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· **Review Article** ·

Advances in single-cell sequencing technology and its application in eye diseases

Zheyao Chen[1](陈哲垚), Youjin Hu[1, 2](胡友金)

State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Ophthalmology and Visual Science,Guangzhou 510060, China

HIGHLIGHTS

- This article reviews the frontier progress of single-cell technology and its application in the precision diagnosis of eye diseases and drug discovery.
- Within comparison to traditional RNA-seq, single-cell sequencing technology exhibits superior accuracy and efficiency, particularly in gene expression pattern analysis at the single-cell level, which yields greater insights into drug and biomarker discovery.
- Consequently, single-cell sequencing technology possesses broader application prospects, particularly in the field of drug discovery and precision diagnosis for ocular diseases.

Abstract: Vision serves as the cornerstone of rountine human life activities, wherein approximately 80% of information is perceived visually. Eye diseases, however, frequently culminate in vision impairment or blindness, severely affecting the quality of life. Due to the obscurity of the underlying molecular mechanisms, therapeutic outcomes for various blinding eye diseases remain suboptimal. Over the past decade, the development of single-cell genomics technology has made it possible to obtain multi-dimensional insights into genomes, epigenomes, transcriptomes, and proteomes of tissues and organs at the single-cell level, providing a potent tool for elucidating the molecular mechanisms of eye diseases and advancing precision diagnosis. Meanwhile, single-cell genomics technology has also been harnessed in drug discovery and screening, promising to transform traditional drug development paradigm that is often characterized by high cost [1], time-consuming [2], and substantial failure rate. This review aims to describe the cutting-edge advances in single-cell omics technology

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Corresponding author: Professor Youjin Hu, State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Jin sui Road 7#, Guangzhou, 510060, China.
E-mail address: huyoujin@gzzoc.com.

Full Text

and its applications in precision diagnosis of eye diseases as well as drug discovery and screening.

Keywords: single-cell; multi-omics; eye diseases; drug discovery

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THE DEVELOPMENT OF SINGLE-CELL SEQUENCING TECHNOLOGIES

Single-cell Transcriptomic Technology

Single-cell RNA sequencing represents the most advanced technique in the realm of single-cell sequencing methods and has emerged as a pivotal tool in molecular biology research [3]. In 2009, Tang et al. [4] were the first to report the methodology of single-cell whole-genome mRNA sequencing (scRNA-seq). Subsequently, the technology of single-cell transcriptomic sequencing has rapidly evolved into high-throughput methodologies enabling the parallel profiling thousands of single cells, encompassing droplet-based sequencing and microwell-based sequencing methods. Both methods involve the reverse transcription of RNA into cDNA, which is subsequently amplified, followed by library construction and sequencing analysis. The primary distinction between these techniques lies in the methodology utilize for capturing individual single cells. Currently, single cell isolation methods include limiting dilution method [5], micromanipulation [6], laser capture microdissection (LCM) [7], fluorescent activated cell sorting (FACS) [8] and microfluidic technology [9]. Among them, limiting dilution and micromanipulation are characterized by low-throughput, and high-labor-intensity for single-cell isolation. LCM allows for rapid and precise isolation of single cells or cells from most solid tissue samples after sectioning and staining. Compartmentalization effectively preserves cell morphology, structure and spatial location information but with low throughput. While FACS enables high-throughput separation of single cells, its reliance on stable fluorescent markers and stains, as well as the need for preparing a cell suspension, renders it unsuitable for micro-volume samples. Droplet-based transcriptomics methods, including Drop-seq [10], employ microfluidics to generate droplets containing a single cell and a unique molecular identifier, facilitating cell lysis and reverse transcription within the droplet. Similar to Drop-seq, inDrop [11] also uses microfluidics to create droplets but introduces a more complex barcode system to improve data quality. Furthermore, 10x Genomics Chromium [12] employs microfluidic technology to partition single cells, encapsulating them in droplets with distinct barcode tags, capable of processing thousands to tens of thousands of cells in a single experiment. Alternatively, microwell-based transcriptomic methods such as CEL-seq [13] and SMART-seq [14], utilize microwell plates for single-cell isolation, processing each cell individually. Nevertheless, CEL-seq [13] utilizes linear amplification to heighten the sensitivity of mRNA detection, making it suitable for identifying low-abundance transcripts, albeit with reduced throughput. Conversely, SMART-seq [14] employs PCR-based amplification of cDNA, delivering high-quality full-length transcriptomic data. The Fluidigm C1 [15] platform, along with its compatible technologies like Smart-seq2 [16] and CEL-Seq2 [17], provides an automated solution for single-cell preparation and analysis based on

microfluidic technology. This platform enables efficient separation and processing of single cells. On the other hand, MARS-seq [18] utilizes an automated microwell plate system for parallel processing of multiple samples, offering high throughput that is suitable for large-scale analysis of different cell types and states. Furthermore, SCRB-seq [19] enhances the capabilities of MARS-seq by introducing barcodes to label each single-cell sample. This not only simplifies the data analysis process but also increases the throughput and efficiency of sample processing, making it a powerful tool for large-scale single-cell sequencing applications.

In summary, droplet-based methods, leveraging microfluidic devices, offer higher throughput in single cells analysis facilitating the simultaneous processing of thousands of cells in a cost-effective manner. Conversely, microplate-based methods are constrained by the plate size and the number of cells available, resulting in lower throughput [20]. Despite generally being more costly than microdroplet-based techniques, Microplate-based methods such as Smart-seq2, can potentially reduce costs through miniaturization [21] and the employment of non-commercial enzymes [22]. Moreover, the library construction limitations of microdroplet-based methods restrict sequencing to either the 5' or 3' end of the transcript, overlooking the detection of allele-specific expression and other isoforms. In contrast, microplate-based methods generate full-length transcripts, thereby enabling the detection of non-highly expressed genes and other rare transcripts with sufficient information [23].

The Development of Single-cell Multi-omics Sequencing Technologies

With the evolution of advancements in methodology and technology, single-cell multi-omics sequencing techniques have emerged. Single-cell multi-omics sequencing enables the capture of multiple molecules including DNA, RNA, and proteins, from a single cell, followed by parallel analysis of multiple omics, thereby simultaneously obtaining different omics information from the same cell. Single-cell multi-omics technology encompasses the integration of various disciplines, such as the integration of genomics with transcriptomics, transcriptomics with proteomics, transcriptomics with epigenomics, and transcriptomics with spatial transcriptomics. Herein, we provide a concise overview of the current research progress in single-cell multi-omics technology (Table 1).

Integration of Genomics and Transcriptomics

In 2014, Han, L. et al. reported the Co-detection and sequencing of genes and transcripts [24] firstly. They used microfluidic chips to capture single cells, separate the cytoplasm from the nucleus, and amplify both cDNA and gDNA from the same cell on the chip, enabling simultaneous analysis of the genome and transcriptome. In 2015, Macaulay, I.C. et al. Introduced G&T-seq [25], which advanced the smart-seq2 [16] method by using biotinylated oligo-dT primers to isolate full-length mRNA from genomic DNA of a single cell. This approach facilitated parallelled amplification and sequencing of the genome and transcriptome. In the same year, Dey, S.S. et al. presented DR-seq [26], which sequenced genomic DNA and mRNA from the same cell, with DNA and RNA amplification preceding separation, minimizing the loss and contamination. In 2018, Han, K.Y. et al. reported SIDR-seq [27], a technique that employed hypo-osmotic lysis to preserve nuclear integrity while capturing cellular lysates with antibody-coupled magnetic beads. This allowed for the separation of genomic DNA and total RNA from single cells. In 2019, Rodriguez-Meira, A. et al. introduced TARGET-seq [28], which improved throughput by leveraging barcodes and minimizing

Table1 Summary of single-cell multi-omics technologies in recent years

Method	Technology	Data types provided	Resolution	References
Co-detection and sequencing of genes and transcripts	Microchannel-based microfluidics	DNA, mRNA sequence	Single cell	[24]
G&T-seq	Plate-based sequencing	DNA, mRNA	Single cell	[25]
smart-seq2	Plate-based sequencing	DNA, mRNA	Single cell	[16]
DR-seq	Mouth-pipetting sequencing	DNA, mRNA	Single cell	[26]
SIDR-seq	Microplate-based sequencing	DNA, mRNA	Single cell	[27]
TARGET-seq	Plate-based sequencing	Genomic and coding DNA, mRNA	Single cell	[28]
Perturb-seq	Droplet-based microfluidics sequencing	sgRNA, mRNA	Single cell	[72]
scM&T-seq	Bead-based sequencing	DNA methylation, mRNA	Single cell	[31]
scMT-seq	Micro-pipetting sequencing	DNA methylation, mRNA	Single cell	[32]
scTrio-seq	Pipette cell-picking sequencing	DNA, RNA, DNA methylation	Single cell	[33]
scNOME-seq	Plate-based sequencing	DNA methylation, chromatin accessibility	Single cell	[35]
scNMT-seq	Plate-based sequencing	Chromatin accessibility; DNA methylation; transcriptome	Single cell	[36]
scNOMeRe-seq	Plate-based sequencing	Chromatin accessibility; DNA methylation; transcriptome	Single cell	[37]
ScCOOL-seq	Plate-based sequencing	Chromatin status, nucleosome, positioning, DNA , methylation, copy number variation (CNV) and ploidy	Single cell	[38]
scCUT&Tag pro	Droplet-based microfluidics sequencing	Histone modifications, proteins	Single cell	[43]
PLAYR	Mass cytometry	mRNA, protein	Single cell	[44]
CITE-seq	Droplet-based microfluidics sequencing	mRNA, protein	Single cell	[45]
REAP-seq	Droplet-based microfluidics sequencing	mRNA, protein	Single cell	[46]
DBiT-seq	Microchannel-based microfluidics	mRNA, protein	Spatial, 10–50 μm	[49]
Slide-Seq	Microarray-based sequencing	mRNA	Spatial, 10 μm	[50]
Slide-seqV2	Microarray-based sequencing	mRNA	Spatial, 10 μm	[51]

reaction volumes to pool libraries. Additionally, it improved mutation coverage through protein digestion, resulting in more efficient DNA and RNA during cell lysis, while also achieving simultaneous analysis of the genome and transcriptome. In summary, this approach not only facilitated simultaneous analysis of the genome and transcriptome but also provided an effective method to elucidate the intricate relationship between genotype and phenotype.

Integration of Transcriptomics and Epigenomics

The integration of single-cell epigenomic analysis and transcriptomics enables the exploration of DNA's epigenetic attributes, specifically DNA methylation, chromatin accessibility, and histone modifications, along with their correlation to the transcriptome.

DNA methylation on CpG islands frequently plays a crucial role in the regulating gene expression [29]. Single-cell whole-genome methylation analysis techniques, with single-base resolution, can be categorized into two core technologies: Reduced Representation Bisulfite Sequencing (RRBS) and Whole-Genome Bisulfite Sequencing (WGBS) [30]. Both methods rely on bisulfite conversion prior to sequencing. Several research groups have devised methodologies for the concurrent analysis of the DNA methylome and transcriptome. For instance, scM&T-seq [31] is an advancement of established single-cell genomics techniques of G&T-seq [25], which integrates scRRBS [30] with Smart-seq2 [16] to simultaneously generate data on DNA methylation and RNA transcription. The application of scM&T-seq [31] in analyzing 61 mouse embryonic stem cells validated the well-known correlations between DNA methylation and the transcriptome. Akin to scM&T-seq [31], scMT-seq [32] necessitates manual separation of the cytoplasm and nucleus, with the cytoplasm housing for scRNA-seq, while the nucleus contains DNA for scRRBS methylome analysis. Subsequently, genomic methylation techniques for tri-omics analysis were introduced. scTrio-seq [33] is a combined DNA methylation analysis technique for tri-omics, enabling simultaneous analysis of the genome, methylome, and transcriptome. The integration of chromatin accessibility with transcriptional analysis can unveil regulatory elements that drive gene expression. ATAC-seq [34] is a pioneering single-cell multi-omics technique that profiles chromatin accessibility. It utilizes Tn5 transposase to target accessible chromatin regions, inserting DNA sequencing adapters at the cleavage sites, thereby identifying open chromatin regions related to transcription in limited cell numbers. ScNOME-seq [35] enables the determination of the three-dimensional chromatin structure within a single-cell nucleus by assessing the relative positioning of chromatin DNA and the genomic DNA methylation status within the nucleus via affinity chromatography. scNMT-seq [36] integrates the measurement of single-cell chromatin state (nucleosome positioning and DNA methylation) with transcriptomic expression levels, enabling the concurrent analysis of DNA methylation, chromatin accessibility, and transcription of the same DNA molecule. Furthermore, scNOMeRe-seq [37] provides a comprehensive cellular profile by elucidating the positioning of chromatin nucleosomes, DNA methylation, and DNA replication timing within a single cell. ScCOOL-seq [38] (Chromatin Whole Omic Scale Landscape Sequencing) represents the development of a single-cell multi-omics sequencing technology capable of concurrently measuring various layers of epigenomic information from a single cell. Single-cell studies reveal insights into chromatin status, nucleosome positioning, DNA methylation, copy number variation (CNV), and ploidy. Furthermore, analyzing histone modifications at single-cell resolution facilitates understanding of epigenetic programming and cellular

differentiation trajectories. In addition to the established chromatin immunoprecipitation sequencing (ChIP–seq)[39] and CUT&RUN [40] methods, the CUT&Tag method developed in 2019 [41] utilizers Tn5 transposase to directly label antibody binding sites for histone modification analysis. This method has been further optimized by other research groups for single-cell resolution analysis of histone modifications, including scCUT&Tag [42] and scCUT&Tag pro [43]. In summary, techniques that integrate epigenomic and transcriptomic data offer powerful experimental approaches for investigating the epigenetic mechanisms regulating gene expression in single cells.

Integration of Transcriptome and Proteome

Proteins are fundamental to all cellular processes and functions, thus characterizing them at the single-cell level through post-translational modifications and interactions is paramount. Recently, several single-cell multi-omics methods have been introduced, capable of concurrently detecting protein and RNA expression within the same cell. For instance, the Proximity Ligation Assay for RNA (PLAYR) [44] enables the quantification of over 40 types of target mRNAs and proteins in tens of thousands of single cells from various cell types. Conversely, Cellular Indexing of Transcriptomes and Epitopes by sequencing (CITE-seq) [45] marries the transcriptome and protein epitopes of cells with DNA oligonucleotide barcodes and antibodies, respectively, leveraging the 10x Genomics sequencing platform to simultaneously analyze surface protein abundance and transcriptome. Similarly, RNA Expression and Protein Sequencing Analysis (REAP-seq) [46], developed on a microfluidic platform, can also concurrently measure protein epitope abundance and transcriptomic levels. In comparison to single-omics sequencing techniques, the integration of proteomics and transcriptomics facilitates more precise single-cell gene expression and phenotypic detection by analyzing both protein and RNA expression within the same cell.

Integration of Transcriptome and Spatial Transcriptomics

Spatial transcriptomics methods retain spatial information and, when combined with other single-cell omics techniques, offer a comprehensive portrayal of the heterogeneity of individual cells within a tissue. Fluorescent In Situ RNA Sequencing (FISSEQ) method, as the first non-targeted in situ sequencing approach, captures the entire transcriptome by sequencing interconnected single-stranded DNA nanoballs of 200-400 nm[47]. In 2016, the concept of spatial transcriptomics was introduced [48], where intact tissues undergo reverse transcription before dissolution, capturing spatial gene expression information with microarray oligonucleotides on a slide, resulting in sequence-ready cDNA coupled with oligonucleotides. Another spatial transcriptomicstechnique, DBiT-seq [49], utilizes microfluidic methods to spatially deliver DNA barcodes into tissue sections. DBiT-seq has demonstrated the co-localization of numerous proteins with the entire transcriptome and can be further extended to the spatial epigenetic analysis of chromatin accessibility and histone modifications. Two additional methods, Slide-Seq [50] and Slide-seqV2 [51], employ a conceptually similar to spatial transcriptome slicing to barcode-scan mRNA in tissues. However, these methods place barcode reagents directly onto glass slides in solution, creating a single-layer filled array on the glass slide, rather than using arranged barcode reagents printed on a slide In summary, spatial transcriptomics techniques enable the acquisition of transcription information from specific locations within a tissue, providing effective data support for research and diagnostic applications.

APPLICATION OF SINGLE-CELL OMICS TECHNIQUES TO BETTER UNDERSTANDING AND PRECISE DIAGNOSIS OF EYE DISEASES

Molecular Cellular Mapping of the Different Cell Types of the Eye

The advent of single-cell sequencing has revolutionized our ability to conduct high-resolution analyses of the diverse cell types and their corresponding gene expression profiles within the eye. Through the utilization of this technique, we can precisely identify the transcriptomic signatures of various ocular tissues, thereby facilitating the construction of cellular molecular atlases for these tissues. This methodological advancement has significantly enhanced our comprehension of the intricate organization and underlying physiological mechanisms of complex ocular tissues.

Single-cell transcriptomics analysis reveals substantial cellular heterogeneity across development stages and elucidates the transcriptional networks regulating human retinal development. Brian S. Clark et al. [52] employed scRNA-seq to scrutinize retinal cells at 16 developmental stages and four initial differentiation phases in retinal organoids. Their study characterized the gene expression profiles of the seven major retinal cell types throughout the maturation and differentiation of retinal progenitor cells. Additionally, they identified disparities in gene expression between the developing macula and periphery, as well as among varying cell populations. Notably, they found that the loss of ATOH7 during late neurogenesis impedes the differentiation of cone photoreceptors. Furthermore. Macosko, E.Z., et al. [10] constructed a comprehensive molecular atlas of the retina in an animal model, leveraging the drop-seq technique to analyze the transcriptomes from thousands of murine retinal cells. This atlas encompasses 39 distinct cell clusters, providing insights into gene expression of known retinal cell types and potential new cell subtypes.

Lukowski SW et al. [53] conducted a similar research on human retinal tissues, employing single-cell transcriptomic sequencing on retinal samples from three donors. Their analysis revealed the transcriptomic characteristic of the adult retina at the single-cell level, identified all major human retinal cell types, and culminated in the construction of a single-cell atlas for the adult human retina. This atlas serves as a valuable resource for elucidating the transcriptional landscape of human retinal cells and offers profound insights into retinal-related diseases. Notably, single-cell sequencing enables a refined examination of the developmental mechanisms of the retina. Hu et al. [54] leveraged scRNA-seq to establish a comprehensive transcriptomic cell atlas of the human photoreceptor lineage, thereby identifying the IGF1-PHLDA1 axis as a regulator of human photoreceptor development. Tang et al. [55] harnessed scRNA-seq to analyze 2,421 individual cells from the human fetal neural retina (NR) and retinal pigment epithelium (RPE), uncovering a tightly regulated gene expression network in human retinal cells. Their study yielded a comprehensive map of disease-related genes in human fetal retinal cells, identified the principal cell types, and potential key transcription factors for each cell type, and emphasized the significance of retinal progenitor cells as potential therapeutic targets for hereditary retinal diseases. With the emergence of single-cell multi-omics technologies, Chen et al. [56] unutilized these techniques to sequence individual nuclei and cells of the human retina, resulting in the creation of a comprehensive multi-omics single-cell atlas of the adult retina. Additionally, single-cell transcriptomic technology

can also be applied to investigate alterations in cellular composition and the intrinsic gene regulatory network during the retinal aging process. Yi W et al. [57] performed single-cell transcriptome sequencing on the retinas of both human and non-human primate rhesus monkeys of varying ages. This approach revealed the cellular and molecular similarities and differences between human and monkey, culminating in the establishment of human and monkey retinal aging. Their findings revealed alterations in cellular composition and significant molecular features in the human retina during aging, providing an important research foundation for the treatment and intervention of age-related retinal diseases.

Furthermore, single-cell transcriptomic sequencing offers a valuable approach for analyzing the molecular characteristics of limbal stem cells. Dou S et al. [58] conducted single-cell transcriptomic sequencing on limbal tissues procured from six samples derived from four healthy individuals, thereby establishing a comprehensive single-cell transcriptomic atlas of human limbal cells, developing a pseudo-time trajectory depicting the developmental trajectory of limbal epithelial cells.

Molecular Mechanisms and Precise Molecular Diagnosis of Eye Diseases

In ophthalmology, the majority of complex diseases encompass diverse cell types. Furthermore, the exceptional resolution of single-cell transcriptomics significantly enhances our comprehension of the the underlying molecular mechanisms of ocular diseases, paving the way for the development of novel diagnostic and therapeutic strategies (Table 2).

Diabetic retinopathy (DR) represents a prevalent complication of diabetes mellitus , constituting a significant cause of blindness among the working-age population globally. In 2021, Hu et al. [59] employed scRNA-seq on the retinas from diabetic non-human primates with diabetes, revealing distinct molecular alternations among six major cell types. Notably, they discovered that TGFβ2 inhibits microglial activation in the initial stages of DR, with a significant upregulation of TGFβ2 in cone cells attributed to hyperglycemia. Moreover, Yan Wang et al. [60] used scRNA-seq to analyze 35,910 cells from the retinas of diabetic rats, aiming to elucidate mechanisms responsible for the blood-retinal barrier (BRB) breakdown and leakage. They succeeded in developing an early BRB atlas in DR, elucidating the composition and degeneration of Müller cells, as well as their regulatory interactions, thereby providing a panel of potential targets for early intervention in DR. Additionally, iu et al. [61] investigated the pathophysiological mechanisms of fibrovascular membranes (FVM), a defining feature of proliferative diabetic retinopathy (PDR), by employing scRNA-seq on surgically excised PDR-FVMs. Their analysis resulted in a comprehensive cellular atlas of FVM, identifying a GPNMB+ microglial subpopulation exhibiting pro-fibrotic and fibrotic properties.

Autoimmune uveoretinitis is another significant cause of vision loss. Single-cell transcriptomics has significantly enhanced our understanding of the pathogenesis of autoimmune uveoretinitis, facilitating the development of advanced therapeutic approaches. Vogt-Koyanagi-Harada (VKH) disease, a prevalent and severe autoimmune uveitis condition in Asian populations, poses a significant threat to vision Monocytes have been implicated in the initiation of autoimmune diseases, particularly in VKH. Hu et al. [62] employed scRNA-seq to analyze blood monocytes from 11,259 VKH patients, resulting in an accurate characterization of human blood monocytes and identification of ISG15 as a

Table2 Application of single-cell omics technology in diagnosis of ocular diseases and drug prediction

Method	Diseases	Targets or drugs	References
scRNA-seq	Diabetic retinopathy (DR)	Revealed TGFβ2 to inhibit microglial activation in the initial stages of DR.	[59]
scRNA-seq	Diabetic retinopathy (DR)	Found features of early blood-retinal barrier (BRB) in DR.	[60]
scRNA-seq	Proliferative diabetic retinopathy (PDR)	Identification of GPNMB+ microglia subsets with profibrotic and fibrotic properties.	[61]
scRNA-seq	Vogt-Koyanagi-Harada (VKH) disease	Identified proinflammatory monocytes and ISG15 as potential targets fordiagnosis and treatment of VKH.	[62]
scRNA-seq	Uveretinitis	Uncover cell populations and gene expression patterns associated with uveal retinitis.	[63]
scRNA-seq	Keratoconus	IdentifiedCTSD and CTSK as novel markersof keratoconus stromal cells.	[64]
scRNA-seq	Uveal melanoma (UM)	LAG3 is a promising candidate molecule for immune checkpoint blockade in patients with high-risk UM.	[65]
RNA-seq	Age-related macular degeneration (AMD)	Nicotinamide (NAM)was proposed as a potential therapeutic agent for AMD.	[66]
RNA-seq	Uveal melanoma (UM)	DOT1L is a potential drug target for UM.	[67]
scRNA-seq	Glioblastoma multiforme (GBM)	Examined drug response in single-cell transcriptome profiles.	[75]

potential diagnostic and therapeutic biomarker for VKH. Furthermore, mouse models provide invaluable insights into the pathogenesis of uveoretinitis. Specifically, Aire -/- mice, which fail to express self-antigens in the thymus, exhibit g reduced central tolerance and develop spontaneous, chronic, and progressive uveoretinitis. Jeremy Nathans et al. [63] characterized wild-type and Aire -/- retinas through scRNA-seq, revealing cellular populations and gene expression patterns associated with uveoretinitis. Their analysis suggests that the primary effector response in Aire -/- retinas is Th1-driven, with Muller glial cells upregulating specific genes in response to IFN-γ, potentially acting as antigen-presenting cells.

Keratoconus, a prevalent corneal ectatic disorder affecting adolescents and young adults, poses a significant risk for progressive visual impairment and even blindness. Despite its widespread occurrence, the underlying etiology of keratoconus remains incompletely elucidated. Single-cell transcriptomics holds promise in unraveling potential disease mechanisms, facilitating the development of new therapeutic approaches. Gao et al. [64] conducted a comprehensive scRNA-seq analysis on central corneal cells obtained from both keratoconus patients and healthy individuals. This study aimed to decipher the involvement of various cell types during disease progression. Their findings underscored the pivotal role of corneal stromal cells in keratoconus, particularly with regard to the dysregulation of collagen and the extracellular matrix (ECM). Moreover, they identified two novel molecular markers, CTSD and CTSK, specific to keratoconus stromal cells.

Furthermore, uveal melanoma (UM) exhibits a highly metastatic phenotype and, in contrast to cutaneous melanoma, demonstrates limited response to checkpoint immunotherapy. In a study conducted by J William Harbour et al. [65], scRNA-seq was employed on 59,915

tumor and non-tumor cells from eight primary and three metastatic samples, alongside V(D)J immunoglobulin repertoire analysis. Notably, the findings emphasized LAG3 as a promising candidate molecule for immune checkpoint blockade in high-risk UM patients.

In summary, single-cell transcriptomic sequencing enables the analysis of gene expression at the single-cell resolution, facilitating the examination of disparities among cell subtypes and subpopulations, and elucidating intricate molecular mechanisms. This advanced technique provides profound insights into the molecular underpinnings of ocular diseases, facilitating early diagnosis, and informing the development of therapeutic strategies.

SINGLE-CELL OMICS TECHNOLOGY AND THE DISCOVERY OF NEW DRUGS

Application of Transcriptome Technology in Drug Prediction and Evaluation

The traditional paradigms of drug research are confronted with ineffciencies in discovery, high costs, and protracted timelines, stemming from the limitation of predicting and screening drugs solely through testing the activity of vast numbers of candidate compounds for potential therapeutic effects. Conversely, the advent of transcriptomic sequencing offers a novel approach, enabling the detection of drug-induced alteration in genomic expression, which subsequently aids in identifying disease-related biomarkers and provides transcriptional regulation patterns to forecast promising drug candidates (Table 2).

Transcriptomic sequencing enables the comprehensive analysis of gene expression profiles before and after drug treatment, leading to the prediction and discovery of therapeutic targets for drug development. Sally et al. [66] utilized RNA-seq analysis to identify a series of biomarkers and candidate drugs related to age-related macular degeneration (AMD). Their study revealed that nicotinamide (NAM) inhibits the expression of drusen proteins, inflammatory and complement factors, while upregulating nucleosomes, ribosomal, and chromatin modification genes, ultimately mitigating disease-related phenotypes. Based on these findings, NAM was proposed as a potential therapeutic agent for AMD. Moreover, in the context of uveal melanoma (UM), the most common and life-threatening ocular malignancy among adults, fan et al. [67] conducted a histone methylation drug screen and employed RNA-seq to compare gene expression levels of relevant targets between UM and normal melanocytes. This approach led to the identification of DOT1L, a methyltransferaseH3K79, as a potential drug target for UM. In summary, transcriptomic sequencing offers valuable insights into gene expression changes following drug treatment, enabling the discovery of potential drug targets. However, it falls short in supporting high-throughput drug screening and predictive capabilities.

To facilitate high-throughput drug screening via transcriptomic sequencing, researchers have refined the methodology for enhancing screening efficiency. Chaoyang Ye et al. [68] introduced a novel method called DRUG-seq, which incorporates unique barcodes into reverse transcription primers to label cDNA in individual wells. Upon completion of first-strand synthesis, the samples are pooled for subsequent analysis. This methodology enables the analysis of 433 compounds across eight doses, thereby significantly enhancing drug screening throughput and minimizing costs.

Derived from primary tissues, organoids represent 3D ex vivo cellular aggregates that mimic tissue

homeostasis closely. In comparison to two-dimensional cell lines and mouse models, organoids exhibit distinct advantages, particularly in drug screening studies, thus enabling personalized precision medicine. For instance, patient-derived tumor organoids (PDOs) have emerged as a reliable in vitro model for drug discovery. Maxim Norkin et al. [69] introduced TORNADO-seq, a RNA-seq based organoid drug screening method. Liu et al. [70] integrated RNA-seq with a superhydrophobic microwell array chip, resulting in a full-genome RNA output analysis aligned with phenotypic data, =which was designated as Grouped-seq. TORNADO-seq facilitated small molecule drug screening, identifying numerous candidate drugs targeting CRC organoids, previously unexploited for CRC treatment, such as antipsychotic thiazine drugs, cholesterol-lowering statins, antifungal conazoles, and antihistamines. Grouped-seq deduces the mechanism of action of the drug, demonstrating its significant potential pharmacological screening of tumor organoids. Patient-derived iPSC-generated retinal organoids have emerged as exceptional tools for investigating retinal diseases. For instance, retinoblastoma retinal organoids derived from patients with RB1 mutations have been shown to closely mimic their natural counterparts, and analysis of retinal retinoblastoma patient-derived retinal organoids revealed the pathological mechanisms underlying RB1 mutations [71]. Consequently, patient-derived iPSC-generated retinal organoids hold promising potential for drug prediction and evaluation.

In summary, transcriptomic sequencing offers comprehensive insights into gene expression profiles which can facilitate the discovery of drug targets, personalized treatment strategies, drug toxicity assessment, drug screening, and discovery, as well as drug mechanism elucidation, providing significant support and guidance for drug research and development, as well as clinical practice.

Application of Single-cell Omics Technology in Precise Drug Prediction

In contrast to traditional RNA sequencing, scRNA-seq enables the analysis of the individual cell transcriptomes, uncovering intercellular heterogeneity. It allows for the discrimination of signals originating from heterogeneous cell subpopulations and rare cell types, facilitating precise identification of drug target genes and assisting in drug prediction (Table 2).

Single-cell transcriptomic technologies can be seamlessly integrated with other methods to accurately delineate the post-treatment features of individual cells. In 2016, Atray Dixit et al. [72] introduced Perturb-seq, a single-cell omics approach, that integrates CRISPR/Cas9 genome editing with single-cell transcriptomic sequencing. This method enables precise identification of the effects of drug treatment and gene editing on individual gene targets, features, and cell states, ultimately elucidating the intricate relationships between genes and cell phenotypes. Although the emergence of single-cell omics technologies has expanded the analytical capacity and reduced the cost per cell, experiments involving numerous samples under diverse conditions or derived from multiple patients still require individual scRNA-seq analyses for each sample. The substantial cost and time commitment associated with scRNA-seq for multiple samples underscores the urgency for methodologies that facilitate sample pooling for a single scRNA-seq assay. For instance, MULTI-seq [73], and Duhee Bang et al. [74] introduced a universal sample barcoding method for drug screening, significantly enhancing screening throughput while

uncovering unique transcriptional responses to individual drugs and single-cell-level target-specific gene expression patterns. Preclinical research demands models that incorporate the cellular diversity of human tumors and offer insights into drug sensitivity among specific cell populations. Consequently, Zhao et al. [75] employed scRNA-seq to analyze cultures of freshly excised glioblastoma multiforme (GBM) tissue slices treated with a range of drugs. This methodology facilitated the examination of drug responses within the single-cell transcriptomic profiles of individual patients, elucidating cellular responses to therapeutics. Takanori Takebe et al.[76] developed a screening model based on human liver organoid (HLO), capable of assessing 238 marketed drugs at four different concentrations, exhibiting high predictive power. While the aforementioned drug screening methods have not been applied to eye diseases, they remain versatile tools. Given the availability of an appropriate eye disease model, these methods can potentially be employed for drug screening in the treatment of eye diseases.

In summary, single-cell omics technologies offer unprecedented resolution at the single-cell level. Through the analysis of individual cellular responses following drug treatment, these technologies facilitate the assessment of pharmaceutical effects, accelerating the screening process for efficacious drugs. Additionally, these technologies empower the development of personalized treatment strategies tailored to the unique cellular characteristics of individual patients.

Prospective

Single cell omics technology offers profound insights into ophthalmic diseases, enabling the classification of cell types and subtypes at the molecular level. This approach can identify ocular cell-type-specific markers, uncover new and rare cell populations, and provide a comprehensive understanding of individual cell differences. Furthermore, it reveals mechanisms underlying retinal formation across different developmental stages, molecular relationships between disease states and cellular responses, as well as specific cell types implicated in various retinal disorders. Currently, the utilization of single-cell omics technology in ophthalmology remains suboptimal. However, with the continuous advancements, this technology is poised to transform into a comprehensive, high-throughput, cost-effective, and user-friendly tool for both research and clinical applications. Notably, the emergence of generative pretrained transformers has introduced a promising approach for developing foundational models with burgeoning single-cell sequencing data, achieving superior performance in tasks such as cell type annotation, multi-batch integration, multi-omic integration, perturbation response prediction and gene network inference [77]. Incorporating perturbation and temporal data into these models will enable the elucidation of causal relationships and the understanding of how genes and cells to eye diseases in the near future.

Single-cell omics-based high throughput sequencing technology enhances the discovery of disease biomarkers and drug therapeutic targets, enabling its application in drug screening, prediction, and precision diagnosis and treatment of eye diseases. Within comparison to traditional RNA-seq, scRNA-seq exhibits superior accuracy and efficiency, particularly in gene expression pattern analysis at the single-cell level, which yields greater insights

into drug and biomarker discovery. Consequently, scRNA-seq possesses broader application prospects, particularly in the field of drug discovery and precision diagnosis.

Correction notice

None

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Author Contributions

(I) Conception and design: Youjin hu
(II) Administrative support: Youjin hu
(III) Provision of study materials or patients: Zheyao chen
(IV) Collection and assembly of data: Zheyao chen
(V) Data analysis and interpretation: Zheyao chen
(VI) Manuscript writing: All authors
(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.

Patient consent for publication

None

Ethical Statement

All experimental protocols were approved by the ethics committee of Zhongshan Ophthalmic Center (Guangzhou, China).

Provenance and Peer Review

This article was a standard submission to our journal. The article has undergone peer review with our anonymous review system.

Data Sharing Statement

None

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