

Single-Cell Multiomics of Macrophage Polarization in Inflammatory Microenvironments

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Abstract.

Macrophages are highly plastic innate immune cells that adapt their functional phenotypes in response to environmental cues, a process traditionally conceptualized as polarization. However, the binary classification of classically activated and alternatively activated macrophages fails to capture the immense phenotypic diversity observed in vivo, particularly within complex inflammatory microenvironments. Recent advances in single-cell multiomics technologies have revolutionized our understanding of macrophage heterogeneity by enabling the simultaneous profiling of the transcriptome, epigenome, and surface proteome at a single-cell resolution. This paper provides a comprehensive overview of how single-cell multiomics has redefined macrophage polarization from a distinct set of binary states to a multi-dimensional continuum of activation trajectories. We explore the limitations of conventional bulk sequencing approaches and discuss the fundamental principles of multiomic integration. Furthermore, we examine the computational frameworks required to delineate complex cellular trajectories and assess the regulatory gene networks governing macrophage plasticity in various pathological contexts, including the tumor microenvironment and chronic autoimmune diseases. By dissecting the epigenetic priming and transcriptomic execution of macrophage activation, this paper highlights the pivotal role of single-cell multiomics in identifying novel therapeutic targets for immune modulation. Ultimately, these high-resolution analytical frameworks pave the way for precision medicine strategies aimed at reprogramming dysregulated macrophage populations in severe inflammatory disorders.

Keywords:

Macrophage Polarization, Single-Cell Multiomics, Transcriptomics, Inflammatory Microenvironment.

1. INTRODUCTION

1.1 The Role of Macrophages in Inflammation

Macrophages are essential components of the innate immune system, distributed throughout virtually all tissues in the human body. They originate either from embryonic yolk sac progenitors, establishing tissue-resident populations early in development, or from bone marrow-derived monocytes that infiltrate tissues during active inflammation [1]. These versatile cells are responsible for a wide array of physiological functions, ranging from the phagocytosis of cellular debris and invading pathogens to the orchestration of tissue repair and the maintenance of systemic homeostasis. In the context of an inflammatory microenvironment, macrophages exhibit

remarkable functional plasticity, constantly sensing and responding to an intricate network of local biochemical signals. The precise nature of this response is heavily dictated by the specific combination of cytokines, chemokines, microbial products, and metabolic byproducts present in the tissue niche [2]. Historically, the scientific community categorized this phenotypic adaptation into a simplified dichotomy. This classification framework separated macrophages into classically activated phenotypes, which are predominantly pro-inflammatory and microbicidal, and alternatively activated phenotypes, which are generally associated with anti-inflammatory responses, immune resolution, and tissue remodeling. However, this rigid categorization has proven increasingly inadequate for describing the vast array of intermediate and hybrid activation states that macrophages adopt when navigating the dynamic and highly complex conditions of human disease.

1.2 Limitations of Bulk Sequencing Approaches

For decades, the investigation of macrophage biology relied heavily on bulk molecular techniques, such as bulk RNA sequencing and traditional flow cytometry. While these methods provided foundational insights into the transcriptomic signatures and surface marker expression profiles associated with distinct macrophage populations, they possessed inherent limitations [3]. Bulk sequencing approaches measure the average expression of genes across thousands or millions of cells within a tissue homogenate. Consequently, they mask the underlying cellular heterogeneity and obscure the presence of rare, yet biologically significant, macrophage subpopulations. In complex tissues characterized by extensive cellular diversity, the average expression profile generated by bulk sequencing rarely represents the true biological state of any individual cell. Furthermore, bulk analyses fail to capture the asynchronous transitions between different activation states, making it nearly impossible to map the continuous trajectories of macrophage polarization during disease progression. To overcome these fundamental limitations, the scientific community has increasingly turned to single-cell multiomics [4]. By interrogating the molecular characteristics of individual cells across multiple biological modalities simultaneously, researchers can now construct high-resolution maps of the cellular landscape. This approach not only uncovers the spatial and temporal dimensions of macrophage activation but also elucidates the complex regulatory networks that govern cellular decision-making within dense inflammatory microenvironments [5].

2. Background and Literature Review

2.1 Macrophage Polarization Paradigms

The concept of macrophage polarization was initially established through in vitro experiments using defined cytokine stimulation. In these highly controlled environments, stimulation with interferon-gamma and lipopolysaccharide induced a classically activated state, characterized by the robust production of pro-inflammatory cytokines such as tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-6, as well as the generation of reactive oxygen and nitrogen species [6]. This phenotype is crucial for host defense against intracellular pathogens and for driving early inflammatory responses. Conversely, stimulation with interleukin-4 or interleukin-13 induced an alternatively activated state, marked by the upregulation of scavenger receptors, arginase-1, and anti-inflammatory cytokines like interleukin-10 [7]. This phenotype is primarily involved in parasite clearance, allergy, immune tolerance, and wound healing. While this binary framework provided a useful conceptual model, emerging evidence from in vivo studies quickly revealed that macrophages rarely exist in these extreme, polarized states. Instead, the local microenvironment exposes macrophages to a complex and often conflicting milieu of signals, leading to the generation of a multidimensional spectrum of activation states. Single-cell RNA

sequencing has been instrumental in dismantling the rigid boundaries of the traditional polarization paradigm, demonstrating that individual macrophages can co-express markers of both classical and alternative activation simultaneously [8].

2.2 Emergence of Single-Cell Multiomics

The advent of single-cell technologies represents a watershed moment in the field of immunology. Initial breakthroughs focused primarily on single-cell transcriptomics, which enabled researchers to quantify messenger RNA expression at the single-cell level, thereby cataloging the vast diversity of immune cell types and states [9]. However, transcriptomics alone provides a restricted view of cellular function, as it does not account for the regulatory mechanisms occurring at the level of chromatin architecture or the post-transcriptional modifications that dictate ultimate protein expression. To address this, the field rapidly evolved to incorporate additional modalities. Single-cell assay for transposase-accessible chromatin with sequencing allows for the genome-wide profiling of open chromatin regions, providing critical insights into the epigenetic landscape and identifying the specific transcription factor binding motifs that prime macrophages for future activation [10]. Furthermore, cellular indexing of transcriptomes and epitopes by sequencing bridges the gap between transcriptomics and proteomics by utilizing oligonucleotide-conjugated antibodies to quantify the expression of dozens to hundreds of surface proteins alongside the transcriptome of the same cell [11]. The true power of these technologies is realized through multiomic integration, where data from multiple modalities are captured either simultaneously from the exact same cell or computationally aligned from parallel experiments. This integrated approach offers a holistic view of the molecular cascade that dictates macrophage polarization.

2.3 Key Drivers of Inflammatory Microenvironments

The polarization of macrophages is intricately linked to the specific context of the inflammatory microenvironment, which varies drastically depending on the underlying pathology. In chronic autoimmune diseases such as rheumatoid arthritis, the synovial microenvironment is characterized by persistent hypoxia, an abundance of damage-associated molecular patterns, and high concentrations of pro-inflammatory cytokines. In this context, macrophages often adopt a hyper-inflammatory state driven by the sustained activation of nuclear factor kappa-light-chain-enhancer of activated B cells and signal transducer and activator of transcription 1 signaling pathways [12]. These macrophages drive the recruitment of autoreactive lymphocytes and contribute directly to cartilage and bone destruction. Conversely, the tumor microenvironment presents a profoundly immunosuppressive niche. Tumors secrete an array of factors, including transforming growth factor-beta, interleukin-10, and vascular endothelial growth factor, which actively subvert macrophage function. Tumor-associated macrophages are frequently coerced into a pro-tumorigenic state, characterized by the suppression of antigen presentation, the promotion of angiogenesis, and the direct support of tumor cell proliferation and metastasis. The specific metabolic alterations within these microenvironments, such as local depletion of glucose and accumulation of lactate, also serve as potent drivers of macrophage polarization, further emphasizing the necessity of comprehensive multiomic profiling to untangle these complex interactions.

3. Methodology and Analytical Frameworks

3.1 Single-Cell RNA Sequencing Protocols

The foundational methodology of single-cell multiomics involves the precise isolation and processing of individual cells from complex solid tissues. The first and often most critical step is the dissociation of the target tissue into a highly viable single-cell suspension. This process requires a delicate balance of enzymatic digestion, utilizing proteases such as collagenase and

disperse, combined with mechanical agitation [13]. Over-digestion can lead to cell death and the release of ambient RNA, while under-digestion results in the loss of tightly adherent tissue-resident macrophage populations. Once a viable single-cell suspension is achieved, the cells must be partitioned to allow for single-cell resolution. The most prevalent method for achieving this is microfluidic droplet-based partitioning, where individual cells are encapsulated into nanoliter-sized aqueous droplets along with a uniquely barcoded gel bead. Within each droplet, the cells are lysed, and the released messenger RNA is captured by the barcoded primers on the bead. Following capture, reverse transcription generates complementary DNA that carries a cell-specific barcode and a unique molecular identifier, which is crucial for distinguishing genuine biological transcripts from amplification artifacts generated during subsequent polymerase chain reaction steps. Alternatively, plate-based sorting methods utilizing flow cytometry can be employed, offering the advantage of pre-selecting specific macrophage populations based on known surface markers, albeit typically at a lower throughput than droplet-based systems [14].

Table 1: Comparison of Single-Cell Sequencing Modalities

Modality Type	Primary Biological Target	Key Information Provided
scRNA-seq	Messenger RNA	Gene expression levels and cell clustering
scATAC-seq	Chromatin Accessibility	Epigenetic state and transcription factor binding
scCITE-seq	Surface Proteins	Phenotypic markers and post-transcriptional state

3.2 Epigenomic and Proteomic Integration

To move beyond pure transcriptomics, researchers employ advanced protocols to capture paired epigenomic and proteomic data. Single-cell epigenomic profiling heavily utilizes the assay for transposase-accessible chromatin. In this protocol, isolated nuclei are treated with a hyperactive Tn5 transposase enzyme. This enzyme selectively inserts sequencing adapters into regions of the genome where the chromatin is open and accessible, which typically correspond to active promoters and enhancers. By sequencing these specific regions, researchers can infer the regulatory landscape of the macrophage and identify which transcription factors are currently driving its functional state. The integration of surface protein expression is achieved through the use of oligonucleotide-barcoded antibodies. Prior to droplet encapsulation, the single-cell suspension is incubated with a highly multiplexed panel of these specialized antibodies, which bind to their respective target proteins on the macrophage cell surface. During the droplet library preparation process, the oligonucleotide barcodes attached to the antibodies are captured and sequenced alongside the cellular messenger RNA. This powerful combination allows researchers to validate transcriptomic findings at the protein level and distinguish between macrophage subpopulations that may exhibit similar gene expression profiles but divergent surface protein phenotypes, a common occurrence in rapidly changing inflammatory microenvironments [15].

3.3 Computational Analysis and Data Integration

The raw sequencing data generated from single-cell multiomic experiments consists of millions of short reads that require extensive computational processing. The initial steps involve aligning the reads to a reference genome, counting the unique molecular identifiers for each gene in each cell, and generating a massive, highly sparse gene expression matrix. Quality control is paramount at this stage to remove low-quality cells, which are often identified by an abnormally high percentage of mitochondrial gene expression indicative of a ruptured cell membrane, or an unusually low number of detected genes. Furthermore, computational algorithms must be employed to identify

and remove multiplets, which occur when two or more cells are inadvertently encapsulated in the same droplet, creating a hybrid expression profile that does not exist in reality. To deal with ambient RNA contamination from lysed cells, specialized background subtraction algorithms are utilized. Once the data matrix is cleaned, dimensionality reduction techniques are essential for visualization and downstream analysis [16]. Because the dataset contains thousands of dimensions representing individual genes, algorithms such as Principal Component Analysis are first used to identify the axes of greatest variance. Subsequently, non-linear dimensionality reduction techniques like Uniform Manifold Approximation and Projection are employed to embed the data into a two-dimensional or three-dimensional space, visually grouping cells with similar multiomic profiles. Following dimensionality reduction, unsupervised clustering algorithms, most notably graph-based methods like the Louvain or Leiden algorithms, are applied to objectively partition the cells into distinct phenotypic clusters [17]. To understand the dynamic transitions between these clusters, researchers utilize trajectory inference algorithms. These computational tools assume that cells exhibiting similar expression profiles represent continuous stages of a biological process. By connecting cells in the high-dimensional space, trajectory inference constructs a pseudotime axis that models the sequential activation of macrophages from a resting state to a fully polarized phenotype. Spatial transcriptomics adds another layer of complexity by mapping the precise physical location of these sequenced macrophages back onto the original tissue architecture, allowing researchers to correlate specific macrophage activation states with their proximity to other cell types, such as invading tumor cells or localized areas of tissue necrosis [18].

Figure 1: Multiomic Integration Workflow

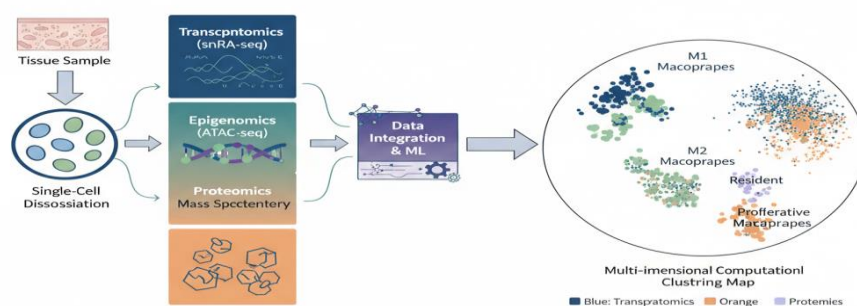


Figure 1: Multiomic Integration Workflow

4. Results and Discussion of Current Applications

4.1 Tissue-Specific Macrophage Heterogeneity

The application of single-cell multiomics has unequivocally demonstrated that macrophage heterogeneity is vastly more profound than previously anticipated, with distinct tissue microenvironments imposing highly specific transcriptional and epigenetic signatures. By profiling healthy and inflamed tissues across multiple organs, researchers have identified core macrophage gene expression programs that are highly conserved, alongside specialized modules that are entirely context-dependent. For instance, microglia, the resident macrophages of the

central nervous system, exhibit a highly specialized molecular profile governed by transcription factors such as Sal-like protein 1 and mothers against decapentaplegic homolog 3. In the context of neuroinflammation, single-cell analysis has revealed the emergence of disease-associated microglia, a subpopulation characterized by the downregulation of homeostatic markers and the upregulation of genes involved in lipid metabolism and phagocytosis [19]. Similarly, in the liver, Kupffer cells play a critical role in clearing blood-borne pathogens and maintaining immune tolerance. Multiomic profiling of liver tissue during acute injury reveals a rapid influx of monocyte-derived macrophages that temporarily adopt a highly inflammatory phenotype to clear necrotic debris, before gradually transitioning into a restorative phenotype that closely mimics resident Kupffer cells. This phenomenon underscores the extreme plasticity of the macrophage lineage and highlights the profound influence of the local tissue stroma in shaping cellular identity and function. The tumor microenvironment serves as arguably the most compelling example of microenvironment-driven polarization. Tumors are highly heterogeneous ecosystems where macrophages are continually reprogrammed by a barrage of tumor-derived signals [20].

Table 2: Key Macrophage Subpopulations and Associated Multiomic Signatures

Subpopulation	Common Inflammatory Context	Defining Transcriptomic and Proteomic Markers
Disease-Associated Microglia	Neurodegenerative Disorders	Upregulation of lipid metabolism genes and specific surface receptors
Lipid-Associated Macrophages	Obesity and Atherosclerosis	High expression of lipid-scavenging receptors and metabolic regulators
Tumor-Associated Macrophages	Solid Malignancies	Expression of immune-suppressive cytokines and pro-angiogenic factors

4.2 Dynamic Trajectories in Disease Models

Beyond merely cataloging discrete cell types, the integration of epigenomic and transcriptomic data has provided unprecedented insights into the temporal dynamics of macrophage activation in chronic disease models. A prime example is the study of atherosclerosis, a chronic inflammatory disease of the arterial wall driven by the accumulation of lipid-laden macrophages known as foam cells. Traditional models suggested a simple linear progression from a circulating monocyte to an inflammatory macrophage and finally to a foam cell. However, single-cell multiomics has revealed a highly branched and complex trajectory [21]. Within the atherosclerotic plaque, researchers have identified a spectrum of macrophage states, including pro-inflammatory subsets that actively drive plaque destabilization, alongside highly specialized lipid-associated macrophages that attempt to sequester excess cholesterol and dampen localized inflammation. Crucially, single-cell epigenomic data has highlighted the concept of epigenetic priming. Analysis of chromatin accessibility has shown that circulating monocytes in patients with systemic inflammatory diseases already possess open chromatin regions at the promoters of key inflammatory genes, long before these genes are actively transcribed. This epigenetic memory, often established in the bone marrow in response to systemic cytokines or microbial products, pre-disposes these monocytes to adopt a hyper-inflammatory phenotype upon entry into the tissue [22]. Understanding these dynamic trajectories and the underlying epigenetic alterations is vital for deciphering why certain inflammatory responses resolve naturally while others progress to chronic, tissue-destructive diseases.

5. Conclusion and Future Directions

5.1 Summary of Key Findings

The integration of single-cell multiomics into immunological research has systematically dismantled the classical binary models of macrophage polarization, replacing them with a highly nuanced, multidimensional understanding of cellular plasticity. By simultaneously analyzing the transcriptomic output, the epigenomic regulatory landscape, and the surface proteomic phenotype of individual cells, researchers have mapped the intricate and highly context-dependent trajectories of macrophage activation within complex inflammatory microenvironments. These advanced analytical frameworks have identified previously unrecognized macrophage subpopulations, such as disease-associated microglia and specialized lipid-associated macrophages, whose distinct molecular signatures are intimately tied to the specific pathology of their localized tissue niche. Furthermore, the combination of spatial transcriptomics and trajectory inference algorithms has illuminated the physical and temporal dynamics of immune responses, demonstrating how localized cell-cell interactions and epigenetic priming events dictate the functional fate of infiltrating monocytes. The massive datasets generated by these technologies underscore the fact that macrophage identity is not a fixed state but rather a continuous adaptation to a constantly fluctuating biochemical environment.

5.2 Translation to Clinical Practice

The detailed molecular maps generated by single-cell multiomics hold immense potential for the future of precision medicine and targeted immunotherapy. By identifying the specific transcription factors and regulatory networks that lock macrophages into pathological states—such as the immunosuppressive tumor-associated macrophages in cancer or the hyper-inflammatory subsets in autoimmune disorders—researchers can pinpoint highly specific vulnerabilities for therapeutic intervention. Current efforts are intensely focused on developing pharmacological agents or utilizing advanced gene-editing techniques to reprogram these dysregulated macrophage populations directly within the patient. For example, strategies aimed at altering the epigenetic landscape of tumor-associated macrophages to restore their inherent phagocytic and antigen-presenting capabilities represent a highly promising frontier in cancer immunotherapy [23]. As spatial multiomics and computational modeling continue to evolve, the ability to predict how individual patients will respond to immune-modulating therapies based on the specific multiomic profile of their tissue macrophages will become increasingly feasible. Ultimately, the transition from high-resolution multiomic discovery to the clinical application of targeted macrophage reprogramming will mark a transformative leap forward in the treatment of complex, refractory inflammatory diseases.

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