changes in the cellular landscape and molecular phenotype throughout the course of IBD, future research should focus on conducting longitudinal studies.

Another obstacle relates to the integration of multiple single-cell and spatial transcriptomics technologies. Though certain studies developing single-cell spatial transcriptomics protocols show their capability in targeting more than 10,000 RNA sequences [130,131], the available commercial systems for single-cell spatial transcriptomics, including the NanoString CosMx SMI [132], Vizgen MERSCOPE [133], and 10x Genomics Xenium [134], are limited to a range of 500 to 980 targets. Therefore, it is often necessary to combine single-cell spatial transcriptomics with traditional single-cell RNA sequencing in IBD research. We anticipate that the advancement of single-cell spatial transcriptomics will lead to the achievement of high-throughput RNA profiling capabilities, similar to what is offered by single-cell RNA sequencing.

It is widely acknowledged that the gut microbiota plays a significant role in the pathogenesis of IBD [135,136]. Over recent years, microbial single-cell RNA-seq has emerged and used to examine the patterns of microbial communities in both health and disorders [137]. To the best of our knowledge, single-cell RNA-seq of the microbiome has not yet been applied to decipher the role of microbial subsets in the temporal and spatial dynamics of IBD. Such an approach could facilitate species-specific targeted therapeutic strategies.

CRediT authorship contribution statement

Liang Liu: Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Zhiheng He:** Writing – review & editing, Writing – original draft. **Peixian Dong:** Writing – review & editing, Writing – original draft, Conceptualization. **Benjamin Davidorf:** Writing – review & editing, Writing – original draft. **Alice Peng:** Writing – review & editing, Writing – original draft. **Qianqian Song:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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