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· Review ·

Application of single-cell sequencing in autoimmune uveitis: a comprehensive review

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HIGHLIGHTS

- Immune cells exhibit diverse functions, acting as both pro-inflammatory and anti-inflammatory agents in the context of autoimmune uveitis. Recent advancements in single-cell research are reshaping and broadening our understanding of their classifications and roles.
- The integration of scRNA-seq with other omics technologies (e.g., proteomics and epigenomics) has advanced understanding of cellular states and regulatory mechanisms at the single-cell level, offering a comprehensive view of cellular interactions and molecular dynamics.
- This emerging technology promises to map the spatial organization of gene expression within tissues, enhancing our understanding of cellular environments and interactions in autoimmune uveitis.

Abstract: Autoimmune uveitis is one of the most common inflammatory eye diseases leading to blindness globally. Its etiology is primarily associated with autoimmune responses. Patients with this condition often exhibit complex and chronic disease courses, with a high propensity for recurrence. Current treatments mainly involve corticosteroids and immunosuppressive agents, which, despite their effectiveness, entail significant side effects that severely impact patients' vision and quality of life. There are still unresolved questions regarding the etiology and immunopathogenesis of autoimmune uveitis, and traditional high-throughput sequencing techniques fall short of adequately elucidating

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Full Text

its pathogenic mechanisms at the cellular level. With the continuous advancement of single-cell sequencing technology, an increasing number of studies are leveraging this approach to deeply investigate the pathogenesis of autoimmune uveitis, thereby offering new insights for identifying novel diagnostic and therapeutic targets. This paper reviews the latest applications of single-cell sequencing technology in exploring the pathogenesis of autoimmune uveitis. Through the utilization of this technology, researchers can gain a more comprehensive understanding of cellular-level changes in patients, providing robust support for the search for new therapeutic avenues. These studies offer new directions for the diagnosis and treatment of autoimmune uveitis and provide valuable information for the development of future therapeutic strategies and approaches.

Keywords: Single-cell sequencing; Autoimmune uveitis; ScRNA-seq; Uveitis; Inflammatory eye diseases

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INTRODUCTION

Autoimmune uveitis (AU) is a prevalent blinding ocular disease triggered by immune system dysregulation.[1] Its etiology is intricate and multifaceted, with a broad spectrum of clinical presentations often accompanied by systemic symptoms. The disease course is typically chronic with a high propensity for recurrence, leading to substantial visual impairment. The pathogenesis of autoimmune uveitis remains inadequately understood, and current therapeutic approaches predominantly involve prolonged administration of high-dose corticosteroids and immunosuppressants.[2] These treatments, however, are associated with significant systemic complications, including hyperglycemia, obesity, and osteoporosis, thereby substantially diminishing patient quality of life.[3] Consequently, there is an urgent need to elucidate novel pathogenic mechanisms and develop innovative treatment strategies to mitigate complications and reduce the risk of therapeutic failure, marking a pivotal direction in the clinical management of autoimmune uveitis.

Recent investigations into the immunopathogenesis of autoimmune uveitis have primarily employed traditional high-throughput sequencing methodologies, including transcriptomics, proteomics, and epigenomics, to extensively examine its underlying mechanisms. Previous studies have identified immune cells such as Th1 and Th17 as critical contributors to the pathogenesis of uveitis.[4-12] However, these studies have been limited by biases and the utilization of bulk cell populations for sequencing analysis, often neglecting cellular heterogeneity within individuals. Such approaches predominantly reflect the characteristics of the most abundant cell types or the average cell population, thereby overlooking specific gene or protein expressions in distinct cell states or subtypes. Consequently, current findings fall short in fully elucidating the pathogenesis of autoimmune uveitis.

Advancements in sequencing technology now enable the analysis of the transcriptome, genome, epigenome, and proteome at the single-cell level, facilitating precise differentiation of cell types and subtypes. This technological progress represents the most effective approach for investigating cellular heterogeneity and cell lineage tracing in complex biological systems.

Single-cell multi-omics sequencing offers profound insights into the genotype-phenotype relationship at the cellular level, allowing exploration of gene regulatory mechanisms, protein expression dynamics, and epigenetic variations.[13] Increasingly, researchers are leveraging single-cell multi-omics sequencing to probe the mechanisms driving autoimmune uveitis, thereby identifying novel diagnostic and therapeutic targets.

This review systematically examines the recent applications of single-cell multi-omics sequencing in elucidating the mechanisms of autoimmune uveitis, summarizing key findings related to marker genes and potential therapeutic targets reported in the literature. These advancements provide crucial insights for the clinical diagnosis and treatment of autoimmune uveitis, paving the way for future therapeutic strategies and improved patient outcomes.

SINGLE-CELL SEQUENCING TECHNOLOGIES: FROM TRANSCRIPTOMICS DISCOVERY TO MULTI-OMICS INSIGHTS IN DISEASE PATHOGENESIS

Single-cell sequencing (SCS), including genomics, transcriptomics, and epigenetic sequencing, have revealed the genetic architecture and gene expression states of individual cells. These technologies highlight the heterogeneity of genetic material and protein information within cells, offering insights into their various stages, functions, and characteristics. Since the advent of the first single-cell RNA sequencing (scRNA-seq) in 2009, the field of single-cell research has experienced rapid development.[14] SCS has found extensive application in immunological research. SCS is used for the effective diagnosis of autoimmune uveitis, assessing the molecular susceptibility of specific cells, and identifying potential therapeutic targets. Recent studies have used scRNA-seq to identify distinct immune cell subsets and their roles in the disease's pathogenesis.[15-19]

Beyond scRNA-seq, Single-cell sequencing assay for transposase-accessible chromatin (scATAC-seq) provides a detailed view of chromatin accessibility, helping to elucidate the regulatory elements that drive gene expression changes in diseases.[20-21] This technique has been instrumental in identifying key transcription factors and regulatory networks involved in immune cell activation and inflammation.

Integrative approaches combining scRNA-seq with other omics technologies, such as epigenomics and proteomics, offer a comprehensive understanding of cellular states and regulatory mechanisms.[22-24] These multi-omics approaches are particularly valuable for studying the complex interactions between different molecular layers in autoimmune uveitis.

IDENTIFYING PATHOGENIC IMMUNE CELL SUBSETS

CD4+ T Cell

T cells are believed to play a central role in the pathogenesis of human uveitis.[4] Retinal antigen-specific T cells that escape elimination in the thymus encounter activating stimuli and costimulatory "danger" signals, evade Treg control, and differentiate into pathogenic effector T cells.[4,25] These cells undergo clonal expansion, migrate to the eye, break down the blood-retinal barrier, and recruit inflammatory leukocytes from the circulation.[4,25] The inflammation damages the tissue and releases ocular antigens. However, the specific T-cell subtypes involved remain unclear.

SCS has been pivotal in delineating the diverse

cell populations involved in AU. In 2019, Heng et al.[26] published a pioneering study utilizing scRNA-seq to characterize the cellular and immune landscapes within the retinas of mice with experimental autoimmune uveitis (EAU). Using a spontaneous EAU model, they identified Th1 cells as the primary drivers of immune inflammation. Additionally, they observed various retinal cells exhibiting responses to interferon-gamma (IFN-γ). Moreover, single-cell TCR sequencing of CD4+ T cells specific for a predominant interphotoreceptor retinoid-binding protein (IRBP) epitope in spontaneous EAU model revealed a remarkable diversity of autoantigen-specific TCRs, with greater clonal expansions in diseased mice.[27] This highlights the complexity of the immune response in autoimmune uveitis and underscores the utility of single-cell technologies in identifying pathogenic immune cell subsets and potential therapeutic targets.

In another study, the mechanism of gingival mesenchymal stem cells (GMSCs) in treating EAU was explored using scRNA-seq. The treatment reduced the generation of $CCR6^{-}CCR2^{+}$ phenotype Th17 cells and increased the generation of $CCR6^{+}CCR2^{+}$ phenotype Th17 cells, which secrete IL-10 for immunoregulation in the lymph nodes of treated EAU mice.[28] A single-cell study reported that aging Th17 cells weaken pathogenicity and ameliorate experimental autoimmune uveitis in mice.[29] These studies found that Th17 cells play both positive and negative roles in regulating antitumor immune responses.

Monocytes

Accumulating evidence has also highlighted the role of monocytes in AU progression. In Vogt-Koyanagi-Harada (VKH) patients, elevated levels of $ISG15^{+}$ proinflammatory monocyte subpopulations have been observed.[30] This subgroup displayed a distinctive gene expression profile indicative of inflammation, antiviral activity, and pathological activation, suggesting their involvement in active inflammation during VKH disease. Further supporting this, Zheng et al.[31] used scRNA-seq to analyze peripheral blood mononuclear cells (PBMCs) from Behçet's disease (BD) patients, revealing significant expansion and transcriptional changes in monocytes. Detailed analysis identified a notable accumulation of C1q-high monocytes in BD patients, driven by activated IFN-γ signaling. Notably, treatment with tofacitinib, a Janus kinase inhibitor, effectively reduced C1q-high monocytes, suggesting a promising therapeutic approach.

Neutrophil

Recent studies employing scRNA-seq have also uncovered sex-specific heterogeneity in neutrophil subsets and their role in the male-biased susceptibility to BD.[32] These findings suggest that targeting unconventional neutrophil subsets could offer novel, sex-specific therapeutic strategies to mitigate the progression of inflammatory diseases.

CD8+ T cell

Further utilizing scRNA-seq and single-cell TCR sequencing (scTCR-seq), researchers have identified clonal transitions between skin-resident and circulating $CD8^{+}$ T cells in skin samples from BD patients, potentially linked to inflammation recurrence.[33] Additionally, current research has focused on the pathogenic role of intraocular $CD8^{+}$ T cells in human AU.[34]

Microglia

Microglia, the resident immune cells of the central nervous system, including the retina, are

crucial for maintaining the homeostasis of the retinal microenvironment.[35] Microglia in AU can help leukocytes cross the blood-retinal barrier.[36] However, their exact role in the pathogenesis of AU remains uncertain.SCS study targeted CD74 and CCL5 in retinal microglia, which could offer new strategies for treating AU by providing insights into the cellular dynamics and mechanisms of immune responses, highlighting potential therapeutic targets to mitigate disease progression and retinal damage.[37]

Dendritic Cell

Kasper et al.[38] first reported a single-cell intraocular immunological atlas of patients with HLA-B27-associated acute anterior uveitis, highlighting the infiltration of dendritic cells (DC). Building on this, Hiddingh et al. [39] further characterized the immunological features of $CX3CR1^{+}CD1C^{+}$ DCs in autoimmune uveitis patients in 2023, noting their secretion of high levels of inflammatory cytokines. Other study also reported that DCs might be related to pro-inflammatory states in VKH patients.[40]

Macrophages

Finally, research highlights the significant role of ocular macrophages in uveitis through the production of CCL2 and CXCL10, which are crucial for recruiting inflammatory cells to the eye.[41] Understanding these chemokine-mediated mechanisms of immune cell recruitment offers potential targets for novel therapeutic strategies to manage uveitis more effectively.

In summary, $CD4^{+}$ T cells, particularly Th1 and Th17 subsets, are key drivers of inflammation in AU, with Th1 cells predominating in experimental models and Th17 cells modulating immune responses. Monocytes, especially pro-inflammatory subpopulations, are involved in diseases like VKH and BD, while neutrophils exhibit sex-specific roles affecting disease susceptibility. $CD8^{+}$ T cells contribute to inflammation recurrence, and microglia and dendritic cells offer potential therapeutic targets due to their roles in immune response and cytokine production. Macrophages also play a significant role by producing chemokines that recruit inflammatory cells to the eye. These findings highlight the complex interplay of immune cells in AU and identify key areas for potential therapeutic intervention.

UNCOVERING MOLECULAR PATHWAYS AND TARGETS

SCS has revealed novel molecular pathways and potential therapeutic targets in AU. Utilizing scRNA-seq and scATAC-seq, researchers have identified specific cytokines and signaling pathways that could be leveraged for targeted interventions.

Id2/Pim1 axis

For instance, Li et al. [42] using the EAU mice model and scRNA-seq, discovered that PIM1 might inhibit the pathogenicity of Th17 cells by regulating the protein kinase B-FOXO1 pathway, suggesting PIM1 as a potential therapeutic target. Additionally, Li et al.[29] found immunological differences between aged and young EAU mice, indicating that aging might reduce the pathogenicity of Th17 cells, thus enhancing the understanding of Th17 cell immunological roles across different ages in EAU mice. Progesterone has also shown potential in modulating pathological processes related to inflammatory cell migration, activation, and differentiation in the EAU model.[43] It was found to regulate the Th17/Treg imbalance by enhancing the regulatory mediators of Tregs and reducing the

overactivation of pathological Th17 cells. Additionally, progesterone treatment reversed the Id2/Pim1 axis, IL-23/Th17/GM-CSF signaling, and the enhanced pathogenicity of Th17 cells, leading to reduced EAU inflammation and providing a potential treatment for AU.

Glycolysis

Zhu et al.[44] identified that HIF1α plays a significant pro-inflammatory role in the EAU model, which has important implications for clinical diagnosis and treatment. Additionally, the glycolysis-associated gene LDHA was found to be related to AU, suggesting that modulating LDHA could present a novel therapeutic approach, highlighting the role of anaerobic metabolism in AU.[45]

GM-CSF/IL-23R/IL-23 Pathway

Li et al.[46] utilized scRNA-seq of lymph nodes in EAU mice to elucidate that IL-38 confers protective effects via the GM-CSF/IL-23R/IL-23 pathway, demonstrating IL-38's protective role in EAU. Moreover, Liu et al.[47] established a sleep deprivation mouse model and an EAU model, creating a scRNA-seq atlas of cervical lymph nodes. They found that sleep deprivation enhances Th17 cell pathogenicity and the development of AU via the GM-CSF/IL-23 pathway, suggesting that targeting this pathway with anti-GM-CSF could mitigate the pathological immune response in sleep-deprived EAU mice.

UNVEILING EPIGENETIC REGULATION

ScATAC-seq provide valuable insights into chromatin accessibility and the epigenetic regulation of gene expression in autoimmune uveitis. By mapping open chromatin regions in individual cells, researchers can identify regulatory elements and transcription factors that drive the inflammatory response. This epigenetic information is crucial for understanding how gene expression is controlled in uveitis and for identifying potential epigenetic targets for therapy. Combining scATAC-seq with scRNA-seq often offers a more comprehensive understanding of epigenetic changes in disease states.

Nuclear Factor-kappa B Transcriptionfactor

In 2021, Shi et al.[40] created the first single-cell chromatin accessibility map of PBMCs from VKH patients. They reported that the nuclear transcription factor NF-κB was highly enriched in conventional dendritic cells, potentially associated with poor disease prognosis. In a 2023 study, researchers conducted scATAC-seq on PBMCs from BD patients and, through integrated analysis with scRNA-seq, predicted that the AP-1, NF-κB, and ETS transcription factor families might act as pro-inflammatory transcription factors in BD.[48] They also identified cytotoxic $CD8^+$ T cells as critical components of the inflammatory pathogenic mechanisms in BD, consistent with findings from other scRNA-seq studies on BD patients.

Signal Transducer and Activator of Transcription

Additionally, another scATAC-seq study on VKH patients found significant enrichment of the JAK/STAT signaling pathway in $CD4^+$ T cells, highlighting the potential of JAK inhibitors as a treatment strategy for uveitis[49]. However, research on single-cell epigenetics in autoimmune uveitis is still in its early stages, necessitating further extensive studies.

These studies underscore the importance of

understanding epigenetic regulation in autoimmune uveitis, revealing potential therapeutic targets and advancing the field toward more effective treatments.

IMPLICATIONS FOR DIAGNOSTIC AND THERAPEUTIC PRACTICE

The detailed cellular and molecular insights gained from SCS studies have profound implications for the development of therapies and diagnosis for autoimmune uveitis. By identifying specific cell populations and molecular pathways involved in disease pathogenesis, SCS facilitates the design of precision medicine approaches. For instance, targeting specific cytokines or signaling pathways identified through scRNA-seq can lead to more effective and tailored treatments. Additionally, understanding clonal expansions and genetic variations can guide the use of personalized immunotherapies.

Dimethyl Fumarate

One example is the use of dimethyl fumarate (DMF), which has been shown to effectively ameliorate EAU by reversing immune cell alterations and gene expressions associated with the disease.[50] Inhibition of PIM1 and CXCR4 plays a critical role in reducing ocular effector T cell infiltration and restoring immune cell balance, highlighting DMF's potential as a therapeutic strategy for autoimmune uveitis.

Cyclosporin A

Researchers also treated EAU mice with Cyclosporin A (CsA) and provided a comprehensive single-cell immunological atlas of peripheral lymph nodes under CsA treatment for EAU.[51] This study offers new insights into the mechanism of CsA in treating autoimmune diseases. Additionally, the mechanism of action of mycophenolate mofetil in the EAU model was explored, revealing that it reduces the antigen-presenting and antibody-producing capabilities of B cells in EAU mice.

Liquid Biopsies

Integrating proteomics of liquid biopsies with single-cell transcriptomics allows tracing the cellular origins of proteins in the eye, revealing disease mechanisms and accelerating molecular aging in specific cell types.[52] This integration transforms diagnostics and prognostics for ocular and other organ diseases.

Kurarinone

A study utilized single-cell sequencing to create a high-resolution atlas of the immunoregulatory effects of Kurarinone (KU) treatment on EAU.[53] The findings revealed that KU significantly modulates immune responses and reduces inflammation in EAU. By elucidating the potential therapeutic mechanisms of KU, the research suggests that KU holds substantial promise for treating autoimmune disorders. KU's ability to target specific immune pathways and cells involved in uveitis highlights its potential as an effective therapeutic strategy.

Mesenchymal stem cells

Mesenchymal stem cells (MSCs) also show great promise in treating AU.[28,54] Recent study also provides valuable insights into the molecular characteristics of EAU model, highlighting the role of Müller glia as antigen-presenting cells and the Th1-driven immune response.[54] The findings suggest that overexpressing CCR5 in MSCs could be a promising therapeutic strategy, improving their efficacy in preventing EAU by modulating the immune response and reducing

inflammation. GMSCs show significant promise as a therapeutic strategy for autoimmune uveitis and potentially other autoimmune diseases. Further research is warranted to fully understand their mechanisms and optimize their clinical use.

Mycophenolate mofetil

Additionally, researchers have explored the mechanism of action of mycophenolate mofetil (MMF) in the EAU model and found that MMF can reduce the antigen-presenting and antibody-producing capabilities of B cells in EAU mice[55].

In conclusion, the application of single-cell sequencing in autoimmune uveitis has uncovered numerous therapeutic targets and mechanisms, paving the way for innovative and personalized treatment strategies that could significantly improve patient outcomes.

CONCLUSION

Autoimmune uveitis is a multifaceted disease characterized by diverse ocular and extraocular manifestations. While the exact pathogenic mechanisms remain partially understood, recent advances suggest that genetic and epigenetic alterations lead to dysregulated immune responses, contributing to disease development. Single-cell multi-omics technologies have significantly enhanced our understanding by revealing intricate molecular networks and cellular heterogeneity associated with autoimmune uveitis. These technologies allow for detailed insights into disease onset and progression at a single-cell level, providing a robust foundation for clinical diagnosis and treatment.

Despite these advancements, challenges remain, particularly in single-cell epigenomics and proteomics, which limit our comprehensive understanding of the disease's molecular mechanisms. Current research is constrained by the availability of patient tissues and cell types studied, often focusing on peripheral blood, aqueous humor, and EAU model tissues. Future studies should aim to integrate data from various pathological tissues to better track immune cell dynamics and developmental trajectories, offering deeper insights into disease pathogenesis.

The dynamics and interactions of immune cells within the ocular microenvironment in autoimmune uveitis patients remain unclear. Enrichment analysis of single cells in tissue and peripheral blood revealed that the pathogenic effect of VKH might be related to monocyte-myeloid cells in peripheral blood through interaction with RPE cells.[40] However, the mechanisms and interactions of peripheral blood immune cell migration into the eye require further study using single-cell trajectory analysis.[56]

Key areas of focus include the roles of Th17 cells, CD8+ T cells, and dendritic cells, which have shown significant involvement in different forms of uveitis. However, further research is needed to fully explore these roles and integrate emerging data. Notably, single-cell spatial transcriptomics holds promise for advancing our understanding of autoimmune uveitis but remains underutilized in this context.

In summary, ongoing advancements in single-cell multi-omics technologies and computational methods are crucial for elucidating the pathogenic mechanisms of autoimmune uveitis. These advancements will help refine disease treatment strategies, develop novel therapies, and guide precision medicine, ultimately improving clinical outcomes.

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Author Contributions

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(III) Provision of study materials or patients: None

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(VII) Final approval of manuscript: All authors

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Conflict of Interests

None of the authors has any conflicts of interest to disclose. All authors have declared in the completed the ICMJE uniform disclosure form.

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None

Ethical Statement

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