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Application of Single Cell Transcriptome Sequencing Technology in Ophthalmology Related Research

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Abstract: Single-cell transcriptome sequencing (scRNA-seq) is an emerging single-cell sequencing technology in recent years, which can achieve a comprehensive and complete complete transcriptome expression profile at the level of a single cell, and play an important and key role in further discovering rare cell types and subtypes, and clarifying more effective cell-specific markers. The application of this sequencing technology in the medical field can better observe and clarify the differences between each single cell and each subtype of single cell. At present, the technology of scRNA-seq has been widely used in clinical tumor diagnosis and treatment, reproduction, obstetrics and gynecology, immunization, neuroscience, microbiology and other fields. In recent years, the application of this technology has been increasingly extensive. At present, the application of this technology in the field of ophthalmology has also gradually increased, and it has played a certain role in the human visual system, especially in the research of retinal development, related cell differentiation, and related organ formation process, suggesting that this technology can explore relevant molecular mechanisms in eye injury repair. Providing help for the discovery of targets and markers of ophthalmology-related diseases may provide new ideas for subsequent ophthalmic diagnosis and treatment. In this paper, we will review the application progress of scRNA-seq technology in ophthalmology and disease research.

1 Introduction

Cells are the most basic units that constitute living bodies and improve their related functions. The heterogeneity of cells in living bodies contains a large amount of biological information, and the development and growth of cells are dynamic. The same cells also have different genetic characteristics at different stages [1]. Most previous transcriptional sequencing of specific tissue samples has been done using high-throughput transcriptome sequencing (RNA Seq), a technique in which tens of thousands of cells are sequenced simultaneously and their average values are calculated. It should be noted that every cell has heterogeneity, and measuring the average value of multiple cells gathered together will mask the differences in gene expression between cells, making it difficult to explore the heterogeneity of individual cells in depth [2]. scRNA-seq technology can isolate tens of thousands of cells at a time, and the mRNA contained in each cell after isolation can be discovered and efficiently amplified, and finally sequencing can be completed. More comprehensive gene expression data of single cells can be obtained, and analysis of cell clustering, subgroup expression characteristics and marker screening can be provided. It is an efficient detection technique for cell gene expression level [3-5]. At present, the technology of scRNA-seq has been widely used in clinical tumor diagnosis and treatment, reproduction, obstetrics and gynecology, immunization, neuroscience, microbiology and other fields. In recent years, the application of this technology has been increasingly extensive. At present, the application of this technology in the field of ophthalmology has also gradually increased, and it has played a certain role in the human visual system, especially in the research of retinal development, related cell differentiation, and related organ formation. Therefore, this paper reviews the application of scRNA-seq in ophthalmic research. This will help to clarify the pathogenesis of eye diseases and provide a new means for the diagnosis and treatment of clinical eye diseases in the future.

2 Application of scRNA-seq in cornea-related studies

The cornea is located in the front of the outer wall of the eye, which is an important barrier covering the iris, pupil and anterior chamber. The visual quality will be seriously affected after the lesion of the cornea. He et al. [6] first described the phenotype of adult embryonic stem cells transforming into corneal epithelial cells and the gene expression profiles that changed with different differentiation stages by using scRNA-seq technology, and finally found that TP63, Pax6 and KRT14 genes were dynamically and highly expressed in the differentiation process of eye surface epithelial cells. At the same time, Wnt, Notch, Hippo and Hedgehog signaling pathways are activated, suggesting that tissue engineered corneal epithelium induced by clinical human embryonic stem cells has important therapeutic significance in improving limbal stem cell deficiency. Other scholars obtained relatively rare stem cells in the corneal epithelium of wild-type and autophagy damaged mice, and applied the RNA-seq technology to detect these cells and the early transport and expansion cell populations. Based on this technology, a new gene TXNIP was discovered, which was found to play an important role in maintaining cell cycle stasis [7]. The above research results suggest that the application of RNA-seq technology in the field of ophthalmology can provide more support for the discovery of related new genes, and explore the direction and ideas of targeted therapy.

3 Application of single-cell transcriptome sequencing technology in the study of retinal development

The development process of mammalian retina is complex, and retinal progenitor cells will produce different neurons in the differentiation process. The biological behaviors involved in the

development process include retinal progenitor cell proliferation, cell outcome and specific neuron differentiation [8]. The retina is an important part of maintaining visual function, and in order to ensure the normal function of the retina, the retina is accurately stratified. Retinal neurons, including cones, rods and retinal ganglion cells, are derived from pluripotent retinal progenitor cells [9]. Previously, some foreign scholars established cell research, and applied the RNA-seq technology to mature retinal ganglion cells for transcripome sequencing, and finally identified 40 subtypes. Meanwhile, enriched markers were collected during the implementation of this experiment, and it was finally determined that retinal ganglion cells differentiated into 4 main subgroups and then 10 intermediate clusters during the differentiation process. It was eventually differentiated into 40 subtypes [10]. Daniszewski et al. [11] performed RNA-seq technology on retinal ganglion cells differentiated from embryonic stem cells to complete transcripome sequencing. During the experiment, researchers obtained 1235 cells and identified three different subgroups from these cells. Collin et al. [12] studied human pluripotent stem cells and found that photoreceptor precursors of CRX (fluorescent labeling of cone and rod cells) were visible in the differentiation process of these cells. With the support of this technology, scholars confirmed that their transcriptomes were highly similar to those of fetal photoreceptors. The results of this study provide evidence for the origin and transplantation of human pluripotent stem cells and photoreceptor cells.

Previous studies on the early formation of retina focused on the molecular change mechanism of different types of retinal neurons in the development process, and the understanding of how human retinal progenitor cells acquire neuron-related functions in the formation of neurons was limited. In this context, Collin et al. [13] conducted RNA-seq transcriptome sequencing at different time points after human embryonic stem cells differentiated into various retina-related organs, and finally identified 9 cell types. The results of cell cluster analysis showed that the number of retinal ganglion cells would decrease gradually with the passage of time, while the photoreceptor cells would gradually differentiate into cone and rod cells with the passage of time. This research result provides effective support for clarifying the sequence of emergence of various types of retinal cells, and also confirms the feasibility of the application of scRNA-seq technology in the field of retina and the analysis of the heterogeneity of different types. Lu et al. [14] identified the whole process of human retina from early development to adulthood, observed its different stages, and obtained the relevant single-cell transcription map by relying on the RNA-seq technology. The conserved and diverse gene expression in humans and mice illustrates the importance of obtaining gene expression data directly from human cells and the limitations of animal models for studying human disease.

4 Application of single-cell transcriptome sequencing in the study of ophthalmic diseases

4.1 Myopia

Banerjee et al. [15] established a study and sequenced the retinal samples of mice 40 days after form deprivation with the application of RNA-seq technology, so as to clarify the correlation between visio-related signaling pathways and myopia. The final results showed that the expression of dopaminergic synapses and gap junction path-related proteins in the aphotic cells (AC) in the retina in myopia state down-regulated the phosphorylation of connexin 36 (Cx36), but Cx36 expression remained unchanged. Zhao et al. [16] used RNA-seq and other technologies to explore genes related to retinal bipolar cells, and found that the functional level of VIPR2 is closely related to high myopia, and found that the loss of VIPR2 function is likely to have a negative impact on the normal function of retinal bipolar cells. This research result also provides new diagnosis and treatment targets and ideas for clinical prevention and control and treatment of myopia.

4.2 Diabetic retinopathy

Diabetic retinopathy (DR) is a common adverse complication in type 1 and type 2 diabetes, and a chronic progressive visual impairment lesion in the eye caused by terminal organ damage in such patients [17]. Niu et al. [18] established a model and sequenced the type 2 DR Model by using the scRNA-seq technology. The final results showed that RLBP1 protein was down-regulated in the retina of mice in this model, and its high expression in Muller glial cells inhibited DR-related neurovascular lesions to a certain extent. The results of this study provide new ideas for the targeted therapy of DR And the molecular mechanism of related dysfunction. Sun et al. [19] established a model, extracted the retinas of healthy mice and diabetic mice respectively, and constructed the transcriptome map of single cells to further clarify the molecular mechanism related to the pathological changes of DR. The final results showed that stress genes such as Cirbp, Rmb3, Mt1 and Mt2 could be induced in most retinal cells, but the number of bipolar cells and gene expression were not affected. The sequencing results provided a new therapeutic direction for DR Pathology research. Becker et al. [20], relying on the RNA-seq technology, collected retinal samples from DR Patients and sequenced them to obtain the relevant mRNA and miRNA expression profiles for analysis. The final result found that the cause of significant differences in expression in the transcription profiles was related to late DR, hippo pathway and gap junction signaling pathway. This reveals disease-associated miRNA/mRNA associations as a potential mechanism for post-transcriptional regulation. scRNA-seq provides a means to investigate the molecular pathways and cell-specific mechanisms of change in the development of DR, and provides a potential pathway for future therapeutic interventions.

4.3 Age-related macular degeneration

Age-related macular degeneration (AMD) is a kind of eye disease which mainly affects middle-aged and elderly people. It is blinding and seriously affects the quality of vision. Previously, some scholars [21] used the Seq-Well-scRNA-Seq platform to analyze the macula and retinal organ of human retina, and the results showed that the expression of multiple AMD-related genes increased in the macroglia-specific cell network cell subtypes of subACTIONet. Included were type IV collagen a3, vascular endothelial growth factor A, and high-temperature essential protein A1, which revealed changes in cell type composition and cell type-specific gene expression associated with AMD progression. The above research results to a certain extent confirmed that the application of RNA-seq technology in the field of ophthalmology can further explore the related molecular mechanisms of AMD pathogenesis, and provide support for the follow-up medical field to better clarify the new treatment ideas of AMD.

4.4 Retinal degeneration

Voigt et al. [22] collected retinal samples with retinal degeneration characterized by visual field loss and pigment change, and sequenced them by using RNA-seq technology. They found that there were astrocytes different from healthy human retinas in these retinal samples, providing a new target for the study of retinal degeneration. Jones et al. [23] established a rat model of retinal degeneration, injected neural progenitor cells into the retina of the rat model and conducted RNA-seq sequencing, and finally found 68 rescue genes. This study provided evidence of gene expression changes after treatment with neural progenitor cells in retinal degeneration.

4.5 Retinoblastoma

Retinoblastoma (RB) is a common intraocular malignant disease in children. Early and accurate diagnosis and effective interventional therapy are the key to improve the cure rate. Yang et al. [24] conducted RNA-seq sequencing on cells from two RB samples, aiming to explore the influence mechanism of related molecules in the progression of the disease and the heterogeneity of related cells, which can provide a new prognosis marker for RB. Collin et al. [25] sequenced scRNA-seq and single-cell chromatin open region of RB with mutation characteristics of two pathogenic genes RB1, respectively. The results showed that the two tumors were caused by biallelic mutation of RB1 gene, and G2/M pyramidal precursor cells were identified as the origin cells of RB. scRNA-seq plays an important role in revealing the heterogeneity and molecular pathogenesis of RB, and this technique opens up new ways for patients to develop effective treatment options.

5 Summaries

From the existing literature analysis, it can be seen that single-cell transcriptome sequencing is more and more widely used in ophthalmology, which can be used to identify retinal or corneal cell types or subsets and analyze gene differential expression. It can also be used in the treatment of eye diseases, such as the discovery of new biological targets in the pathogenesis of AMD, providing a new therapeutic direction for the subsequent treatment of the disease. Embryonic stem cells or pluripotent stem cells can also be used to induce retinal differentiation, study the early formation and development mechanism of human retina, the time course of cell differentiation, and identify the cell types and subtypes at each stage of retinal formation. It can also be used to study the molecular mechanism of eye injury repair. At present, the application direction of single-cell transcriptome sequencing technology in the field of ophthalmology is limited, and it can be extended to other tissues of the eye such as lens and conjunctiva. Cell function analysis during retinal development and trajectory analysis of retina development; To study the pathogenesis of eye related diseases; Molecular mechanism of eye injury repair.

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Data Availability

Data sharing is not applicable to this article as no new data were created or analysed in this study.

Conflict of Interest

The author states that this article has no conflict of interest.

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