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Integrating Single-Cell RNA Sequencing and Microbial Metabolomics for Predictive Biomarker Discovery in Inflammatory Bowel Disease

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Abstract

Background and Aims: Inflammatory bowel disease (IBD), encompassing Crohn's disease (CD) and ulcerative colitis (UC), represents a significant global health challenge, with substantial variability in patient responses to treatment. Personalized medicine offers a promising approach to optimizing therapeutic strategies by addressing this heterogeneity. Single-cell RNA sequencing (scRNA-seq) and microbial metabolomics are emerging technologies that provide high-resolution insights into immune and microbial factors influencing IBD pathogenesis and therapeutic outcomes. This review explores the integration of scRNA-seq and microbial metabolomics to identify predictive biomarkers for IBD management.

Methods: This literature review synthesizes findings from peer-reviewed studies on scRNA-seq and microbial metabolomics in IBD. Relevant articles were identified through database searches using keywords such as "Inflammatory bowel disease," "Crohn's disease," "Ulcerative colitis," "Single-cell RNA sequencing," "Microbial metabolomics," "Precision medicine," "Biomarkers," "Gut microbiota," "Short-chain fatty acids," and "Bile acids." The inclusion criteria prioritized studies reporting advancements in biomarker discovery, integrative analytical techniques, and insights into the molecular and microbial mechanisms of IBD. Key methodologies and findings from these studies were critically analyzed, highlighting current challenges and opportunities in leveraging multi-omics approaches for precision medicine.

Results: Integration of scRNA-seq and microbial metabolomics has revealed novel insights into the immune and microbial drivers of IBD. scRNA-seq studies have identified pro-inflammatory T cell populations, macrophage subtypes, and fibroblast markers associated with disease activity and treatment resistance. Concurrently, metabolomics has highlighted dysregulated metabolic pathways, including short-chain fatty acids and bile acids, which influence immune responses and therapeutic efficacy. The combination of these datasets enables the identification of robust biomarkers, such as COL5A2 and selenium-related pathways, that predict treatment outcomes and inform personalized therapeutic strategies.

Conclusions: The integration of scRNA-seq and microbial metabolomics offers a powerful approach to biomarker discovery and personalized medicine in IBD. These technologies provide complementary insights into cellular and microbial interactions that drive disease progression and treatment response. Future research should focus on longitudinal studies and multi-centre collaborations to validate these biomarkers and establish standardized protocols for their clinical application.

Keywords
Inflammatory bowel disease; Single-cell RNA sequencing; Microbial metabolomics; Precision medicine; Gut microbiota.

Introduction

Inflammatory bowel disease (IBD), encompassing Crohn's disease (CD) and ulcerative colitis (UC), represents a significant global health challenge [1, 2]. These chronic inflammatory conditions of the gastrointestinal tract affect millions worldwide, imposing a substantial socioeconomic burden [1]. For example, the annual healthcare costs associated with IBD in the United States alone exceed $6 billion, with additional losses attributed to reduced workplace productivity and absenteeism. While various therapies exist, treatment efficacy varies considerably among patients [2, 3], highlighting the urgent need for personalized approaches that optimize treatment selection and improve outcomes [4, 5]. This review explores the potential of integrating single-cell RNA sequencing (scRNA-seq) and microbial metabolomics to identify predictive biomarkers for IBD, paving the way for truly personalized medicine.

Crohn's Disease and Ulcerative Colitis: A Comparative Overview

Definition, Symptoms, and Clinical Manifestations

CD and UC, the two major forms of IBD, share some overlapping symptoms but differ significantly in their clinical presentation and disease course [6–8]. CD is characterized by transmural inflammation that can affect any part of the gastrointestinal tract, often leading to complications such as fistulas, strictures, and abscesses. UC, in contrast, involves continuous inflammation confined to the colonic mucosa [8]. Symptoms common to both include abdominal pain, diarrhea, weight loss, and rectal bleeding [8, 9]. However, the severity and location of these symptoms can vary significantly between individuals and disease subtypes [5, 9]. The heterogeneity in symptom presentation and disease progression underscores the complexity of IBD and the need for individualized treatment strategies [1, 5]. The inflammation pattern in UC is uninterrupted and restricted to the colonic mucosa, while in CD, it is patchy and can affect any part of the gastrointestinal tract, often leading to fibrotic complications.

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These differences likely reflect distinct cellular mechanisms and pathways underlying their pathogenesis. Despite extensive research, the pathogenesis of UC remains incompletely understood, highlighting a need for a more comprehensive understanding of the complex interactions between genetic, immune, microbial, and environmental factors [9].

Epidemiology and Prevalence Rates

The global prevalence of IBD is increasing [3, 9], with regional variations reflecting diverse genetic, environmental, and lifestyle factors [10]. Developed countries generally exhibit higher prevalence rates compared to developing nations, suggesting a role for environmental factors such as diet, urbanization, sanitation, and antibiotic use in disease onset [11]. However, the exact prevalence varies across studies due to differences in diagnostic criteria, study populations, and methodologies. Factors like diet, access to healthcare, and genetic predisposition contribute to regional variations. For instance, studies have shown differences in microbial composition and metabolic profiles between IBD patients from different geographical regions [10], suggesting that environmental factors play a significant role in shaping the gut microbiome and influencing the development of IBD. Further research is needed to fully elucidate the intricate interplay of genetic predisposition and environmental triggers in IBD development across different populations [10, 11]. The increasing global incidence of IBD necessitates further research into the environmental and lifestyle factors that contribute to its development [10].

Current Therapeutic Options and Their Limitations

Current IBD treatments aim to induce and maintain remission, focusing on managing inflammation and preventing complications [5, 11]. These include aminosalicylates, corticosteroids, immunomodulators (such as thiopurines), and biologic therapies (e.g., anti-TNF agents, integrin inhibitors, and JAK inhibitors) [5, 12]. However, a substantial proportion of patients do not respond adequately to these therapies [4, 5] or experience adverse effects. The heterogeneity of IBD and the complexity of its pathogenesis contribute to these limitations [5, 13]. For example, some patients may not respond to anti-TNF therapy due to the presence of specific immune cell subsets that are resistant to this treatment [4]. The lack of reliable predictive biomarkers makes it difficult to select the most appropriate therapy for each individual, resulting in treatment failures and increased healthcare costs. The need for personalized medicine approaches is evident, tailoring therapies based on individual patient characteristics to improve treatment success and minimize adverse effects [5, 13].

Current Treatment Challenges in Inflammatory Bowel Disease

Variability in Patient Responses to Therapies

A major hurdle in IBD management is the significant variability in patient responses to therapies [4, 5, 12]. Even within the same IBD subtype (CD or UC), patients may exhibit markedly different responses to the same treatment regimen [5]. This heterogeneity stems from complex interactions between genetic predisposition [11], environmental factors [11], the gut microbiome [13, 20], and immune system dynamics [14, 15]. For example, studies have shown that the composition and function of the gut microbiome can significantly influence the efficacy of biologic therapies [14]. Understanding these individual variations is crucial for developing personalized treatment strategies [1, 4]. The identification of predictive biomarkers could help stratify patients into subgroups based on their likelihood of responding to specific therapies [4, 5].

Challenges in Predicting Treatment Outcomes

Predicting treatment outcomes in IBD remains challenging [1, 5, 12]. Current clinical markers, such as C-reactive protein (CRP) and fecal calprotectin, offer limited predictive power [12, 16]. These markers reflect general inflammation but do not capture the complex interplay of factors influencing treatment response [12]. The lack of reliable biomarkers hinders the ability to tailor treatment to individual patients [1], leading to potentially ineffective therapies and increased healthcare costs [5]. Studies have attempted to identify more specific biomarkers using multi-omics approaches, but these findings often require further validation in larger, independent cohorts [1, 5]. The development of robust predictive models requires a deeper understanding of the complex interactions between host genetics, the microbiome, and the immune system [1, 5].

Impact of Ineffective Treatments on Patient Quality of Life

Ineffective IBD treatments can have profound consequences on patient well-being [5, 12, 17]. Patients may experience prolonged symptoms, including abdominal pain, diarrhea, and fatigue, significantly impacting their daily lives and quality of life [5]. Moreover, delayed or inappropriate treatment can lead to complications such as strictures, fistulas, and an increased risk of colorectal cancer [5]. The psychological toll of chronic illness and ineffective treatment can also be substantial, adding another layer of complexity to the patient's experience [5]. The economic burden associated with treatment failures and adverse effects underscores the urgency of developing more effective and personalized treatment strategies [5]. Studies have shown that patients with IBD often experience significant emotional distress, depression, and anxiety, which can be exacerbated by ineffective treatment [5]. Improved treatment strategies that focus on individual patient needs are crucial to improving both physical and mental health outcomes [5].

Importance of Predictive Biomarkers

Enhancing Treatment Efficacy Through Personalized Medicine

Personalized medicine offers the potential to revolutionize IBD treatment by tailoring therapies to individual patients based on their unique characteristics [1, 4, 6]. By identifying predictive biomarkers, clinicians can select the most effective treatment strategy for each patient, minimizing the risk of treatment failure and adverse effects [4, 6]. This approach is already showing promise in other areas of medicine and holds the potential to significantly improve IBD management [6]. The use of predictive biomarkers can lead to earlier intervention, improved treatment outcomes, and a better quality of life for patients with IBD [1, 4]. The development of predictive biomarkers is therefore a crucial step towards achieving truly personalized medicine in IBD [1, 4].

Potential Benefits of Personalized Treatment Based on Biomarkers

The identification of robust predictive biomarkers would allow for the development of personalized treatment strategies that maximize efficacy and minimize adverse effects [4-6]. Clinicians could select the most appropriate therapy for each patient based on their individual biomarker profile, potentially reducing the need for trial-and-error approaches [4, 5]. This

approach could lead to earlier achievement of remission, mucosal healing, and sustained disease control [4, 5]. Improved treatment outcomes would translate into enhanced quality of life, reduced healthcare utilization, and decreased economic burden associated with IBD [4, 5]. Moreover, personalized medicine could facilitate the development of new therapeutic targets and strategies based on the specific molecular mechanisms underlying disease pathogenesis and treatment response [4, 5].

Examples of Successful Biomarker-Driven Therapies in Other Diseases

The success of biomarker-driven therapies in other diseases provides a compelling case for their application in IBD [6, 13]. In oncology, genomic profiling has enabled the development of targeted therapies that selectively target cancer-specific mutations [6]. Similarly, in autoimmune diseases, biomarkers are used to predict treatment response and minimize adverse events [6]. These examples demonstrate the potential of biomarker-driven therapies to improve patient outcomes across various disease areas [6]. The use of biomarkers to personalize treatment is gaining traction in other chronic diseases, and IBD is poised to benefit from similar advancements [13]. The successful integration of biomarkers into clinical practice in other areas of medicine provides a roadmap for the development and implementation of biomarker-driven therapies in IBD [13].

Reducing Treatment Failures Through Predictive Biomarkers

Predictive biomarkers can significantly reduce treatment failures in IBD by guiding treatment selection [4-6]. By identifying patients who are likely to respond to specific therapies, clinicians can avoid ineffective treatments and minimize delays in achieving remission [4, 5]. This approach can reduce the emotional distress and economic burden associated with treatment failures [5]. The development of predictive biomarkers can improve the efficiency and effectiveness of IBD treatment, leading to better patient outcomes and reduced healthcare costs [4, 5]. This could also lead to the development of novel therapeutic strategies targeted at specific mechanisms of disease pathogenesis and treatment resistance identified through biomarker studies [4, 5].

Consequences of Treatment Failures and Adverse Effects

Treatment failures and adverse effects in IBD can have significant consequences [5, 12, 17]. Patients may experience prolonged symptoms, impacting their quality of life. Delayed or inappropriate treatment can lead to complications such as strictures, fistulas, and increased risk of colorectal cancer. Adverse effects of medications can range from mild to severe, further impacting quality of life and increasing healthcare utilization. The economic burden associated with treatment failures and adverse effects underscores the need for improved, personalized treatment strategies [5]. The development of predictive biomarkers can help mitigate these negative consequences by improving treatment selection and monitoring. Early identification of treatment failure or adverse effects can also allow for timely intervention and prevent serious complications [5, 12].

Need for Improved Methods to Tailor Treatments to Individual Patients

The significant variability in patient responses to IBD therapies necessitates improved methods for tailoring treatments to individual patients [4-6]. For example, integrating genetic profiling and gut microbiome analysis has shown promise in predicting individual responses to biologic therapies. The development of predictive biomarkers is crucial for achieving this goal. By identifying specific molecular characteristics associated with treatment response, clinicians can select the most effective therapy for each patient, minimizing the risk of treatment failure and adverse effects. This approach requires a multi-disciplinary effort involving clinicians, researchers, and bioinformaticians to integrate diverse data sources and develop robust predictive models. The implementation of personalized medicine approaches in IBD requires careful consideration of ethical and practical aspects, including access to testing, cost-effectiveness, and data privacy [4, 5].

Single-Cell RNA Sequencing (scRNA-seq)

scRNA-seq is an innovative method for exploring RNA diversity within individual cells, providing insights into cell functions and compositions in complex tissues and organisms. Historically, cells were identified by morphology or protein markers, but next-generation sequencing (NGS) now allows for precise cellular-level detection and analysis [21]. Over the past two decades, technological advancements have enhanced experimental protocols and computational workflows, enabling comprehensive and high-throughput transcriptome analysis [22]. The scRNA-seq workflow includes three steps: single-cell isolation, library preparation, and sequencing.

Overview of Technology

To preserve their viability, single-cell isolation segregates individual cells into subpopulations by mechanical and enzymatic methods. Common techniques include fluorescence-activated cell sorting (FACS), microfluidic systems, and laser capture microdissection (LCM) [21]. LCM uses a laser to precisely isolate cells of interest but is costly and may compromise tissue integrity. FACS employs flow cytometry to sort cells by molecular markers, enhancing specificity but requiring tissue dissociation, which may affect cell viability. Microfluidic systems offer a low-volume alternative that reduces reagent use and are increasingly preferred over FACS [23]. These systems label and capture individual cells into nanopores [24], ensuring unique barcoding of transcripts in high-throughput settings [21].

Library preparation begins with complementary DNA (cDNA) synthesis from RNA transcripts via reverse transcription. The cDNA is amplified using polymerase chain reaction (PCR) or in vitro transcription (IVT) [21]. Unique molecular identifiers (UMIs) are incorporated during reverse transcription to barcode each mRNA transcript, improving quantification accuracy and minimizing amplification errors [24]. The barcoded cDNA is then sequenced using platforms such as single-cell RNA barcoding and sequencing (SCRB-seq), cell expression by linear amplification and sequencing (CEL-seq), split-pool ligation-based transcription sequencing (SPLit-seq), massively parallel single-cell RNA sequencing (MARS-seq), switching mechanism at the 5' end of the RNA transcript sequencing (Smart-seq), and Drop-sequencing. These methods vary in RNA capture efficiency, bias, cell size compatibility, and library

construction costs, producing large datasets for analyzing gene expression, cell types, and functional states [24].

Advantages for IBD Research

Historically, the colonic microbiome and its surrounding immune function in IBD have been poorly understood. However, scRNA-seq offers new advancements in IBD research, providing several advantages. Unlike bulk RNA sequencing, which identifies genes in mixed-cell populations, scRNA-seq provides high-resolution gene expression profiling at the single-cell level, offering insights into how individual cells express certain genes. Assessing the gene expression profile of each inflammatory cell enables a better understanding of the cellular pathogenesis underlying IBD. Investigating the genetic and metabolic characteristics of tissues and cells involved in IBD pathogenesis helps elucidate cell-to-cell interactions. Furthermore, scRNA-seq enables the identification of rare cell types and specific metabolic states relevant to IBD, facilitating the discovery of novel therapeutic targets for minimizing inflammation [25]. Moreover, scRNA-seq reveals the heterogeneity of nonhematopoietic intestinal cells, allowing for the identification of novel biomarkers and the characterization of immune cells [26].

Applications of scRNA-seq in IBD

Immune Cell Analysis

Resident intestinal immune cells play a vital role in maintaining gastrointestinal homeostasis and serving as a molecular barrier against infectious microorganisms. scRNA-seq enables extensive immune cell analysis, providing detailed profiles of immune cell populations in intestinal biopsies. Additionally, scRNA-seq offers greater insights into T-cell and macrophage subtypes involved in inflammation. Macrophages residing in the intestinal tract gain a regulatory role that limits the inflammatory response and maintains a protective barrier against harmful agents. scRNA-seq has allowed for the discovery of a subset of resident macrophages with transcriptional signatures distinct from inflammatory macrophages previously identified in Crohn's disease (CD) patients [26].

For example, scRNA-seq technology analyzed native macrophages exposed to butyrate, identifying a transcriptomic signature marked by antimicrobial gene expression from a subset of differentiated macrophages. This technology revealed that butyrate promotes a protective mechanism involving LC3-associated host defense, potentially serving as prophylaxis against proinflammatory consequences. scRNA-seq has also been used to investigate T-cell subsets involved in the intestinal tract, further elucidating the interplay between these cells and IBD. Specifically, scRNA-seq has demonstrated that intestinal stem cells abundantly express MHC-II molecules, which bind to CD4+ T cells. Dysregulation of the gastrointestinal microenvironment influences intestinal cell differentiation into Th1, Th2, and Th17 cells. scRNA-seq has shown that preferential Th17 expression decreases stem cell renewal, whereas Th1 and Th2 cells inhibit stem cell renewal and promote Paneth cell and tuft cell differentiation, respectively [26].

Identifying Cellular Markers

scRNA-seq has provided an innovative approach for identifying cellular markers predictive of treatment response. For instance, Zhong et al. analyzed inflamed and non-inflamed tissues using scRNA-seq and identified distinct cell types. The study identified a novel biomarker, **COL5A2**, with diagnostic and predictive value for anti-tumor necrosis factor (TNF) treatment responsiveness in CD patients [27]. Another study combined scRNA-seq with spatial molecular imaging in IBD patients and discovered novel intestinal macrophages, distinguishing between resident and inflammation-related macrophages (M0 and M1) [28]. Of note, the inflammatory subtype exhibited an alternative activation pattern with multiple markers such as **EGFR ligands, NRG1, HBEGF, CLEC10A, and ASGR1**, distinct from the native M1 signature [17]. Another study utilized scRNA-seq, metabolomic profiling, bulk RNA-seq, and mass spectrometry to investigate the immune profile and metabolic environment of untreated IBD patients. This study found that **selenium**, a Crohn's disease-specific metabolite, drives type 1 T helper (Th1) cell differentiation, highlighting selenium as a potential therapeutic target [25].

Microbial Metabolomics

Metabolomics is an area of precision medicine that analyzes metabolites and is coined as the study of metabolites present within a biological system. It is used clinically to characterize novel biomarkers associated with the disease and prognosis and give a better idea of the response to treatment [29]. There are two different approaches: targeted and untargeted approach. The targeted metabolomics approach is considered a quantitative analysis with concentrations already determined whereas the untargeted approach is considered qualitative.

Overview of Technology

The metabolomics workflow consists of three key steps: sample preparation, metabolite extraction, and comprehensive analysis. The sample type (e.g., cell, tissue, or fluid) and the chosen metabolomics approach (targeted vs. untargeted) dictate the preparation method. Techniques used to analyze and identify key metabolites include **nuclear magnetic resonance (NMR) spectroscopy** and **mass spectrometry (MS)**.

- **NMR Spectroscopy**: NMR detects and quantifies metabolites based on their chemical environment. It is advantageous due to its reproducibility, minimal sample preparation, and preservation of sample integrity. However, it lacks sensitivity and has extensive signal overlap. Methods such as spatial editing and increasing scan numbers improve detection, but the number of identifiable metabolites remains limited [30].
- **Mass Spectrometry (MS)**: MS is widely used for metabolomics due to its high sensitivity and ability to identify and quantify a diverse range of molecules in biological samples [31].

For **untargeted metabolomic** workflows, metabolites are extracted and analyzed using liquid chromatography (LC) coupled with MS. The resulting data are processed using specialized software (e.g., XCMS), which aligns retention times and identifies metabolite features. These features are then cross-referenced with metabolite databases for identification, with validation occurring through MS/MS spectral matching [32].

For **targeted metabolomics**, known metabolites are quantified using standard compounds, and concentrations are determined through calibration curves. This method is commonly used for absolute quantification of key metabolic markers.

Metabolomics offers functional insights into microbial activity in the gut, in contrast to traditional microbiome sequencing, which only assesses community composition. This functional analysis helps identify key metabolic pathways affected in IBD [31].

Advantages for IBD Research

Throughout the years, advances in metabolomics have also been used to better understand the host microbiota through the lens of the intestinal microbiome [33]. Moreover, metabolites are created by enzymes encoded by the genes involved within the organism and if there are genetic mutations present that alter the enzymatic function of certain proteins, this can be sensed by metabolomic analysis and therefore be a source of context into disease pathogenesis. Finally, metabolomics can help identify the role of metabolites in influencing immune responses and IBD disease progression. Since IBD is noted to be a result of a dysregulated intestinal immune system and dysbiosis of the gut microbiome, a decrease in microbial diversity is easily detected by metabolomics [34].

Applications in IBD

Metabolite Profiling

In IBD, aberrant energy metabolism has been identified by metabolomics, involving lipid and TCA-related metabolites that were demonstrated by serum samples collected from IBD patients [35]. Interestingly, many studies note a decrease in TCA intermediates such as citrate, aconitate, α-ketoglutarate, succinate, fumarate, and malate in IBD patients compared to healthy patients in a variety of extracted samples. Furthermore, metabolomics helped show an increase in serum ketone bodies as a consequence of impairment in energy metabolism in IBD patients. Ketone bodies are mainly made in the liver through fatty acid oxidation, which is derived from acetyl-CoA. Here, glucose was shown to be elevated in IBD patient serum samples showing that these patients may not be able to utilize glucose [36].

Influence on Therapeutic Efficacy

Further applications of metabolomic analysis have also investigated the influence metabolite profiles have on therapeutic efficacy. Clinical metabolomics is used to assess how metabolite profiles affect the efficacy of multiple IBD therapies including TNF inhibitors (infliximab) and IL-23 inhibitors (ustekinumab). For example, recent studies have looked at serum bile acid (sBA) profiles in large groups of IBD to assess changes under anti-TNF alpha treatment quantified by high-pressure liquid chromatography-electrospray tandem MS (HPLC-ES-MS/MS) showing that such treatment returns sBA levels back to baseline in IBD patients. As such, sBAs were considered to be non-invasive biomarkers for clinical remission in IBD [37]. Furthermore, another study identified serum tryptophan levels elevated in IBD patients who responded to TNF inhibitor therapy but were not affected in those who did not respond to such therapy [38]. Although metabolomics has been the steadfast method of highlighting the roles of metabolites in therapy response, more studies need to be practiced to explore other therapies involved in the treatment of IBD.

Rationale for Integrating scRNA-seq and Microbial Metabolomics

Comprehensive Biomarker Discovery

Integrating single-cell RNA sequencing (scRNA-seq) and microbial metabolomics enhances our understanding of IBD pathogenesis and improves the prediction of treatment responses. By simultaneously examining cellular transcriptional profiles (e.g., TNF-α, IL-6, IL-1β, and CCL2 upregulation in immune cells) and the metabolic signatures of gut microbiota (e.g., increased lipopolysaccharides, trimethylamine-N-oxide, and decreased butyrate production), it provides a comprehensive view of the complex host-microbiome interactions driving disease progression.

This integration yields richer biomarkers for IBD. scRNA-seq reveals cell-type-specific transcriptional changes that may serve as early indicators of disease, such as reduced ***Faecalibacterium prausnitzii*** and ***Roseburia***, which are associated with gut inflammation and immune dysregulation. Additionally, mutations in bacterial recognition genes like NOD2, strongly linked to Crohn's disease, alter immune responses to gut microbiota, leading to chronic inflammation. Meanwhile, microbial metabolomics can identify key metabolic shifts within the gut that may precede or accompany these cellular alterations, such as increased levels of lipopolysaccharides (LPS), which contribute to metabolic endotoxemia and intestinal inflammation, and changes in bile acid metabolism, which affect gut barrier integrity in IBD. Furthermore, a dysbiosis-driven shift toward gram-negative bacteria leads to increased intestinal permeability and microbial translocation, exacerbating immune activation [39]. By integrating these data, researchers can build more robust models that capture both cellular and microbial components of disease.

Enhancing Predictive Accuracy

Integrating scRNA-seq and metabolomic data enables more precise predictive models for patient treatment responses. A dual-layered approach that considers both cellular (genetic, transcriptional, immune) and metabolic (microbiome, metabolomic) signatures provides a nuanced understanding of disease pathophysiology. For example, metagenomic sequencing of Mycobacterium tuberculosis can detect early-stage isoniazid resistance, allowing clinicians to adjust treatment before MDR-TB fully develops. Similarly, microbiome analysis in IBD has identified specific bacterial species linked to IBD progression, improving differentiation between IBD and IBS and prediction of treatment response [40].

Methodology for Integration

Dual Integration Techniques

To effectively integrate scRNA-seq and microbial metabolomics data, researchers employ various statistical methods. Correlation-based integration identifies links between datasets, while data set concatenation merges gene and metabolite measurements into a single table for analysis. However, these techniques have limitations—correlation often fails due to different time scales, while concatenation methods must address varying underlying data distributions.

Multivariate-based integration uses variations of standard techniques like partial least squares and principal component analysis to model relationships between variables. Pathway-based integration leverages existing biological knowledge from databases [41]. These methods map measured metabolites and transcripts to pathways, identifying statistically significant changes in behavior or correlation to phenotypic endpoints. The combination of these integrative techniques allows researchers to take a multi-pronged approach, extracting meaningful insights from the complex web of interactions between host cells, gut microbiota, and their metabolic outputs.

Advanced techniques like network analysis and machine learning-based modeling provide deeper insights into interactions between host cells and the gut microbiome. Co-expression and co-abundance networks reveal intricate

connections, highlighting the key pathways driving IBD. Additionally, machine learning-based integrative modeling captures nonlinear and synergistic effects between the host and microbial components, enhancing predictive accuracy [42].

Analysis and Interpretation

Interpreting integrated data is crucial for identifying predictive biomarkers and validating their clinical relevance. Strategies include statistical correlation analysis, pathway enhancement analysis, and machine learning-based feature selection to highlight critical disease signatures. Ultimately, the ability to effectively integrate and interpret these multi-omics datasets holds immense promise for advancing personalized diagnosis, prognosis, and treatment strategies for IBD patients, as it can provide an accurate understanding of the disease drivers. In summary, the integration of scRNA-seq and microbial metabolomics data offers a powerful approach for comprehensive biomarker discovery and enhanced predictive modeling in inflammatory bowel disease.

Summary of Current Evidence and Research

Single-Cell RNA Sequencing in IBD

scRNA-seq studies have identified key pathogenic dysregulations in CD and UC. These include expansion of pro-inflammatory T cell populations, altered activation states of innate lymphoid cells, and aberrant polarization of myeloid cells. These cell subsets, along with their unique transcriptional signatures, have emerged as potential biomarkers to predict disease activity, monitor treatment response, and guide personalized therapeutic strategies for IBD patients.

Notable biomarkers identified include IL-17A-producing Th17-like T cells and IL-17A+ regulatory T cells (Tregs) in ulcerative colitis. In contrast, IL-1β-secreting cells (e.g., T cells, B cells, dendritic cells, and macrophages) and IFN-γ-expressing Th1-like T cells, are prominent in Crohn's disease, which drive chronic inflammation. Additionally, CD-specific intraepithelial CD39+ Th17 cells have been discovered, with subsets exhibiting both protective and pathogenic roles, distinguished by IL-17A/IL-26 versus GZMB/CCL4 expression profiles, respectively. In UC, BEST4+ enterocytes and WFDC2+ goblet cells have been identified as critical for mucosal homeostasis and pH balance, while their depletion correlates with disease severity. Meanwhile, CD features CEACAM7+ colonocytes and a shift in epithelial stem cell dynamics, contributing to intestinal barrier dysfunction.

Further, HLA-DR+SIRPα+CD14+ macrophages in CD, display distinct subsets producing IL-1β and IL-23, amplifying Th1/Th17 inflammation [43]. In UC, MRGPRX2+ mast cells have emerged as novel therapeutic targets, with a loss-of-function mutation conferring protection against disease progression.

Additionally, GIMATS (IgG plasma cells, inflammatory mononuclear phagocytes, activated T cells, and stromal cells) has been associated with anti-TNF therapy resistance in CD, while IL1B+ /LYZ+ myeloid cells have been linked to non-responsiveness to anti-integrin therapy in UC. These findings underscore the power of single-cell transcriptomics in refining biomarker discovery and advancing precision medicine approaches for IBD [43].

Microbial Metabolomics in IBD

Parallel advances in microbial metabolomics have provided critical insights into how gut microbial metabolites regulate inflammation and immune homeostasis. Metabolic disturbances revealed imbalanced microbial production of short-chain fatty acids (e.g., decreased butyrate from *Faecalibacterium prausnitzii* and *Roseburia hominis*), bile acids (e.g., reduced secondary bile acids like deoxycholic acid and lithocholic acid due to impaired 7α-dehydroxylation), and tryptophan metabolites (e.g., diminished indole derivatives such as indoleacetic acid and indole-3-propionic acid, leading to reduced activation of the aryl hydrocarbon receptor and impaired mucosal immunity) [46]. Such metabolic shifts are potential therapeutic targets, as modulating the gut microbiome and thus its metabolic outputs could help restore homeostasis and mitigate inflammation in IBD.

Identification of Key Findings

Consensus and Insights

Integrating single-cell transcriptomics and microbial metabolomics, a more comprehensive understanding of IBD pathogenesis has emerged. As discussed, microbial metabolomics has identified key metabolic disturbances in short-chain fatty acids, bile acids, and tryptophan metabolites. The interplay between the host immune system and the gut microbiome has emerged as a central driver of disease heterogeneity and treatment outcomes in IBD. For instance, the expansion of pro-inflammatory Th17 cells and Treg dysregulation reveal mechanisms of chronic intestinal inflammation. Th17 cells, which secrete IL-17A, disrupt the intestinal barrier and drive immune hyperactivation, while impaired Tregs compromises immune tolerance, exacerbating inflammation [46]. These findings clarify immune dysregulation in IBD and highlight therapeutic targets, such as modulating the Th17/Treg balance to restore immune homeostasis and reduce disease severity. These investigations highlight the role of dysregulated immunity and aberrant microbial metabolism in intestinal inflammation. Moreover, identifying specific immune cells and metabolic biomarkers paves the way for personalized treatments and predictive therapeutic models.

Research Gaps

Despite significant progress, key knowledge gaps remain, warranting further investigation. The interplay between the host immunity, gut microbiome, and environmental factors is not fully elucidated. While single-cell and metabolomic studies have identified novel biomarkers, their clinical utility requires validation in larger, diverse cohorts. Ongoing research is needed to translate these findings into actionable precision medicine strategies for IBD management.

As discussed, microbial dysbiosis in IBD is linked to altered metabolite profiles, including reduced butyrate production (e.g., *Faecalibacterium prausnitzii*, *Roseburia hominis*), impaired bile acid metabolism (decreased 7α-dehydroxylation), and diminished tryptophan-derived indole metabolites, weakening acyl hydrocarbon receptor activation and mucosal immunity. However, it is still unclear whether these metabolic shifts initiate or perpetuate chronic intestinal inflammation. Also, future studies should leverage the integrative power of multi-omics approaches to uncover these gaps and novel diagnostic and therapeutic targets for IBD.

Proposed Research Design

Study Design and Methodology

The pathophysiology of IBD is intricate and involves a combination of genetic, environmental, and immune-related factors. IBD is characterized by recurring and significant events, including dysfunction of the epithelial barrier and an imbalance between pro- and anti-inflammatory mediators secreted by immune cells or intestinal epithelial cells [47]. Due to the complex nature of IBD, it is challenging to develop disease models that can accurately cap the diversity of disease development and progression [48]. This demonstrates the necessity to explore the cellular and molecular mechanisms driving therapeutic responses in Crohn's disease and ulcerative colitis.

Experimental Approach

In order to identify the mechanisms and diversity of therapeutic responses in IBD patients we propose a prospective cohort study that integrates scRNA-seq and microbial metabolomics to identify and validate predictive biomarkers of therapeutic value in IBD patients. The study will track microbial and gene expression changes in patients before and after therapy initiation. This data will allow markers to be categorized as either diagnostic or prognostic and allows for further research into therapies tailored to the presence of these markers. The integration of scRNA-seq and microbial metabolomics to categorize IBD presentations in patients allows researchers to account for the relationship between environmental factors and immune response, two of the most important factors impacting disease progression and treatment.

By integrating scRNA-seq and metabolomic data, this study aims to identify a biomarker signature that predicts individual patient responses to therapy, providing a foundation for precision medicine in IBD treatment. This approach will need to be validated in larger multi-center trials to confirm its clinical utility.

The study will enroll patients diagnosed with either CD or UC. Inclusion criteria include individuals ages 18-65 with UC classified as moderate to severe by the Truelove and Witts Severity Index or moderate to severe CD as classified by the Crohn's Disease activity Index. Patients must be currently having an acute flare-up that is set to be maintained with Biologics and/or Immunomodulators once remission is achieved. Exclusion criteria will include patients with a history of intestinal surgical resection, recent antibiotic use, or concurrent use of therapeutics known to affect IBD progression including but not limited to Aminosalcylates, Steroids, Immunomodulators, and Biologics. Patients must also have no prior history of taking Immunomodulators or Biologics for the induction or maintenance of remission in CD or UC.

Patients will provide intestinal biopsy samples and stool samples before and during therapy. Therapy will follow current guidelines outlined by the American College of Gastroenterology. Biopsy samples will be immediately processed by scRNA-seq analysis and then preserved in appropriate media. Stool samples will immediately undergo metabolomic analysis and then be frozen for storage.

Pre-Induction Therapy: Intestinal biopsy samples will be obtained during endoscopic examination before management of the acute flare-up occurs. Stool samples will also be collected for microbial metabolomics analysis.

Pre-Maintenance Therapy: Once endoscopic and clinical remission is achieved Intestinal biopsy samples will be obtained during endoscopic examination before maintenance therapy occurs. Stool samples will also be collected for microbial metabolomics analysis.

Post-Therapy: Additional biopsy samples will be collected at Months 6 and 12 post-therapy initiation to monitor molecular and microbial changes over time. Stool Samples will be collected every 4 weeks.

Patients who develop a relapse in remission during the study period will continue in the study and follow the sampling criteria outlined in Pre-Induction Therapy. Once maintenance is achieved, the Biologic and/or Immunomodulator therapy will be changed and the effect will be compared to the original therapy.

Advanced Techniques

Advanced Techniques that will be used during the study include: High-Resolution scRNA-seq: We will use high-resolution scRNA-seq to profile gene expression in immune cells obtained from intestinal biopsies. The transcriptomic data will provide insights into cellular drivers of inflammation and potential treatment outcomes. The quality of scRNA-seq data will be increased by filtering low-quality cells and performing normalization.

Comprehensive Metabolomic Analysis: Stool samples will be used to analyze the gut-derived metabolites, including short-chain fatty acids (SCFAs) and bile acids, to understand their influence on immune modulation and therapeutic efficacy. These metabolites will be quantified to correlate their abundance with the patient's immune profile and therapeutic responses.

Clinical Outcomes and Measurements

Biomarker Evaluation

Biomarkers will be evaluated based on their ability to distinguish between responders and non-responders to specific IBD therapies. Markers will include a combination of specific immune cell gene expression profiles and metabolite levels. Utility of these markers will then be determined using receiver operating characteristic curves to assess the sensitivity and specificity of identified biomarkers in predicting response to therapy. Biomarker levels will be tracked long term and correlated with clinical outcomes to further validate their predictive utility.

Tracking Therapeutic Responses

Clinical response to therapy will be monitored using the Crohn's Disease Activity Index for Crohn's disease and the Truelove and Witts Severity Index for ulcerative colitis. Endoscopic evaluations will be performed at baseline and follow-up to assess mucosal healing. Changes in disease activity will be correlated with alterations in scRNA-seq-derived immune cell profiles and metabolomic changes. The effectiveness of therapy will be determined by improvements in clinical scores, endoscopic findings, and normalization of biomarker levels.

Gaps in Knowledge and Future Research Directions

Despite advancements in understanding the multifactorial nature of IBD, gaps remain in identifying predictive biomarkers for therapeutic response. Multi-omics approaches have shown potential in identifying molecular phenotypes associated with disease progression and treatment outcomes [49].

Comprehensive Studies on Biomarkers

While studies have identified various biomarkers, most research is limited by cross-sectional designs. This is not as useful for understanding dynamic changes in immune cell populations, microbial diversity, and metabolite profiles. There is a need for more longitudinal studies that track biomarkers over extended periods, both before and after therapy initiation if we desire to better predict therapeutic outcomes based on these markers. For example, other studies have found that fluctuations in the gut microbiome, particularly changes in the abundance of butyrate-producing bacteria, can predict treatment response [49]. However, to establish the clinical utility of these biomarkers, future studies must adopt a longitudinal approach to correlate these molecular shifts with long-term disease outcomes.

There also exists another limitation in the current studies. Most existing studies have been conducted in small, single-center settings, which limits the generalizability of findings due to demographic, genetic, and environmental differences in patient populations. Multicenter trials are needed to validate these markers across diverse populations, as genetic and microbial variations significantly influence treatment response in IBD patients [50]. By integrating data from patients of different ethnicities, we can improve the accuracy of biomarker-based models for predicting therapeutic outcomes [49]. Thus, multi-center collaboration is crucial for developing a universal biomarker panel applicable in various clinical settings.

While scRNA-seq and metabolomic profiling have been used to identify potential biomarkers, the mechanisms by which these markers predict and influence therapeutic responses remain poorly understood. The recent IBD literature is full of reports claiming that such biomarkers have been identified, but most reports do not include clear definitions of biomarkers, nor do they use strict criteria. To be reliable, any biomarker must meet strict standards for validation and reproducibility [51].

Mechanisms of Biomarker Effects

Recent studies employing scRNA-seq have identified specific immune cell subsets, such as IL-13Rα2+ inflammatory fibroblasts, that are enriched in patients resistant to anti-TNF therapy [50]. However, how these cellular profiles interact with metabolites in the gut environment to affect treatment outcomes is not yet fully understood. Metabolomics data have shown that specific metabolites, such as short-chain fatty acids (SCFAs) and bile acids, can influence immune cell function and the efficacy of biologic treatment. Therefore, future research should focus on integrating these multi-omics data to map the interactions between cellular states, metabolites, and therapeutic responses.

Identifying the pathways and biological processes driving biomarker changes is important for personalized treatment. Schierova et al, 2021 highlighted that gut microbiome composition can predict disease relapse after infliximab withdrawal, emphasizing the need to understand microbial-host interactions and their effects on the immune system. This is why further research should also investigate how specific gut microbial species contribute to therapeutic resistance or response. Further understanding these mechanisms will enhance our ability to develop targeted therapies and improve the precision of IBD treatment [52].

Multi-Center Trials

As briefly mentioned previously, validating the clinical utility of biomarkers across diverse patient populations is essential for translating research findings into practice. Conducting research across multiple centers allows for the inclusion of diverse patient populations, capturing variations in disease phenotypes, genetic backgrounds, and environmental exposures. A study combining Western and Asian cohorts found that microbial diversity and specific microbial signatures could predict response to anti-TNF therapy, exemplifying the importance of including varied populations in future research [50]. These multi-center studies also facilitate the development of standardized methodologies for sample collection and outcome measurement, allowing researchers to improve the reproducibility and clinical applicability of findings.

Future studies should employ a multi-omics approach, integrating scRNA-seq, metabolomics, and microbiome profiling to obtain a comprehensive understanding of disease progression and treatment responses. Collaborative efforts are needed to establish standardized protocols, including consistent sampling times, data analysis pipelines, and definitions of therapeutic response [53]. In addition, prospective interventional trials are necessary to compare biomarker-guided treatment strategies against standard care to determine the effectiveness of personalized medicine vs current guidelines in IBD management. However, the expectation that multi-omics will yield accurate diagnostic, prognostic and predictive biomarkers to help clinicians is likely not going to be fulfilled in the near future. Only carefully performed and reproducible multi-omics data in well-defined IBD patient cohorts will allow reliable grouping of patients based on molecular subgrouping. This is essential to clinically group UC or CD patient subgroups that can be individually targeted to promote personalized medicine [54].

By addressing these gaps and implementing multi-center, multi-omics research designs, future studies can pave the way for precision medicine in IBD, reducing treatment failures and improving patient outcomes through tailored therapeutic strategies.

Implications for Clinical Practice and Public Health

Due to the wide spectrum of genetic causes and environmental triggers involved in IBD, the exact pathogenesis of IBD remains unknown. For instance, the pathology of Crohn's disease has been classically studied by examining mucosal biopsies. However, single-cell techniques have provided unique perspectives in exploring these tissues on a cellular level. scRNA-seq can be used to identify novel cell types and compare healthy and disease-related tissues at a single-cell level [17]. For example, scRNA-seq identified the presence of specific surface biomarkers (ie, CD39+ and PD-1+) on CD8 T-cells associated with disease progression in Crohn's disease, while the limited presence or absence of CD39+ PD-1+ CD8 T-cells correlated with remission. Additionally, scRNA-seq has improved our understanding of the heterogeneous cellular changes involved in Crohn's disease, which would have been more difficult to appreciate from gross specimens. For instance, it was found that fibroblast-specific cell surface receptor markers (eg, Cadherin-11) may play a major role in the development of Crohn's disease fibrosis. Given that there are currently no specific anti-fibrotic therapies available for Crohn's-related fibrosis, single-cell sequencing can help determine key biomarkers that can be used as novel therapeutic targets, which serve as a personalized

treatment tailored to the individual patient [55]. Furthermore, the addition of microbial metabolomics allows us to pinpoint specific metabolites found in the gut microbiome, which provides an even more targeted treatment approach for complex autoimmune disorders such as IBD. However, the molecular signature of the gut microflora in IBD has yet to be discovered, as no reliable biomarker for IBD has yet to be found to guide diagnosis and treatment [56].

Although scRNA-seq is a powerful tool that allows for increased understanding of the role of individual cells and cell types, this technology still has limitations [57]. However, with additional time and increased data mining, there will be more clinical trials that will integrate scRNA-seq to effectively target and treat diseases [17]. Additionally, healthcare providers can be involved by conducting these precision medicine clinical trials and perusing publicly available multi-omics data along with atlas and clinical data [17, 55].

Conclusion

The integration of scRNA-seq and metabolomics represents a groundbreaking approach in understanding and treating IBD. This combination offers unprecedented insights into cellular mechanisms and metabolic pathways, revealing complex interactions between host immune responses and microbial metabolism. The integration enables identification of novel biomarkers, provides deeper understanding of disease heterogeneity, and allows for more accurate prediction of treatment responses. The synergy between these technologies has illuminated previously unknown cellular subsets and metabolic signatures specific to IBD, creating a more comprehensive picture of disease pathogenesis.

The advancement toward precision medicine in IBD treatment marks a pivotal shift from traditional one-size-fits-all approaches. By leveraging integrated multi-omics data, clinicians can potentially develop personalized treatment strategies based on individual patient profiles. This approach could significantly improve treatment outcomes by enabling early identification of optimal therapeutic strategies, reducing trial-and-error approaches in treatment selection, minimizing adverse effects through better-targeted interventions, improving cost-effectiveness of treatments, and enhancing patient quality of life through more effective disease management.

The integration of scRNA-seq and metabolomics has significant implications for both clinical practice and research. In clinical practice, this integration promises to enable more accurate diagnostic tools, implement personalized treatment protocols, better predict disease progression and treatment outcomes, and enhance monitoring of treatment effectiveness. From a research perspective, there remains a critical need for larger, multi-center validation studies, development of standardized protocols for data integration, investigation of novel therapeutic targets, and exploration of mechanistic relationships between cellular and metabolic markers.

To fully realize the potential of this integrated approach, several key actions are necessary. These include the establishment of large-scale, multicenter clinical trials to validate preliminary findings, development of standardized protocols for sample collection and analysis, and investment in technological infrastructure to support integrated data analysis. Additionally, healthcare providers must be trained in the interpretation and application of multi-omics data, while collaborative networks need to be established to share data and expertise. Implementation of cost-effective screening methods for routine clinical use will also be crucial.

The successful integration of scRNA-seq and metabolomics in IBD research represents a promising step toward precision medicine. However, continued research, validation, and implementation efforts are essential to translate these findings into improved patient outcomes. The field must now focus on bridging the gap between research findings and clinical application to revolutionize IBD treatment and management. This integrative approach holds immense promise for transforming our understanding and treatment of IBD, ultimately leading to better patient care and outcomes.

Conflict of Interest Statement

The authors of this manuscript declare that they have no conflicts of interest that are directly or indirectly related to the work submitted for publication. Specifically:

1. Financial Interests: None of the authors have received any financial compensation, funding, grants, or other monetary support that could be perceived as influencing the research, analysis, or conclusions presented in this work.
2. Professional Relationships: The authors have no employment, consultancy, board membership, or other professional relationships with organizations that could be perceived as influencing the work presented here.
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4. Personal Relationships: The authors have no personal relationships with individuals or organizations that could inappropriately influence or bias the work presented here.
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