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The Application of Single-Cell Omics Technologies in Neuroscientific Research

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Abstract With the deepening of neuroscientific research, traditional research methods have become difficult to meet the comprehensive analysis of the complexity and heterogeneity of the nervous system. The rise of single-cell omics technologies has brought new opportunities to neuroscientific research. This review summarizes the heterogeneity of nerve cells and the basic principles, advantages, and limitations of single-cell omics technologies. Through specific cases, it delves into the application of single-cell omics technologies in the study of neurons and synapses, the analysis of the pathogenesis of neurodegenerative diseases, as well as neural regeneration and repair research, and analyzes their contributions to neuroscience. In addition, this review also looks forward to the future development direction of single-cell omics technologies in neuroscientific research and discusses the current and future technical and ethical challenges and their solutions. This review aims to comprehensively sort out and evaluate the application of single-cell omics technologies in neuroscientific research, hoping to provide useful references and insights for researchers in related fields.

Keywords Single-cell omics technologies; Neuroscientific research; Neurons and synapses; Neurodegenerative diseases; Neural regeneration and repair

Neuroscience, as an interdisciplinary field exploring the structure and function of the human nervous system, has always been at the forefront of scientific research. The complexity and intricacy of the nervous system make it crucial for understanding human cognition, emotions, behaviors, and numerous neurological disorders. The importance of neuroscience research is self-evident; it not only deepens human understanding of cognition but also directly relates to the effective prevention and treatment of numerous neurological disorders such as Alzheimer's disease, Parkinson's disease, and depression (O'Banion and Yasuda, 2020).

In recent years, with the rapid development of biotechnology, single-cell omics technologies have emerged, bringing new hope to neuroscience research. These technologies allow for in-depth studies at multiple omic levels—genomics, transcriptomics, epigenomics—of individual cells, thereby revealing the unique functions and roles of single cells within the nervous system (Cadwell et al., 2017). The rise of this technology not only significantly enhances the precision and depth of neuroscience research but also provides new ideas and methods for the study of neurodegenerative diseases, and neuroregeneration and repair (Shen et al., 2020).

This review aims to explore the applications, significance, and prospects of single-cell omics technologies in neuroscience research. It reviews the single-cell omics features of neuroscience, analyzes the heterogeneity of neural cells, and discusses the basic principles and technical processes of single-cell omics technologies. By reviewing related research cases, it provides a detailed display of the specific applications of single-cell omics technologies in the study of neurons and synapses, the mechanisms of neurodegenerative diseases, and neuroregeneration and repair research, and assesses their contributions to neuroscience. The review also discusses the technical and ethical challenges faced by single-cell omics technologies in neuroscience research and explores possible solutions.

The emergence of single-cell omics technologies brings new opportunities to neuroscience research. By thoroughly exploring the applications and significance of single-cell omics in neuroscience, this review hopes to provide useful references and insights for advancing the field of neuroscience, as well as pave new paths for the effective prevention and treatment of neurological disorders.

1 Single-Cell Omics Characteristics in Neuroscience

1.1 Heterogeneity of neuronal cells

The heterogeneity of neuronal cells is one of the core characteristics of the nervous system, which bestows the brain with unparalleled complexity and functionality. This heterogeneity is reflected at multiple levels including the morphology, structure, electrophysiological properties, and molecular composition of neuronal cells. At the genomic level, the heterogeneity of neuronal cells manifests as variability and complexity in gene expression across different cell types (Song et al., 2020).

The nervous system contains various cell types, including neurons, astrocytes, oligodendrocytes, and microglia, each exhibiting significant genomic heterogeneity. For example, neurons are responsible for the transmission and processing of information, and their genomes contain numerous genes related to synaptic transmission, ion channels, and the synthesis and release of neurotransmitters. In contrast, glial cells mainly provide support, protection, and nourishment to neurons, and their genomes are enriched with genes related to metabolism, immunity, and repair (Tavakolian-Ardakani et al., 2019).

Furthermore, even within the same type of neuron, genomic characteristics can vary between different brain regions and developmental stages. For instance, dopaminergic neurons play crucial roles in motor control, emotional regulation, and cognitive functions, containing numerous genes associated with these functions. However, the expression patterns and regulatory mechanisms of these genes can differ among dopaminergic neurons in different brain areas.

1.2 Overview of single-cell technologies

Single-cell omics technologies are methods capable of deeply investigating molecular information such as the genome, transcriptome, and epigenome at the level of individual cells. The advent of these technologies has enabled a more detailed understanding of the heterogeneity among different cell types within an organism, as well as the molecular mechanisms of cells under physiological and pathological conditions (Clark et al., 2018).

Single-cell omics primarily includes single-cell sequencing and single-cell epigenomics. Single-cell sequencing technology allows for high-throughput sequencing of the genome or transcriptome of individual cells, revealing each cell's gene expression profile and variations (Jeongwoo et al., 2020). On the other hand, single-cell epigenomics focuses on studying the chromatin structure, DNA methylation, and histone modifications of individual cells, which are crucial for understanding cell differentiation, development, and disease mechanisms (Figure 1). Hu et al. (2018) summarized the current single-cell multi-omics methods, such as scG&T-seq (single-cell genome and transcriptome sequencing), scMT-seq (single-cell methylome and transcriptome sequencing), scM&T-seq (single-cell methylome and transcriptome sequencing), scTrio-seq (single-cell tri-omics sequencing), and scCOOL-seq (single-cell chromatin overall landscape sequencing), and measured different combinations of omics data (Figure 1).

The basic principle involves isolating individual cells, lysing them, and tagging molecules, followed by high-throughput sequencing and analysis of these molecular data. The workflow typically includes steps such as cell isolation, reverse transcription and amplification, library construction, sequencing, and data analysis. Among these, cell isolation is a crucial first step, which can be achieved through methods such as flow cytometry, micromanipulation, or tissue digestion.

1.3 The role of single-cell genomics in neurogenesis and neurodegenerative diseases

Single-cell genomics offers a unique perspective and powerful tools for revealing the molecular mechanisms behind neurogenesis, synaptic plasticity, and neurodegenerative diseases. During neurogenesis, single-cell genomics can intricately depict how neural stem cells gradually differentiate into various types of neurons and glial cells, along with the dynamic changes in gene expression during these processes. By analyzing the genomic, transcriptomic, and epigenomic information of individual cells, we can gain a deeper understanding of the regulatory networks in neurogenesis and identify the roles of key genes and signaling pathways.

In terms of synaptic plasticity, single-cell genomic technologies can capture the molecular changes at synaptic sites when neurons are stimulated externally. These changes include the synthesis and degradation of synaptic proteins, opening and closing of ion channels, and regulation of gene expression. By comparing genomic information from synaptic sites in different states, key genes and signaling pathways related to synaptic plasticity can be identified, thus enhancing our understanding of the molecular basis of synaptic plasticity (Jeongwoo et al., 2020).

For neurodegenerative diseases, single-cell genomics can reveal genomic changes in different cell types as the disease progresses. For example, in Alzheimer's disease, single-cell sequencing technologies can detect abnormal gene expression in neurons and glial cells, which may be closely associated with the onset and progression of the disease. Additionally, single-cell epigenomics technologies can provide insights into the epigenetic mechanisms behind these gene expression abnormalities, offering new ideas and methods for the diagnosis and treatment of the disease.

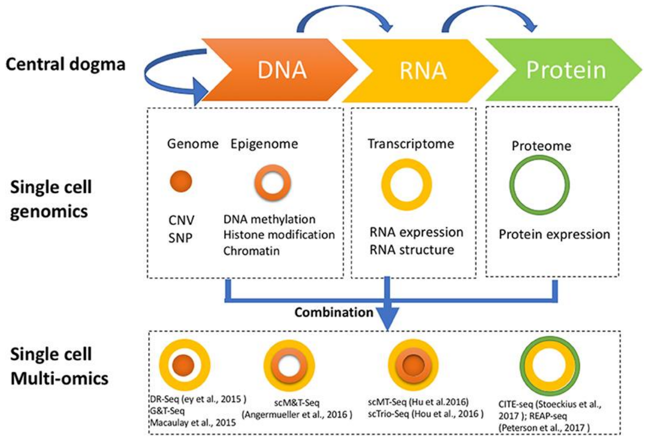

Figure 1 Analysis strategies for single-cell-omics techniques (Hu et al., 2018)

Note: Three major types of molecules relating to biological central dogma (TOP). Single cell genomics methods profiling the genome, epigenome, transcriptome, and proteome are shown by different shapes with variable colors (MIDDLE). Single cell multi-omics methods are built by combining different single cell sequencing methods to simultaneously profile multiple types of molecules of a single cell genome wide (BOTTOM). For example, G&T-seq was built by combining genome (orange) and transcriptome (yellow) to simultaneously detect DNA and RNA of the same cell genome wide

2 Applications of Single-Cell Omics Technologies in Neuroscience Research

2.1 Research on neurons and synapses

Single-cell omics technologies have played a crucial role in the identification of neuron types and the analysis of synaptic connections. By studying the genome, transcriptome, or epigenome of individual neurons in depth, scientists can more accurately identify and classify neurons, revealing their unique biological characteristics and functions (Musk, 2019).

2.1.1 Neuron type identification

In the field of neuron type identification, single-cell omics technologies have provided unprecedented precision. For example, using single-cell RNA sequencing (scRNA-seq) technology, researchers are able to map the gene expression profiles of neurons in different brain regions and distinguish various neuron subtypes. These subtypes differ not only in morphology and electrophysiological properties but also in gene expression patterns. This detailed classification is crucial for understanding the complexity and diversity of brain functions (Viswam et al., 2019).

2.1.2 Synaptic connection analysis

In the area of synaptic connection analysis, single-cell omics technologies offer a new perspective on the communication mechanisms between neurons. Researchers use these technologies to precisely analyze the genomics and transcriptomics of pre- and post-synaptic cells, thereby revealing the molecular basis of synaptic connections. For instance, by comparing the synaptic connection strength and stability between different neurons, scientists have discovered key genes and signaling pathways related to synaptic plasticity. These findings not only help understand how neurons cooperate to perform complex brain functions but also provide new insights into the treatment of neurodegenerative diseases.

2.2 Mechanisms of neurodegenerative diseases

Single-cell genomics has played a significant role in unveiling the pathogenic mechanisms of neurodegenerative diseases such as Alzheimer's Disease (AD) and Parkinson's Disease (PD). By analyzing the genome, transcriptome, and epigenome of individual neurons in brain tissues of patients, scientists have been able to more precisely understand the molecular level pathological changes in these diseases. These case studies demonstrate the immense potential of single-cell genomics in researching the pathogenic mechanisms of neurodegenerative diseases (Vasaikar, 2019).

2.2.1 Pathogenic mechanism of Alzheimer's disease

Taking Alzheimer's Disease as an example, researchers have used single-cell RNA sequencing technology to discover a plethora of gene expression abnormalities in the neurons of AD patients. These abnormalities include genes related to synaptic function, energy metabolism, and inflammatory responses. By comparing single-cell data across different disease stages and different brain regions, scientists have also revealed the progressive degeneration and death of neurons in the course of AD, as well as the response and role of glial cells in the disease progression. These findings not only provide new insights into the pathogenic mechanisms of AD but also support the development of therapeutic strategies targeted at specific pathological processes (Viswam et al., 2019).

2.2.2 Pathogenic mechanism of Parkinson's disease

In the case of Parkinson's Disease, single-cell genomics has also revealed the degenerative death process of dopaminergic neurons in the brains of PD patients. Scientists have identified specific gene expression abnormalities and epigenetic modifications in dopaminergic neurons, which are closely related to core pathological mechanisms of PD, such as mitochondrial dysfunction and oxidative stress. Additionally, single-cell genomics has helped researchers uncover the responses and changes of other types of neurons during the progression of PD, as well as the interactions between glial cells and dopaminergic neurons.

2.3 Research on neural regeneration and repair

Single-cell genomics has achieved remarkable results in the fields of neural regeneration and neural stem cell differentiation. These studies have not only deepened our understanding of the mechanisms of neural system development and repair but also provided new strategies for the treatment of neurodegenerative diseases (Rochford et al., 2020).

Taking neural regeneration as an example, scientists have used single-cell RNA sequencing technology to conduct in-depth studies on the regeneration process of neural stem cells after injury. They discovered that certain specific genes and signaling pathways are activated in the injured environment, promoting the proliferation and differentiation of neural stem cells. These findings not only reveal the molecular mechanisms of neural regeneration but also provide potential targets for developing drugs that promote neural regeneration (Aqrawe et al., 2018).

In the area of neural stem cell differentiation, single-cell genomics technology also plays a crucial role. Researchers have used single-cell sequencing technology to perform detailed analyses of gene expression changes during the differentiation process of neural stem cells. They found that neural stem cells at different differentiation stages possess unique gene expression patterns, which are closely associated with the direction and function of cell differentiation. These findings not only help to understand the differentiation mechanisms of neural stem cells but also provide an important theoretical basis for cell replacement therapies for neurodegenerative diseases.

3 Prospects and Challenges of Single-Cell Omics Technologies in Neuroscience Research

3.1 Future directions of single-cell omics technologies

Single-cell omics sequencing technologies have already achieved substantial results in the field of stem cell biology. However, the current technologies are still not ideal for studying stem cells in living organisms. These technologies are expected to be further developed and improved over the next few years. The current single-cell omics sequencing technologies exhibit both strengths (well-developed aspects) and weaknesses (developing aspects) as illustrated in Figure 2. Therefore, the future development direction of single-cell omics technologies in neuroscience research is particularly promising. With continuous innovation and optimization of these technologies, single-cell omics is expected to provide a deeper and more comprehensive perspective for neuroscience research (Leopold et al., 2019).

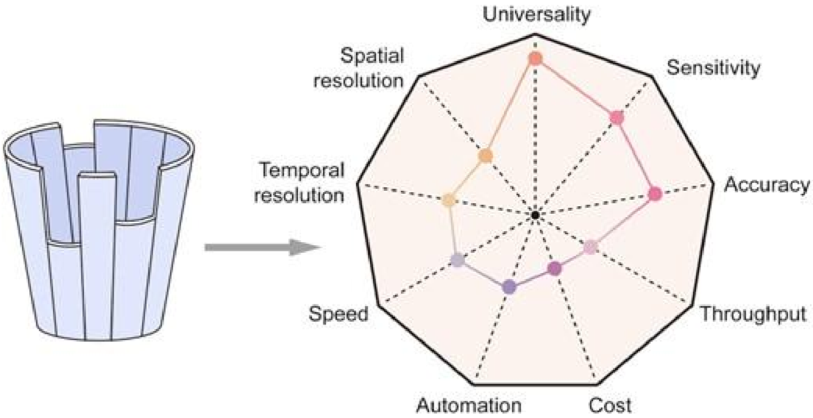

Figure 2 Characteristics of the current single-cell omics sequencing technology (Lu and Tang, 2022)

Note: Single-cell omics sequencing technology is shown shaped like a barrel with both long (developed) and short (developing) boards (left panel), and a radar chart shows the current technical states of nine major characteristics, with the more developed state of the character indicated by its being positioned more peripherally (right panel)

Further integration of technologies will be an important direction in the future. By combining single-cell omics with other advanced technologies such as imaging techniques and genetic methods, it will be possible to study the genome, transcriptome, proteome, and intercellular interactions at the level of individual cells, thus revealing the complex mechanisms of the nervous system more comprehensively. Deep analysis and interpretation of data will be another key direction. As single-cell omics data continue to accumulate, how to effectively integrate, analyze, and interpret these data to extract meaningful biological information will become a focal point of research (Lin et al., 2017).

Moreover, single-cell omics technologies are also expected to play a significant role in the early diagnosis and treatment of neurodegenerative diseases. Through single-cell analysis of patient brain tissue, pathological changes can be detected earlier, providing possibilities for early intervention and treatment. Single-cell omics technologies are also expected to provide more insights for basic neuroscience research. For instance, in processes such as neurogenesis and synaptic plasticity, single-cell omics can provide a deeper understanding of the heterogeneity and dynamic changes between cells, thereby revealing fundamental laws of the nervous system.

3.2 Current and future technological and ethical challenges

Although single-cell genomics technology has made significant progress in neuroscience research, it still faces a series of technical and ethical challenges. On the technical level, the experimental process of single-cell genomics is complex and requires high-quality samples, which demands high technical skills and experience from researchers. Additionally, the vast amount of data generated by single-cell genomics requires strong computational power and advanced algorithms for data analysis and interpretation, posing challenges for data processing and analysis. Moreover, the accuracy and reliability of the technology also need continuous attention and improvement to avoid misleading conclusions caused by technical errors (Abraham et al., 2020).

On the ethical level, the application of single-cell genomics technology in neuroscience research involves numerous ethical issues. Firstly, the acquisition and handling of samples must adhere to strict ethical and regulatory requirements to ensure the protection of the rights of the research subjects. Secondly, stringent security measures must be taken to manage and protect data involving personal privacy and sensitive information. Additionally, when applying single-cell genomics technology in clinical diagnostics and treatment, it is necessary to consider its potential ethical and societal impacts, such as discrimination and bias.

3.3 Solutions strategy

In response to the technical and ethical challenges that single-cell genomics technology may currently and potentially face in neuroscience research, future studies should comprehensively consider factors such as technology, ethics, and regulations to promote the healthy development of single-cell genomics in neuroscience. This includes continuously optimizing experimental procedures, enhancing data processing and analysis capabilities, strengthening ethical review and regulation, and fostering interdisciplinary collaboration. Technically, ongoing investment in research and development can optimize experimental procedures and improve the accuracy and reliability of single-cell genomics technology (Hu et al., 2018). Moreover, enhancing data processing and analysis capabilities, using advanced algorithms and computational methods, can deeply mine the biological information of single-cell data. Additionally, promoting interdisciplinary cooperation and communication, and integrating other fields' techniques and methods, such as imaging technologies and genetic methods, can provide a more comprehensive and in-depth perspective for neuroscience research.

Ethically, it is essential to strictly adhere to ethical and regulatory requirements, ensuring the lawful acquisition and handling of samples. Strengthening ethical review and regulatory mechanisms is crucial to ensure the compliance and fairness of research processes. Meanwhile, attention should be paid to the protection of personal privacy and sensitive information, with necessary security measures taken to manage and protect research data. When applying single-cell genomics technology to clinical diagnosis and treatment, it is necessary to fully assess its potential ethical and social impacts and take corresponding measures to minimize potential negative effects.

Furthermore, enhancing scientific outreach and public communication is important to improve the public's awareness and understanding of single-cell genomics technology. Through various channels such as science lectures, academic papers, and media reports, scientific knowledge and research progress can be communicated to the public, thereby enhancing public trust and support for scientific research.

4 Summary and Outlook

Over the past several years, single-cell genomics has become a dazzling gem in the field of neuroscience research, offering unprecedented opportunities to unravel the mysteries of the nervous system. By delving into the genomic, transcriptomic, and epigenomic information of individual neurons or glial cells, single-cell genomics has brought revolutionary changes to neuroscience.

In the area of neuron type identification, single-cell genomics enables researchers to more precisely identify and classify neurons, thereby revealing their unique biological characteristics and functions. This not only enhances our understanding of brain complexity but also provides a basis for studying the roles of specific types of neurons in behavior, cognition, and emotion. In the study of neurodegenerative diseases, single-cell genomics offers robust support for uncovering the pathogenesis of diseases such as Alzheimer's and Parkinson's (Lia et al., 2018). Through single-cell analysis of patient brain tissue, scientists can discover gene expression anomalies related to the disease and interactions between cells, providing a basis for developing treatment strategies targeted at specific pathological processes.

The contribution of single-cell genomics to neuroscience research is immeasurable. It provides a deeper, more detailed perspective for neuroscience research, enabling a more comprehensive understanding of the structure and function of the nervous system. It offers new tools and methods for the diagnosis and treatment of neurodegenerative diseases, potentially leading to breakthroughs for these difficult-to-cure diseases. Single-cell genomics also promotes the integration of neuroscience with other fields, providing a broader stage for neuroscience research.

Looking ahead, the application prospects of single-cell genomics in neuroscience research will be even broader. With continuous innovation and optimization of the technology, we can expect the emergence of more precise and efficient single-cell genomics techniques. These technologies will provide the possibility to reveal more secrets of the nervous system, thereby promoting the deep development of neuroscience research. Meanwhile, with the continuous accumulation of single-cell genomics data, effectively integrating, analyzing, and interpreting this data will become a focus of research. This will require us to invest more effort in data processing and analysis to unearth more biological information.

Furthermore, as single-cell genomics continues to evolve, it is expected to be applied in more areas of neuroscience research, such as neurodevelopment, neuroplasticity, and neuroimmunity. This will provide possibilities to reveal more aspects of the nervous system, thereby promoting the comprehensive development of neuroscience research.

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