ECCM 2024 Conference
The 2nd International Electronic Conference on Clinical Medicine
13–15 November 2024 | Online
MDPI

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

Suzie Al-Absi[1], Muhammad Hassan MD[2], Andrew Zarzar[1], Lily Marie Anton[1], Nina Huynh[1], Aparna Naik[3]
[1]A.T. Still University School of Osteopathic Medicine in Arizona
[2]Nuvance Health/Vassar Brothers Medical Center, Poughkeepsie, NY
[3]Noorda College of Osteopathic Medicine, Provo, Utah

INTRODUCTION & AIM

Inflammatory Bowel Disease (IBD) is a multifactorial chronic disease that involves repeated alternating cycles of active inflammation and remission, affecting different areas of the gastrointestinal tract. Current treatments aim to control inflammation and maintain remission, including anti-inflammatories, corticosteroids, immunomodulators, and biologics, but no curative therapy exists. One of the biggest challenges in IBD management is the unpredictability of response to treatment. Response variability necessitates a trial-and-error approach, often prolonging disease activity. This analysis explores combining single-cell RNA sequencing (scRNA-seq) and microbial metabolomics to identify biomarkers predictive of therapeutic response, providing insights into cellular inflammation drivers and immune modulation through gut metabolites. This integrated method offers a precise approach to predicting individual responses to therapies like TNF inhibitors and IL-23 blockers.

sc-RNA seq: is an innovative technique for studying RNA diversity at the cellular level, providing insights into cell functions and compositions in complex tissues and organisms. The scRNA workflow (figure 1) consists of three steps. Single Cell Isolation: Techniques such as fluorescence-activated cell sorting (FACS), microfluidic systems, and laser capture microdissection (LCM) are used to separate individual cells. Each methods has trade-offs in specificity, tissue integrity, and reagent usage. Library preparation: RNA transcripts are converted into complementary DNA (cDNA) and barcoded with unique molecular identifiers (UMIs) to improve accuracy and reduce errors. Sequencing: Various platforms, such as Smart-seq, SCRB-seq, and Drop-seq are used to analyze gene expression, offering different levels of efficiency and bias, based on experimental needs.

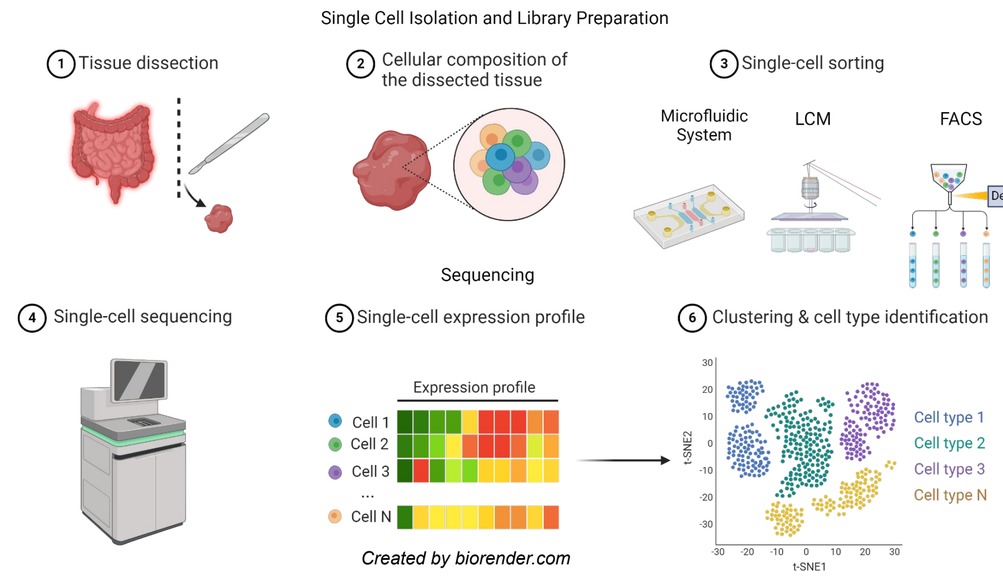

Figure 1: Illustrating scRNA workflow.
scRNA-seq allows for detailed gene expression analysis of immune cells, such as T cells and macrophages, within intestinal biopsies, providing insights into cellular drivers of inflammation and potential treatment outcomes. This technique paves the way for understanding the cellular interplay behind IBD by uncovering the genetic and metabolic characteristic of individual inflammatory cells involved in pathogenesis.

Microbial Metabolomics: analyzes metabolites to identify biomarkers for disease, prognosis, and treatment response. It uses two approaches: targeted (quantitative) and untargeted (qualitative). The process (figure 2) includes sample preparation, metabolite extraction, and analysis using techniques like nuclear magnetic resonance (NMR) and mass spectrometry (MS). NMR offers minimal sample prep but lower sensitivity, while MS provides high sensitivity and identifies a wide range of metabolites. Untargeted metabolomics involves liquid chromatography and MS, with results validated against databases, while targeted methods use standard compounds for precise metabolite quantification.analyzes gut-derived metabolites like short-chain fatty acids (SCFAs) and bile acids, which influence immune modulation and therapeutic efficacy.

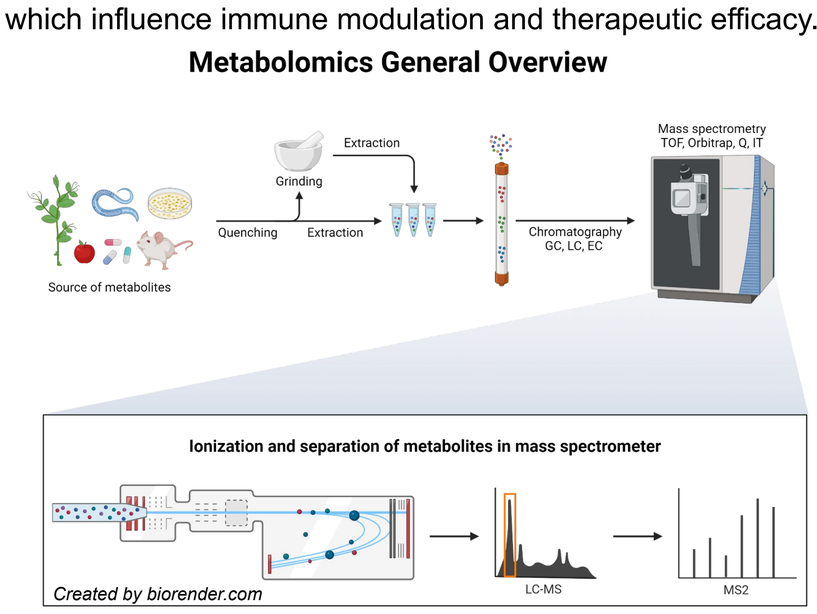

Figure 2: A general overview of the metabolomics workflow

RESULTS & DISCUSSION

Integration of scRNA-seq and Microbial Metabolomics: Integrating single-cell RNA sequencing (scRNA-seq) and microbial metabolomics data enhances our understanding of IBD pathogenesis by offering a holistic view of host-microbiome interactions. Single-cell transcriptomics reveals cell-type-specific gene expression changes, while microbial metabolomics identifies key metabolic shifts in the gut. This combined data can improve predictive models for patient treatment responses, aiding personalized strategies. Recent scRNA-seq studies have highlighted immune dysregulations in IBD, such as pro-inflammatory T cell expansion and altered myeloid cell states, identifying potential biomarkers for disease prediction and management. Advances in microbial metabolomics show imbalances in the production of short-chain fatty acids, bile acids, and other molecules in IBD patients, linked to their immunomodulatory effects and therapeutic implications.

Proposed Research Design: We propose a prospective cohort study integrating scRNA-seq and microbial metabolomics. This study aims to identify and validate predictive biomarkers by tracking microbial and gene expression changes in patients before and after therapy initiation. We will enroll patients aged 18–65 diagnosed with moderate to severe CD or UC, classified by the Crohn's Disease Activity Index and the Truelove and Witts Severity Index, respectively. Inclusion criteria include individuals experiencing an acute flare-up slated for maintenance with biologics and/or immunomodulators upon achieving remission. Exclusion criteria encompass prior intestinal surgical resection, recent antibiotic use, concurrent use of IBD-affecting therapeutics, and any previous use of immunomodulators or biologics for induction or maintenance of remission.

Participants will provide intestinal biopsy and stool samples at specific intervals: before induction therapy, before maintenance therapy (post-remission), and at 6 and 12 months after therapy initiation. Stool samples will also be collected every four weeks. Biopsy samples will undergo scRNA-seq to profile gene expression in immune cells, while stool samples will be analyzed for gut-derived metabolites.Biomarkers will be assessed based on their ability to differentiate responders from non-responders, utilizing receiver operating characteristic curves to evaluate sensitivity and specificity. Longitudinal tracking of biomarker levels will be correlated with clinical outcomes to validate their predictive utility. Clinical responses will be monitored using standardized disease activity indices and endoscopic evaluations to assess mucosal healing. Changes in disease activity will be correlated with alterations in immune cell profiles derived from scRNA-seq data and metabolomic changes.

The effectiveness of therapy will be determined by improvements in clinical scores, endoscopic findings, and normalization of biomarker levels. This integrated approach aims to identify a biomarker signature that predicts individual patient responses to therapy, providing a foundation for personalized IBD treatment strategies.

Current Limitations and Prospects for Future Study: Despite advancements, gaps remain in identifying predictive biomarkers for therapeutic responses in IBD. Most studies are limited by cross-sectional designs and small, single-center cohorts, hindering the understanding of dynamic changes in immune cell populations and metabolite profiles. In order to enhance prediction accuracy, we need longitudinal, multicenter studies that can track biomarkers over time and across diverse populations.

While multi-omics approaches like scRNA-seq and metabolomics have identified potential biomarkers, their mechanisms in predicting and influencing therapeutic responses are not fully understood. Integrating their data can help better map interactions between cellular states and treatment outcomes. Also, by creating standard methods and using strict criteria to validate biomarkers, researchers can provide reliable, reproducible results. Future research should focus on collaborative, multi-center, multi-omics studies to address these limitations. Such efforts will allow for precision medicine in IBD that improves patient outcomes.

CONCLUSION

Given the unpredictable therapeutic responses in patients with CD and UC, treatment for IBD remains a significant challenge. However, two innovative techniques have recently emerged that could provide a breakthrough in understanding the complex pathogenesis of IBD. The integration of scRNA-seq and microbial metabolomics can help identify specific biomarkers and gut metabolites to predict disease activity, monitor treatment response, and guide development of novel, targeted therapies for IBD.

FUTURE WORK / REFERENCES

Future research directions could expand on the integration of scRNA and microbial metabolomics for IBD such as biomarker refinement for specific IBD subtypes. With this approach, researchers can finely parse through the differences of UC and CD and accurately tailor treatment strategies within each disease type.