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REVIEW

Single-Cell Sequencing: High-Resolution Analysis of Cellular Heterogeneity in Autoimmune Diseases

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Abstract

Autoimmune diseases (AIDs) are complex in etiology and diverse in classification but clinically show similar symptoms such as joint pain and skin problems. As a result, the diagnosis is challenging, and usually, only broad treatments can be available. Consequently, the clinical responses in patients with different types of AIDs are unsatisfactory. Therefore, it is necessary to conduct more research to figure out the pathogenesis and therapeutic targets of AIDs. This requires research technologies with strong extraction and prediction capabilities. Single-cell sequencing technology analyses the genomic, epigenomic, or transcriptomic information at the single-cell level. It can define different cell types and states in greater detail, further revealing the molecular mechanisms that drive disease progression. These advantages enable cell biology research to achieve an unprecedented resolution and scale, bringing a whole new vision to life science research. In recent years, single-cell technology especially single-cell RNA sequencing (scRNA-seq) has been widely used in various disease research. In this paper, we present the innovations and applications of single-cell sequencing in the medical field and focus on the application contributing to the differential diagnosis and precise treatment of AIDs. Despite some limitations, single-cell sequencing has a wide range of applications in AIDs. We finally present a prospect for the development of single-cell sequencing. These ideas may provide some inspiration for subsequent research.

Keywords Single-cell sequencing · Autoimmune diseases · Heterogeneity · Multi-omics analysis

Introduction

Single-cell sequencing, including genomics, epigenomics, and transcriptomics, is a significant technology for deciphering cellular and molecular mapping at the level of single cells, whereas bulk sequencing provides average data [1]. The technology enables cell biology research at an unprecedentedly enormous resolution and scale. With the robustness and accessibility of the technology increasing annually, it has become an important tool for life science research [2]. This technique has been used in a wide range of diseases including tumors, infections, and AIDs. In the field of oncology, it can be used to uncover the heterogeneity of the tumor microenvironment and identify subpopulations of immune cells that are relevant to immune surveillance and could be potential therapeutic targets [3]. With the help of single-cell sequencing, more breakthroughs have also been made in research on infectious diseases. For example, the viral RNA responsible for COVID-19 was identified using scRNA-seq [4]. What's more, scRNA-seq has multiple applications in studies such as intercellular communication analysis,

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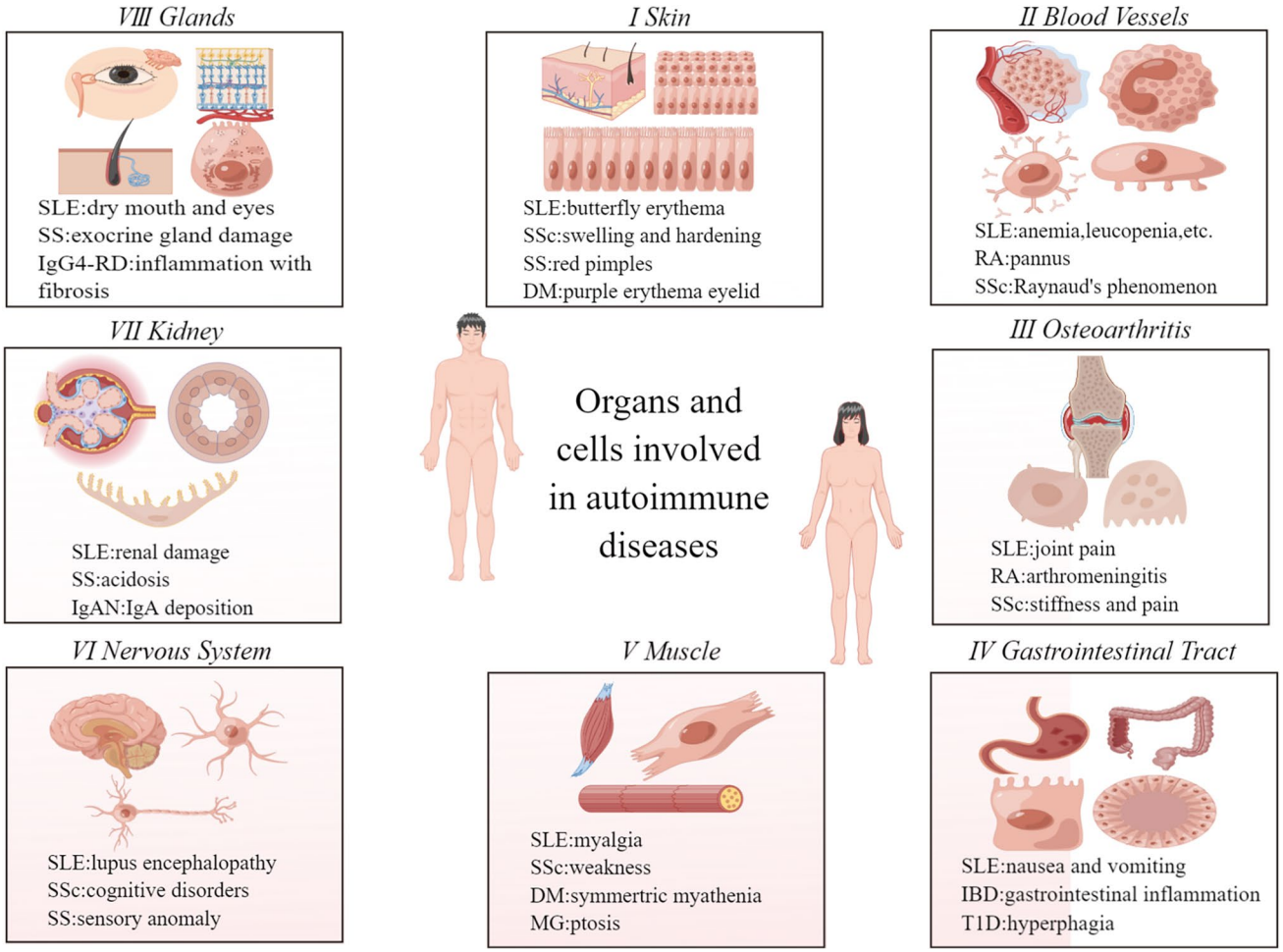

Fig. 1 Organs and cells involved in AIDs (By Figdraw.)

regulatory single-cell states, and immune cell distribution [5]. It is also a powerful tool to promote personalized treatment by defining subpopulations of cells with hidden therapeutic targets [6]. The wide range of applications for single-cell sequencing will be described in more detail later on.

AID is a disease state resulting from the abnormal immune response against the body's own components. The immune response against foreign antigens usually ends with the clearance of those antigens. However, when the immune response occurs to the body's own cells or tissues, these own components are not easily cleared but constantly attacked, resulting in the body a disease state. The causes of AIDs are complex and variable, ranging from genetic factors involving mutations in family genes or one's own genes to external environmental factors such as infections, medications, and daily diet [7]. The different types and factors determine the different clinical symptoms. Common AIDs include systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), systemic sclerosis (SSc), systemic vasculitis (SV), sicca syndrome (SS), inflammatory bowel disease (IBD), dermatomyositis (DM) [8], etc. The etiology and clinical manifestations are so diverse and complex that they are difficult to study [9]. Some organs and cells involved in AIDs are shown in Fig. 1. Immune cell heterogeneity is one of the common features of AIDs, and single-cell sequencing technology can analyze the cellular genome or transcriptome at the level of single cells. It helps eliminate the heterogeneity of the immune system by identifying different immune cell subpopulations between healthy individuals and patients, characterizing the random heterogeneity among them, and constructing the developmental trajectory of immune cells [10]. Therefore, single-cell sequencing has tremendous applications in the field of AIDs. In this paper, we will introduce single-cell sequencing and its application in different diseases especially AIDs in detail to provide some help for clinical practice and related research.

Single-Cell Sequencing

Sequencing technology has been developed through the first [11], second [12], and third [13] generations, gradually reaching large-scale, high-throughput, and high-resolution. Single-cell sequencing is based on the second-generation sequencing technology [14]. It is divided into four main steps, including single-cell sorting, nucleic acid extraction and amplification, high-throughput sequencing, and data analysis [15]. Unlike bulk sequencing, which provides an aggregate measure of genetic variation across a population of cells and cannot accurately represent an individual cell, single-cell sequencing is a technique that specifically targets and sequences the genetic material of a single cell. It analyzes the genome or transcriptome from the single-cell level; thus, it can accurately measure the gene structure and expression of a single cell. Therefore, single-cell sequencing can analyze the heterogeneity of cells with similar phenotypes. The technology allows biological research to reach an unprecedented resolution and scale and provides a new vision for life science research. Single-cell sequencing has undergone significant innovations in recent years. We show these innovations combined with the basic procedure of single-cell sequencing in Fig. 2, list the key points of each technology (Table 1), and summarize the different omics-based methods (Table 2) at the end of this section.

Single-Cell Genome Sequencing

Single-cell genome sequencing means the amplification and sequencing of genomes at the single-cell level. This technology can reveal differences between various cell populations and elucidate the process of cell evolution [25]. The current mainstream focus of single-cell genome sequencing is on copy number variation (CNV). CNV is the result of genomic rearrangements and is mainly manifested as sub-microscopic duplications or deletions. Such variation is an important submechanism in many human diseases and has become a hot topic of research, and single-cell sequencing has provided an advanced tool and perspective for analyzing CNV [79]. To investigate the role of CNV in hepatocellular carcinomas (HCCs), Guo et al. performed scDNA-seq and scRNA-seq to clarify the evolutionary model followed by CNV accumulation and identified CAD, a gene that participated in pyrimidine synthesis, as a biomarker of early recurrence in HCC [80]. In addition, single-cell whole genome sequencing (scWGS) is also an important technology. Hong et al. demonstrated the oncogenic properties of cGAS-STING signaling by this technology. Their work provided a strategy for the

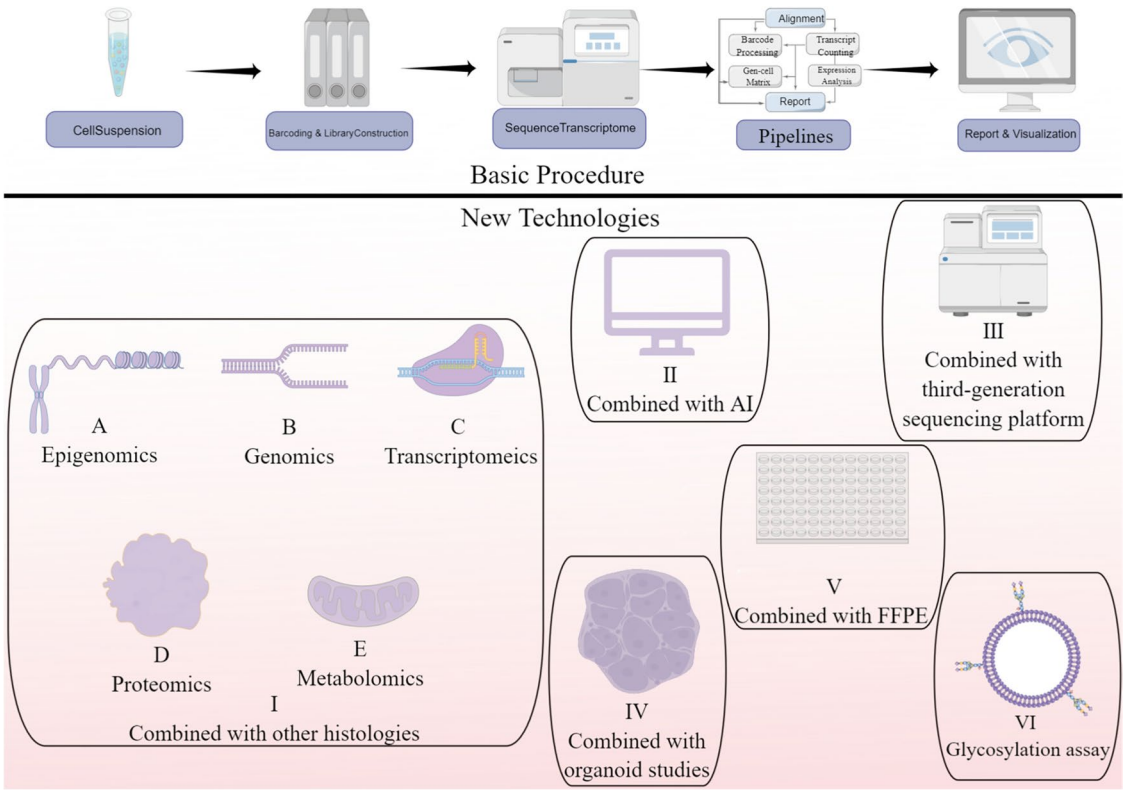

Fig. 2 Single-cell sequencing procedure and new technologies (By Figdraw.)

Table 1 A summary of different technologies

Technology	Description	Focus	Applications
Single-cell genome sequencing	Amplification and sequencing of genomes at the single-cell level	Copy number and whole genome	Lineage tracing [16]
Single-cell transcriptome sequencing	Sequencing the transcriptome at the single-cell level	Gene expression and cellular heterogeneity	Helping to solve the problems like low sample volume or cellular heterogeneity in cancer, immunology, etc. [1]
Single-cell epigenome sequencing	Dissecting the regulation of transcriptional activity at epigenetic levels as a complement to the transcriptome	Methylation, chromatin, histones, etc	Cellular gene regulatory networks and the study of genetic factors and disease risk [17]
Single-cell spatial transcriptome sequencing	Sequencing technology that measures gene expression while preserving spatial information	Spatial location of cells	Studies for which genetic labeling techniques are not applicable [18]
Single-cell ribosome sequencing	Combining nuclease footprint with the construction and expansion of small RNA libraries to measure translational dynamics in individual cells	Translational process	Studying the translation process in high resolution [19]
Single-cell multi-omics integration analysis	Techniques linking genetic, epigenetic, transcriptional, protein, and metabolic information in individual cells	Integration analysis	Lineage tracing, specificity mapping, cellular spatial information mapping, etc. [20]
Single-cell sequencing combined with AI	Analyzing single-cell sequencing data using AI tools	Data analysis	As a powerful tool for analyzing single-cell sequencing data [21]
Single-cell sequencing based on TGS platform	Methods for single-cell sequencing developed based on the TGS platform	High-fidelity reads	Identifying allele-specific gene expression patterns within individual cells [22]
Single-cell sequencing technology combined with organoid studies	Single-cell sequencing aids organoid studies with its ability to comprehensively classify cell types	Comprehensive classification of cell types	Disease and organ development modeling, drug development, and stem cell physiology [23]
FFPE in single-cell sequencing	Preservation of single-cell sequencing data with FFPE	Data preservation	Helping to select the best NGS method [24]

Table 2 Methods based on different omics to single-cell sequencing technologies

Technology		Method
Name	Feature	
Single-cell genome sequencing	Amplification and sequencing of genomes at the single-cell level [25]	SMOOTH-seq [26] DOP-PCR [27] MDA [28] MALBAC [29] LIANTI [30] PEP-PCR [31] dd-scCNV-Seq [32]
Single-cell epigenome sequencing	Resolving the mechanisms that regulate transcriptional activities at the epigenetic level [33]	scRRBS [34] scATAC-seq [35] scChIC-seq [36] scNOME-seq [37] Smart-RRBS [38] PBAT [39] scBS-Seq [40] scWGBS [41] scPBAT [42] scChIP-seq [43] CUT&Tag [44] Paired-Tag [45] MERFISH [46] sciMAP-ATAC [47]
Single-cell transcriptome sequencing	Sequencing the transcriptomic information at the level of single cells [48]	10X Genomics Chromium [48] Smart-seq2 [48] VASA-seq [49] CEL-seq2 [50] Drop-seq [51] inDROP [52] MARS-seq [53] SCRB-seq [54] Smart-seq [55] scCARE-seq [56]
Single-cell spatial transcriptome sequencing	Preserving spatial information while measuring the expression of genes [57]	MIA [6] Cell2location [58] SPOTlight [59] RCTD [60] Seurat [61] DestVI [62] spatialDWLS [63] DSTG [64]
Single-cell ribosome sequencing	Measuring translational dynamics and ribosome behavior on specific transcripts in single cells [65]	STAMP [19] Ribo-ITP [66] scSLAM-seq [67]

Table 2 (continued)

Technology		Method
Name	Feature	
Single-cell multi-omics sequencing	Linking multi-omics information such as genomics, transcriptomics, and epigenomics in single cells [68]	scCOOL-seq [69]
		scTrio-seq [70]
		G&T-seq [71]
		TARGET-seq [72]
		scM&T-seq [73]
		SNARE-seq [74]
		scCAT-seq [75]
		scNMT-seq [76]
		CITE-seq [77]
		LIGER [78]

treatment of chromosomally instable cancers that overexpress IL-6R [81]. And Huang et al. discussed the ability of the technology in terms of genome coverage, homogeneity, reproducibility, etc. This work facilitates the researcher in selecting a suitable kit [27].

Single-Cell Transcriptome Sequencing

Single-cell transcriptome sequencing is a technology that sequences the transcriptomic information at the level of single cells. It is applied to studying gene expression in individual cells. At the same time, it solves the problem of cellular heterogeneity that cannot be addressed by sequencing tissue samples. This technology includes full-length RNA sequencing and non-full-length RNA sequencing, with the hottest applications currently being Smart-seq2 for the former and 10X Genomics for the latter. They all have their own advantages. For example, Smart-seq2 has better coverage, can detect rare transcripts, and has a wider range of applications, while 10X Genomics is high throughput, is affordable, and can capture single cells more accurately [48]. In the following section, we are going to introduce the research progress made by single-cell transcriptome sequencing, using full-length RNA sequencing as an example.

Full-length scRNA-seq can yield highly complex libraries that contain thousands of different genes with excellent sensitivity and specificity for transcriptional quantification [49]. These full-length libraries have the advantage of detecting transcriptional isoforms, dissecting SNV, and allowing the assembly of VDJ regions of TCR and BCR [82]. Compared with non-full-length RNA sequencing, full-length RNA sequencing can discover more new genes and is more suitable for low-abundance transcripts and variable splicing transcripts. And full-length RNA sequencing has lower data noise for mRNAs with low expression levels [48]. Anand et al. investigated the mechanisms of drug resistance in a type of leukemia (ETP-ALL) with NOTCH1 mutations by full-length scRNA-seq analysis of tumor and microenvironmental cells. This study uncovers interactions between signaling, cellular plasticity, and immunity, illustrating the multidimensional nature of tumor heterogeneity. On this basis, they proposed combination therapies targeting different cancer states and immune functions, which may be successful in eradicating tumor cells that undergo immune evasion through simultaneous transcriptional programs [83]. In a paper published in 2020, Hagemann-Jensen M et al. described Smart-seq3, a technology that combines a full-length transcriptome counting strategy. Compared to Smart-seq2, Smart-seq3 is much more sensitive. It can detect thousands of transcripts in each cell. The authors also anticipate that Smart-seq3 will be able to describe the cellular features of different tissues and organisms on a large scale [84].

Single-Cell Epigenome Sequencing

Single-cell epigenome sequencing complements single-cell transcriptome sequencing. It resolves the mechanisms that regulate transcriptional activities at the epigenetic level [33]. The stable pattern of gene expression is maintained in part by epigenetic modifications of DNA and histones. The five main categories of mechanisms that regulate gene expression without altering DNA sequence are DNA methylation, chromatin accessibility, histone modifications, DNA–protein interactions, and chromatin steric structure [85]. Corresponding research methods are now available for each type of mechanism. Examples include scRRBS, a bisulfite-based sequencing method to detect DNA methylation at the level of single cells

[34], scATAC-seq, which identifies DNA regulatory elements involved in repressing or activating gene expression [35], scChIC-seq, which analyzes histone modifications at single-cell resolution [36], and so on. There are also single-cell epigenomic multi-omics techniques such as scNOME-seq that can analyze chromatin accessibility and DNA methylation status of individual cells [37]. Recently, Gu et al. also introduced Smart-RRBS, a new method for single-cell methylome and transcriptome analysis [38]. Dynamic regulation of the epigenome facilitates the establishment and maintenance of cellular identity and may support cellular plasticity and behavior. The single-cell epigenome complements the transcriptome by providing insights into cell type-specific gene expression regulation. It is widely used in studies that dissect cellular gene regulatory networks and link genetic factors to disease risk. Yu et al. analyzed peripheral blood mononuclear cells (PBMCs) from SLE patients using methods including scATAC-seq and identified several transcription factor (TF) activation patterns and key TFs, seven of which showed important binding sites in SLE patients. Their findings revealed key TFs in PBMCs in patients with SLE and provided helpful insights for epigenetic therapy [17]. Zhang et al. performed single-cell chromatin accessibility assays on a large number of adult human tissues and, in conjunction with previous studies, systematically explained non-coding variants associated with specific human characteristics and diseases, providing advanced insights for analyzing gene regulatory programs in human cell types across tissues, organs, systems, and life stages [86].

Single-Cell Spatial Transcriptome Sequencing

The spatial organization of cells plays a central role in normal development, homeostasis in vivo, and pathophysiology. Spatial transcriptomics can preserve spatial information while measuring the expression of genes. Srivatsan et al. introduced sci-Space into this technique, which resolves larger spatial heterogeneity while retaining single-cell resolution. Their study applied to mouse embryonic development revealed differences in spatial patterns of different cell types, and they anticipated that sci-Space would facilitate the establishment of single-cell spatial maps of mammalian development [57]. In addition, Baccin et al. systematically constructed the molecular and cellular atlas of the bone marrow (BM) niche in the bone, sinus, and small arteries combining single-cell and spatially resolved transcriptomics. Their study reveals the cellular and spatial organization of the BM niche, providing a new perspective for systematically dissecting the complex organization of BM [87]. In the context of tumor therapy, Qi et al. found a positive correlation between a type of fibroblast (FAP+) and a type of macrophage (SPP1+) in colorectal cancer. In addition to single-cell analyses, they verified their close association by immunofluorescence staining and spatial transcriptomics. The results of this study provide a potential therapeutic option to improve treatment outcomes by disrupting the associated fibroblast-macrophage interaction [88].

Single-Cell Ribosome Sequencing

Single-cell ribosome sequencing (scRibo-seq) combines nuclease footprint with the construction and expansion of small RNA libraries to measure translational dynamics in individual cells, providing further evidence for the translational process and significant differences between cells that appear to be identical. It also provides a ribosome analysis method that measures ribosome behavior on specific transcripts in a single cell population with high sensitivity and high resolution to individual codons. These advantages allow the translation process to be resolved in greater detail and provide evidence for widespread alterations in translational regulation during mitosis [65].

Although this new technology has not yet been widely applied to the study of clinical diseases, researchers have been refining the method to maximize its benefits. For example, Brannan et al. developed STAMP (Surveying Targets by APOBEC-Mediated Profiling) to explain the role of RNA-binding proteins in gene expression and RNA processing in individual cells. It enables the study of translational landscapes at unprecedented cellular resolution [19]. Ozadam et al. developed ribosome profiling via ITP (Ribo-ITP). This method provides high coverage and resolution ribosome occupancy results from low input samples like single cells. The authors' team used this method to characterize the translation of individual oocytes and embryos during early mouse development [66]. It is believed that the technology will have a bright future of application with the researchers' explorations.

Single-Cell Multi-omics Integration Analysis

Single-cell multi-omics techniques link genetic, transcriptional, and epigenetic information in individual cells. In addition to the previously mentioned genomics, transcriptomics, and epigenomics, it is also possible to integrate analyses such as proteomics and metabolomics. Nam et al. discussed emerging single-cell multi-omics analysis and experimental techniques. Data captured and integrated suggest that cancer is the result of a complex interaction between genetic and non-genetic factors in the evolution of somatic cells [89]. Stephenson et al. performed a single-cell multi-omics analysis of multiple PBMCs from patients with different severity of novel coronavirus pneumonia.

This study highlighted the coordinated immune response that promoted the understanding of COVID-19 pathogenesis and revealed single-cell components that can be used as therapeutic targets [68]. In addition, Fasolino M et al. also performed a single-cell multi-omics analysis of pancreatic islets, revealing a new cellular state in type 1 diabetes (T1D). Their study revealed cell types and processes that might be involved in the immunopathogenesis of T1D and provided an innovative program for the comprehensive exploration and discovery of human pancreatic functions [90].

Single-Cell Sequencing Combined with Artificial Intelligence

With the development of artificial intelligence (AI), the scope of application of AI tools in the life sciences is expanding, and it is gradually showing great potential in the field of single-cell sequencing. Combined with AI, single-cell data can be analyzed and understood more effectively [21]. Chen et al. created an AI tool that uses single-cell sequencing data from the Drosophila visual system to identify genes expressed only in certain cell types. By feeding scRNA-seq data into their algorithm, they identified genes that are uniquely expressed at different developmental stages in most cell types in the Drosophila visual system and discovered a completely new cell type [91]. Besides, deep learning algorithms developed from AI have emerged as a powerful tool for the analysis of scRNA-seq data, which can identify potential information from scRNA-seq data, favoring the interpretation of heterogeneity between different scRNA-seq experiments [92]. And Bao et al. demonstrated the method's reliability for biomedical applications [93].

Single-Cell Sequencing Based on Third-Generation Sequencing Platform

Third-generation sequencing (TGS) is a single-molecule sequencing technology that does not require PCR amplification for the sequencing process. It has long read lengths, much longer than the second-generation sequencing [13]. To address the challenge of detecting structural variation (SV) and extrachromosomal DNA (ecDNA) in single cells, Fan et al. developed a new scWGS method based on a TGS platform and named it SMOOTH-seq. In their study, they evaluated methods to detect CNV, SV, and single nucleotide variants (SNV) in cancer cells and showed that SMOOTH-seq safely and efficiently detects SV and ecDNA in single cells but showed relatively limited accuracy in detecting CNV and SNV. Overall, however, SMOOTH-seq has enabled scWGS to enter a new phase of development due to its ability to generate high-fidelity reads [26]. Fan et al. also developed a new scRNA-seq technique based on a TGS platform named SCAN-seq, which has higher sensitivity and accuracy. In addition, they used the technique to analyze mouse preimplantation embryos and showed that it distinguished cells at different developmental stages and identified many transcripts that exhibited developmental stage-specific expression patterns. They also found that SCAN-seq has high accuracy in identifying allele-specific gene expression patterns within individual cells. This technology represents a breakthrough in the field of single-cell transcriptome analysis [22].

Single-Cell Sequencing Technology Combined with Organoid Studies

Organoids are 3D cellular collections of organ-specific cell types that develop from stem or progenitor cells. They are capable of self-assembling in a way similar to that in vivo through cellular sequencing and spatially restricted lineage differentiation [94]. Single-cell sequencing technologies, especially scRNA-seq, allow for a comprehensive classification of cell types. In combination with organoid studies, single-cell sequencing facilitates a better understanding of how organoids apply to the development of the corresponding organ and the mechanisms of related diseases [95].

Yoshihara et al. used scRNA-seq in a study to generate functional human islet-like organs for the treatment of diabetes and performed three biological replicates to improve the reliability of the results [96]. Czerniecki et al. used technologies including scRNA-seq to identify parietal, mesenchymal, and partially differentiated compartments in organoids and to identify pathways that can dilate the vascular endothelium. They also developed an automated platform that allows for high-throughput screening in combination with other analytical techniques. This platform unexpectedly revealed the role of myosin in polycystic kidney disease [97]. In a review, Khedoe et al. suggest that combining models such as organoid and organelle technologies with advanced analytical platforms such as single-cell sequencing may help to elucidate the pathogenic mechanisms of systemic sclerosis-related interstitial lung disease (SSc-ILD) and identify new therapeutic targets [98]. In addition, a systematic review of recent advances in scRNA-seq, organoid, and their current application areas was conducted by Yin et al. in 2021, summarizing the advantages of combining scRNA-seq and organoid technologies in modeling disease and organ development, drug development, and stem cell physiology [23].

Formalin Fixation and Paraffin Embedding in Single-Cell Sequencing

Formalin Fixation and Paraffin Embedding (FFPE) sections can detect visual features of disease that can be correlated with valuable clinical data. FFPE technology is a valuable

resource for medical pathology and mechanistic studies, drug discovery, and retrospective studies due to its advantages such as long preservation time and the ability to correlate clinical and multi-omics data. Progress in multi-imaging techniques has greatly enhanced the ability to characterize healthy and lesion tissues at the level of single cells. And CODEX, a method of detection, provides insight into the spatial relationships of single cells in tissues. Black et al. performed a multi-cycle imaging procedure on FFPE and fresh frozen tissue using methods that included both techniques [99]. McDonough et al. evaluated DNA extraction methods using FFPE samples from different tissue types. This work helps to select the best NGS method, and the selection of DNA extraction and library preparation methods affects the capabilities of archival tissues in NGS [100]. However, genetic material (DNA and RNA) used for NGS can be problematic due to fixation. Cazzato et al. discussed the use of FFPE tissue samples in NGS execution, focusing on the problems that arise when using this material for nucleic acid extraction. They also developed the most effective strategies to prevent and decline SNV and other fixation artifacts [24].

Significance of Single-Cell Sequencing

Single-cell sequencing enables heterogeneous analysis, which is the core reason why this technology can be used in research in major medical fields. The significance of single-cell sequencing in the study of AIDs and other diseases will be described in several ways as follows.

Constructing a Cell Atlas

Cell atlas is the digitization of cells. It uses a matrix of numbers to describe the characteristics of each cell and classify them systematically. Mapping various cell types in the human body is currently a major goal in the field of scientific research, which contributes to a deeper understanding of biology, medicine, and diseases. It has many therapeutic implications, with applications as diverse as anti-tumor immunology, vaccine development, regenerative medicine, and so on [101].

Wang et al. analyzed the composition of peripheral blood immune cells in patients with RA, SLE, and primary sicca syndrome (pSS) using scRNA-seq data. The results showed a strong gene expression in megakaryocyte (MK) expansion and identified certain subpopulations of MKs with marked cellular heterogeneity [102]. Zhang et al. used a comprehensive strategy based on typical correlation analysis of scRNA-seq profiles to identify 18 unique cell populations driving inflammation in RA joints. They combined the strategy with bulk cell counting and transcriptomics to identify several cell state expansions in RA synovium. The identification of these cell populations facilitates the elucidation of RA pathogenesis [103]. Other scientists have also conducted animal experiments. Zakharov PN et al. investigated islet-infiltrating cells from autoimmune diabetic mice using scRNA-seq. The data revealed transcriptional heterogeneity between lymphocyte and myeloid subpopulations. A progressive activation program experienced by resident macrophages in the islet microenvironment was also observed. This study reveals that diabetic autoimmunity arises from distinct transcriptional cell populations. It provides a single-cell picture that defines the staging of autoimmune diabetes [104]. To investigate the effects of aging on related AIDs by affecting lymph node function, Li et al. used techniques such as scRNA-seq to map immune cells in the cervical draining lymph nodes of mice associated with experimental autoimmune uveitis (EAU). The results suggest that aging counteracts EAU injury in aged mice by modulating the role of immune cells, especially Th17 cells [105].In addition, the germinal center (GC) response is essential for adaptive immunity as well as for establishing peripheral immune tolerance. Its dysfunction may lead to AIDs. To understand the gene regulation of the GC response, King et al. produced single-cell transcriptomic and epigenomic profiles of human tonsils, characterized different immune cell subpopulations, and constructed trajectories of gene expression during B cell functioning. These analyses provide a new and effective resource for explaining the gene expression and cellular interactions in AIDs [106]. In addition to AIDs, single-cell sequencing has also played a role in the construction of atlas for other diseases. Suo et al. analyzed nine prenatal tissues in conjunction with scRNA-seq and other techniques to create a developmental map of the human immune system spanning nine organs. They revealed the process of blood and immune cell formation, which will help to enhance the understanding of immune diseases. They also identified a novel type of B cell, as well as unique T cells that appear in early life stages. This work provides new research resources and biological insights to inform cell engineering and regenerative medicine research [107]. To further characterize the cellular features of aging and those ameliorated by caloric restriction (CR), Ma et al. have established the most comprehensive single-cell transcriptome sequencing and single-cell nuclear transcriptome sequencing profiles to date. They describe possible mechanisms of action of CR to delay aging and explain the possibility of amelioration of aging through metabolic intervention acting on the immune system [108].

Contributing to the Pathogenesis

Pathogenesis refers to the combination of physiological, biochemical, genetic, and environmental factors that cause the occurrence of a disease. It's important to the prevention and

treatment of diseases. On the one hand, single-cell sequencing can identify cell subtypes, reveal inter-cell interactions, and elucidate the pathogenic process of diseases in terms of abnormalities in physiological and biochemical pathways. On the other hand, it can be used for cytogenetic analysis to search for the origins of diseases from the expression of genetic material.

Wang et al. analyzed samples from non-small cell lung cancer patients with scRNA-seq. They mapped the whole-body immune cell transcriptome and revealed the central role of cytotoxic and effector T cells, NK cells, and macrophages in the immune microenvironment between lung adenocarcinomas and squamous carcinomas. This study deepens the understanding of the pathogenic process of lung cancer, and these cellular interactions can be used to design personalized therapeutic regimens for patients in clinical practice [109]. What's more, Wang et al. identified immune cell subpopulations on PBMCs by techniques such as scRNA-seq and found similar transcriptional profiles in several AIDs such as RA and SLE, with some gene expression profiles being associated with ribosome assembly and hemostasis. This provides advanced insights into the peripheral immune cell profiles of several immune diseases including RA and SLE and suggests that aberrant regulation of MK expansion may be one of the pathogenic mechanisms of these diseases [102]. Wu et al. analyzed CD45 + cells by scRNA-seq to identify immune cell subsets involved in the pathogenesis of RA subtypes. They identified several molecules associated with synovial immune cell abnormalities and revealed the significance of anti-citrullinated peptide antibodies (ACPA) in RA with the help of immunohistochemical staining. Their data suggest differences in cellular and molecular pathways in the pathogenesis of RA subtypes with different serologic responses and highlight the importance of precise treatment based on ACPA status [110]. In addition, numerous studies have shown that virus infection is an important factor in the initiation of AIDs [111]. Single-cell sequencing can reveal the peripheral immune characteristics of patients with virus infection [112, 113], which can also contribute to the pathogenesis of AIDs caused by virus infection.

Helping in Disease Diagnosis

Due to its high resolution, single-cell sequencing can detect characteristic cellular molecules of some diseases, which can be used as a clinical indicator for screening, identification, and diagnosis of diseases.

Zheng et al. analyzed data differences of skin damage in skin tissues from discoid lupus erythematosus (DLE) patients, SLE patients, and healthy controls (HCs) by scRNA-seq. On the one hand, they found that the percentage of T cells, B cells, and NK cells was higher in DLE than in SLE. This facilitated the differential diagnosis between DLE and SLE. On the other hand, amplification of some cell subpopulations was found in DLE and SLE compared to HC, and cellular communication between cell types such as fibroblasts and macrophages/dendritic cells was more complex in DLE and SLE. In conclusion, they elucidated the heterogeneous features of skin lesions between DLE and SLE and identified some specific cell subtypes and ligand-receptor pairs, which provide advantages for the diagnosis and treatment of lupus erythematosus [114]. What's more, Zhang and Lee constructed TCR and BCR sequences from a large amount of peripheral blood RNA-seq data from SLE and RA patients and then analyzed the clonality and diversity of the immune sequences between them, revealing characteristic changes in the proportions of cellular subpopulations in both diseases [115]. Trzupek et al. demonstrated that the presence of LGALS9 and some other components in platelets can be used as a clinical biomarker of RA. This study provides advanced insights into the diagnosis and treatment of these two diseases [116]. In addition, Chang et al. demonstrated the effectiveness of cell-based noninvasive prenatal testing (cbNIPT) by single-cell sequencing and further explored the role of scWGS and haplotype analysis with cbNIPT for various monogenic disorders including genetic deafness, hemophilia, and greater vestibular aqueduct syndrome. It shows that cbNIPT based on scWGS and haplotype analysis has great potential for prenatal diagnosis of various monogenic diseases [117].

Identifying Therapeutic Targets

In the same way that single-cell technologies can be used to elucidate the pathogenesis of disease, research that focuses on certain key genes, molecules, cells, or pathways of action in the pathogenesis of disease can lead to the discovery of new targets for disease treatment.

Liu et al. analyzed peripheral blood samples at the molecular and single-cell level in conjunction with phenotypic, transcriptomic, and BCR profiling and elucidated the mechanism of impaired incompetent B cells in SLE patients, revealing the key role played by IL-4 in reversing incompetent B cells in SLE and improving the understanding of B cell autoimmunity. They established a theoretical basis for the treatment of SLE through the blockade of IL-4 signaling. This work provided a subsequent and potentially clinical therapeutic approach for early intervention and prevention of SLE [118]. Kobayashi et al. identified an inflammatory gene module and a single cell population associated with SSc pathophysiology by combined analysis of bulk and scRNA-seq analyses. They may serve as candidate therapeutic targets for SSc [119]. Martin JC et al. applied single-cell technology to ileal CD lesions and identified a cellular module leading to resistance to anti-TNF therapy. This study points out, on the one hand, the limitations of current diagnostic

assays. On the other hand, it highlights the potential of single-cell tools in identifying new targets for therapy and tailoring therapeutic opportunities [120]. Hua X et al. used single-cell sequencing after constructing a mouse model of experimental autoimmune myocarditis (EAM) to identify genes involved in the inflammatory response to myocarditis and investigated the immune network during the transition from myocarditis to cardiomyopathy. They found that macrophages play an important role in all stages of the disease. They identified clusters associated with inflammation, in which the level of Hif1a-type expression correlated with the degree of inflammation. They suggested that Hif1a-type inhibitors could attenuate inflammatory cell infiltration in EAM and could be potential therapeutic targets in the clinic [121]. There are also researchers having made use of scRNA-seq datasets to analyze the relationship between cancer stemness and immune checkpoint inhibitor response. Zhang Z et al. developed the Stem.Sig gene as a potential therapeutic target and found the potential application of this research in overcoming immune tolerance [122]. Lee et al. applied scRNA-seq to the clinical evaluation of an oncogene homologue (HRAS), demonstrating the efficacy of scRNA-seq in observing the tumor microenvironment and identifying molecular and cellular therapeutic targets in certain refractory cancers [123].

Developing Prognostic Models

Prognostic models are used to predict the probability of a patient's stage of disease progression, whether they are cured, whether they die, and other events at some point in the future. Single-cell sequencing can screen for key genes to help clinicians or researchers develop prognostic models and make more accurate prognostic judgments.

Wang et al. combined machine learning algorithms and bioinformatics of single-cell sequencing analysis to analyze common biomarkers and pathways in SLE and metabolic syndrome (MetS). They successfully constructed prognostic models using the screened genes, which are associated with immune and metabolic processes. They also constructed an effective diagnostic model for the two diseases and found that two biomarkers expressed mainly by monocytes had the highest diagnostic efficacy. This work provides advanced insights into the pathogenesis of SLE combined with MetS and the development of new combination therapies [124]. Zhang et al. investigated immune cell dysregulation in peripheral blood samples from SLE patients by scRNA-seq and some other technologies. The results revealed that the abundance and dysfunction of CD8 + CD27 + CXCR3- T cells could be used as a potential biomarker for SLE diagnosis and prognosis. They experimentally constructed a dynamic network biomarker (DNB) model whose scores accurately predicted SLE disease progression and could provide recommendations for clinical treatment such as drug dosage determination [125]. Mao and Xu used single-cell sequencing data to identify the cell types that contribute to bladder cancer (BC) aggressiveness and combined several analytical techniques to localize the genes involved to further develop a BC prognostic model to analyze feedback on immunotherapy from patients at different stages, and the role of these prognostic genes was validated by techniques such as Western blot [126]. Sun et al. analyzed data by techniques including scRNA-seq from patients with intrahepatic cholangiocarcinoma (ICC) and found a relationship between SPP1 CD68 tumor-associated macrophages (SPP1-TAM) and clinicopathological features of the disease. They also recognized the prognostic significance of SPP1-TAM and pointed out that it can be an independent poor prognostic indicator for survival in ICC [127].

Single-Cell Sequencing Applied to Various AIDs

Systemic Lupus Erythematosus

SLE is an AID in relation to multiple organs, mainly affects young women, and may present with symptoms like fever, sensitivity to light, rash, and so on [128]. The development of SLE is associated with aberrant activation of T and B lymphocytes and the formation of immune complexes in tissues and organs by a large number of autoreactive antibodies and antigens. Many studies have reported that peripheral blood T lymphocyte deficiency may be one of the pathogenic mechanisms of SLE. There is also evidence that B cells play an important role in the pathogenesis of SLE. The diversity of TCR and BCR is also involved in the determination of the autoimmune response [129]. To further understand the pathogenesis of SLE, many researchers have conducted studies with the help of single-cell sequencing.

Nehar-Belaid et al. analyzed PBMCs from children with SLE and control children using scRNA-seq. It was suggested that the expression of interferon-stimulated genes (ISGs) in the cells of pediatric patients with SLE was much higher than that in the cells of HC subjects. The high expression of ISGs was mainly found in immune cells, especially plasma cells. The expansion of such a unique subpopulation allowed for the classification of patients with high disease activity. It was also demonstrated that a similar subpopulation is also amplified in adult patients. This study lays the foundation for exploring the transcriptional signature of SLE and the origin of disease heterogeneity [130]. Zheng et al. revealed peripheral blood cell types in SLE patients, identified the associated TCR/BCR, and characterized the biological process of its pathogenesis by single-cell sequencing. Their findings first elucidated the differences in immune

cell subpopulations between SLE patients and HCs. They explored the transcriptional profiles of these subpopulations to figure out the pathogenesis of SLE. And several TCR/BCR that they identified could be used as diagnostic or therapeutic targets. In conclusion, they used single-cell sequencing to reveal the transcriptomic profile of immune cells from SLE patients and their immune functions [131]. Perez et al. also developed "multiplexed scRNA-seq" to explore cells, transcription, inheritance, and variation associated with SLE [132]. Recently, Dong et al. performed single-cell sequencing and immunoassay analysis of BM and peripheral blood B cells from SLE patients and HCs. They reported for the first time the presence of developmental differentiation disorders of BM early B cells in some SLE patients. This study reveals the abnormal immunological characteristics of BM B cells in SLE patients, which will be helpful for accurate typing and precise diagnosis and treatment of SLE patients, and also provides advanced perspectives for further exploring the pathogenesis and intervention strategies of SLE [133]. Furthermore, Trzupek et al. used a single-cell multi-omics technology with proteins and mRNAs to map high-resolution T cell and NK cell populations in the blood of patients with SLE, providing a new peripheral blood cellular marker of the disease's activity [116]. Cui et al. have also made use of scRNA-seq data, which were integrated and analyzed to identify patterns of transcriptional and epigenetic regulation in SLE and revealed cellular subpopulations associated with these mechanisms [134]. The above studies showed that SLE is closely associated with abnormalities in the number or function of T and B cells and that the abnormal TCR/BCR clonotypes can be used as diagnostic or therapeutic targets. Most current cell atlas of SLE involves only peripheral blood cells, but SLE can also cause damage to parenchymal organs such as kidneys, lungs, and liver [135]. Researchers can try to construct a cell atlas of the tissues and organs involved in SLE and systematically compare the similarities and differences in cell types between them. In addition, attempts can be made to construct a cell atlas of the various subtypes of SLE and to analyze single-cell changes before and after treatment. And most of the current studies are using scRNA-seq to reveal the transcriptomic features of SLE. Perhaps more genomic or epigenomic studies could be conducted in the future.

Rheumatoid Arthritis

RA is an AID with synovitis as the pathological basis. It is characterized by symmetrical, aggressive arthritis of the hands and feet with positive serum rheumatoid factor, which can affect joint function and often involves extra-articular organs [136]. Synovial macrophages and synovial fibroblasts (SFs) are the core target cells of RA. Identifying key cell subpopulations in inflammatory tissues and their activation status is a significant step in identifying new therapeutic targets for RA.

Many investigators have found that a subpopulation of fibroblasts located in the parietal lamina undergoes significant expansion and is associated with RA activity. However, the exact mechanisms underlying the differentiation and expansion of these cells remain unclear. Many scientists have been working to investigate the mechanisms involved. Wei et al. made use of scRNA-seq to identify the key role of NOTCH3 signaling in the differentiation of certain fibroblasts. The signaling can drive transcriptional and spatial gradients in vascular endothelial fibroblasts. They also conducted animal experiments using mice as a model and found that deleting the NOTCH3 gene or blocking NOTCH3 signaling attenuated the inflammatory response and prevented joint damage. These findings suggest that synovial fibroblasts are regulated by endothelium-derived Notch signaling, which underlies the inflammation and pathology in inflammatory arthritis [137]. Cheng et al. have reviewed the breakthroughs achieved by scRNA-seq technology in recent years in the study of two types of synovial cells, SFs and synovial macrophages [138]. In addition, many studies have begun to focus on the role of fibroblast-like synoviocytes in the pathogenesis of RA and have used the new technique of single-cell combined with comprehensive multi-omics analyses to analyze the process of their action [139]. For example, Kenney et al. used multicomponent spatial and single-cell transcriptomics to investigate changes in cellular composition in the sinuses of co-draining lymph nodes to explain the molecular mechanisms by which aberrant lymphatic drainage and B cells lead to severe RA. And they identified that macrophages and CD6 + T cells may play an important role in IgG2b class switching [140]. These studies identified abnormal cell subpopulations in RA and revealed the mechanisms by which inflammatory signals such as NOTCH3 signaling act on targets including synovial fibroblasts and synovial macrophages to cause arthritis. In the future, studies on changes before and after treatment in single cells, especially immune cells, could be initiated more extensively.

Systemic Sclerosis

SSc is a relatively rare chronic AID of connective tissue characterized by cutaneous fibroplasia and vascular onion skin-like changes that may eventually progress to skin sclerosis and vascular ischemia, which can involve the skin and may also affect organs such as the heart, lungs, and digestive tract [141].

Apostolidis et al. identified endothelial cell (EC) markers and characteristic pathways associated with SSc injury with the help of scRNA-seq to explain the mechanism of SSc injury and propagation. The results showed that EC in SSc patients is related to vascular injury, activation, and

generation as well as extracellular matrix production. They also identified two top markers of EC in SSc [142]. Gaydosik et al. made use of scRNA-seq to study immune responses mediated by T cells in the affected skin of SSc patients and identified a special cluster of T cells that may be associated with B cell responses. Single-cell transcriptome analysis has a strong advantage in analyzing specific T cell heterogeneity. It facilitates the identification of functional genes associated with SSc, which could be useful in determining the immune mechanisms of SSc and suggesting more targeted and innovative therapies [143]. Kobayashi et al. also characterized SSc-associated gene network modules and immune cell clusters at single-cell resolution, providing new candidate targets for the treatment of SSc [119]. In addition, to gain more insight into how alterations in the fibroblast phenotype contribute to SSc-ILD fibrosis, Valenzi et al. made use of scRNA-seq of lung tissue samples from healthy individuals and SSc-ILD patients to identify transcripts of the mesenchymal cell population. They compared and found that myofibroblasts underwent the greatest phenotypic changes in SSc-ILD, where the expressions of collagen and other pre-fibrotic genes were also substantially upregulated. This study suggests that myofibroblast differentiation and proliferation are key pathological mechanisms of SSc-ILD fibrosis [144]. These studies explain some of the mechanisms by which SSc causes skin sclerosis and vascular damage. They revealed abnormal T cells in the skin that mediate the immune response causing fibrosis as well as pathways that damage ECs. As fibroplasia is a feature of SSc, future single-cell studies on SSc could focus on myofibroblasts and their subtypes. It is also feasible to analyze cell atlas of different subtypes of SSc.

Systemic Vasculitis

SV is a group of chronic AIDs with inflammatory cell infiltration of blood vessels, vascular destruction, and tissue ischemia and necrosis as the major pathological changes. It can often lead to multi-system and multi-organ dysfunction or can be limited to a particular organ [145]. This group of diseases includes giant cell arteritis (GCA), polyarteritis nodosa (PAN), Kawasaki disease (KD), Behcet disease (BD), ANCA-associated vasculitis (AAV), Henoch-Schonlein purpura (HSP), and so on.

Wang et al. used single-cell techniques to map immune cells in the blood of GCA patients more comprehensively, identifying selected cellular subpopulations that were significantly correlated with clinical phenotypes and treatment response. They found that immature neutrophils can lead to protein oxidation and enhanced endothelial permeability through respiratory bursts. The same population has been detected in other SVs. These findings suggest a relationship between immature neutrophils and pathogenesis and establish the clinical cellular profile of GCA, and the authors sequentially propose different therapeutic approaches for systemic vascular inflammation [146]. Carmona EG et al. have used single-cell transcriptome analysis to reveal the role of cytotoxic CD4 + T cells in the inflammatory and vascular remodeling process of GCA. They also made recommendations for targeting CTL as a potential treatment option [147]. Narsinh et al. applied scRNA-seq with endovascular biopsy to study the course of pathogenic vertebral basilar artery aneurysm in patients with PAN and identified a subpopulation of endothelial cells associated with cerebral aneurysms in PAN [148]. Wang et al. analyzed PBMCs isolated from patients with acute KD using scRNA-seq. They found that monocytes are a major source of pro-inflammatory mediators and that each cell type has a unique global and dynamic immune response throughout the disease. The finding facilitates understanding of the pathogenesis of KD and its treatment. They suggested that PBMC was a therapeutic target for KD [149]. Zheng et al. analyzed PBMCs from BD patients and healthy donors using scRNA-seq. Their analysis demonstrated that C1q-high monocytes can affect the inflammatory response in BD by enhancing phagocytosis and promoting the secretion of pro-inflammatory cytokines, suggesting that C1q could be used as a therapeutic target and an indicator of clinical evaluation for BD [150]. What's more, Shi et al. performed a multi-omics single-cell study of BD, identifying extensive cellular heterogeneity and disease-associated immune responses in terms of transcription and epigenome [151]. In conclusion, abnormal immune cells and cytokines in peripheral blood are suggestive of SV. As SV encompasses a list of diseases, it is necessary to construct a comprehensive atlas to link the various diseases.

Sicca Syndrome

SS is a chronic inflammatory AID. It mainly affects the exocrine glands, often resulting in dry mouth and eyes due to hypoplasia of the salivary and lacrimal glands. In addition to this, it can involve other exocrine glands and organs, with clinical signs of multi-system damage [152].

Horeth et al. made use of scRNA-seq of the submandibular gland (SMG) of an established mouse model of pSS to detect and characterize the molecular and cellular heterogeneity of the SMG cell population. Single-cell transcriptome studies revealed the diversity of immune cell dysregulation in SS, particularly revealing the activation status of salivary gland epithelial cells. In summary, their extensive studies have not only revealed pathway mediators and biomarkers of the disease but also demonstrated the complex nature of the cell populations in the SMG that may promote researches in SS. These new findings facilitate the understanding of the basic molecular mechanisms and cellular states of SS and will provide more effective information for identifying

therapeutic targets [153]. Similar to the above studies, to explore the cellular and molecular mechanisms related to the pathogenesis of SS, Hong et al. analyzed PBMCs from pSS patients and HCs using scRNA-seq, revealing immune cell subsets and susceptibility genes associated with pSS. The results suggested that the number of T cells, the percentage of cytotoxic T cell subsets, the expression levels of interferon, and susceptibility genes such as HLA-DRB5 were higher in pSS patients than in HCs. These data reveal this disease-specific cell subpopulation and provide some new targets for pSS. The investigators also hypothesized that cytotoxic T cells may be related to the pathogenesis of pSS, providing a possible direction for the treatment of pSS [154]. These studies identified abnormal immune cell populations in the peripheral blood and abnormal activation of glandular ECs of SS. The current studies mainly focus on transcriptional characteristics of SS by scRNA-seq. More single-cell technologies could be applied to reveal genomic, epigenomic, and even proteomic information.

Inflammatory Bowel Disease

IBD is an idiopathic inflammatory disease of the intestine. It mainly includes ulcerative colitis (UC) and Crohn's disease (CD) [155]. Common etiologies are related to environmental, genetic, infectious, and immune factors.

To investigate the mechanisms determining mucosal dysfunction in IBD patients, Mitsialis et al. used mass spectrometry to analyze immune cell populations of IBD patients and HCs at single-cell resolution. The results showed increased numbers of T regulatory cells, CXCR3 + plasma cells, and other inflammatory cytokine-producing cells in the IBD patient samples. The findings were then validated by scRNA-seq. They also comparatively investigated the differences in inflammatory responses between patients with UC and CD. The results of this study could help in developing specific treatments for patients with different types of IBD [156]. In addition, a study reported an increase in Gasdermin B (GSDMB) in IBD. Gasdermins belong to a family of structurally related proteins that are often found in studies on febrile diseases. Among them, GSDMB is well associated with genetic susceptibility to chronic mucosal inflammation [157, 158]. This study determined the specificity of epithelial cells to inflammatory colonocytes by single-cell analysis. They found that the proliferative activity of GSDMB-deficient cells was reduced and the adhesion of GSDMB-deficient intestinal epithelial cells was increased, which was detrimental to wound healing. They concluded that GSDMB could regulate epithelial repair independently of apoptosis and that it was a key factor in restoring the function of the epithelial barrier and reducing inflammation. It was of therapeutic importance for diseases with barrier function including IBD [159]. These studies revealed specific inflammatory cells and inflammatory cytokines acting on the intestinal mucosal ECs. More detailed cell atlas with more samples of the two diseases could be constructed in the future.

Dermatomyositis

DM is a non-purulent inflammatory lesion mainly involving the transverse muscle with a predominantly lymphocytic infiltrate and may be associated with a variety of skin lesions. It is mainly characterized by symmetrical weakness of the upper body, often involving multiple organs, and can be combined with other connective tissue diseases and even tumors.

DM lesions are similar to cutaneous lupus erythematosus (CLE) lesions to the extent that they are often indistinguishable. To identify unique features of both, Tsoi et al. compared the transcriptional profiles of DM and CLE lesion tissues to identify a potentially novel molecular signature. Previous studies have shown that DM and CLE share common IFN-I signaling pathways such as IFN-κ upregulation, while at the same time, there are other inflammatory pathways present in CLE. In addition, they noted that DM lesions can be distinguished from CLE by a certain genetic biomarker, which includes upregulated expression of IL-18. Using scRNA-seq, they further identified keratin-forming cells as the primary source of IL-18 increase in patients with DM. This study emphasizes the pathogenic role of IL-18 in DM and has important clinical significance for differentiating DM from CLE [160]. In addition, for a long time, there has been a lack of appropriate biomarkers and therapeutic approaches to control juvenile dermatomyositis (JDM) due to the poor understanding of the cell types that mediate this disease. Neely et al. used single-cell sequencing combined with a proteomic approach to study immunophenotypes and disease-related specific genes in PBMC from JDM patients in different stages of the disease. They yielded a large amount of investigationally significant data. These data provided new insights for probing immune dysregulation in JDM and new resources for follow-up studies in myositis [161]. These studies revealed that upregulation of IL-18 expression is a characteristic manifestation of DM, and the infiltration of specialized lymphocytes is also suggestive. Future single-cell studies could focus on the differences in cell atlas between two different types of DM in adults and juveniles.

IgG4-Related Diseases

IgG4-related disease (IgG4-RD) is a malignant, infectious, and inflammatory disorder mediated by multiorgan immunity. It is characterized by significantly increased serum

IgG4 levels. The main pathological features of the disorder are lymphoplasmacytic infiltration, storiform fibrosis, and occlusive phlebitis [162]. Pharmacological treatments such as glucocorticoids are preferred [163], the degree of fibrosis is strongly correlated with responsiveness to immunosuppressive therapy, and the specific antigens and T cell clones responsible for the disease are paramount in elucidating its pathogenesis [164].

In an attempt to clarify the transcriptional profiles of immune cell subpopulations of IgG4-RD at the level of single cells, Wu et al. assessed specific cellular subpopulations and pathways in IgG4-RD PBMCs by using single-cell sequencing. They identified relevant cell types and isoforms, which strengthened the understanding of transcriptional profiles and cellular heterogeneity of IgG4-RD [165]. Munemura et al. used techniques such as scRNA-seq to study certain immune cells in diseased tissues of IgG4-RD patients to identify cellular factors that promote class switching in this type of fibrotic disease. Their analyses revealed a subpopulation of IL-10-expressing Tfh cells associated with infiltration of affected organs in IgG4-RD patients [166]. Li et al. also made use of scRNA-seq analysis of SMG and PBMC from IgG4-RD patients and controls. They identified three new immune cell subpopulations and a high degree of overlap between the B cell and T cell infection-related pathways of SMG and PBMC. This work reveals the cellular and molecular changes of IgG4-RD at single-cell resolution and provides new perspectives on the etiology and therapeutic targets of this disease and AIDs in general [167]. In conclusion, single-cell sequencing plays an important role in revealing the characteristic transcriptional profiles of the immune cell subpopulations in IgG4-RD. As storiform fibrosis and occlusive phlebitis are also features of IgG4-RD, single-cell studies could focus on fibroblasts and vascular ECs.

Immune-Mediated Kidney Disease

Immune-mediated kidney disease is a group of chronic glomerular diseases with similar immunopathological features. The damage is mainly caused by the deposition of immune complexes. And the clinical manifestations include proteinuria, haematuria, edema, and other symptoms of nephropathy. This group of diseases includes IgA nephropathy (IgAN), lupus nephritis (LN), membranous nephropathy (MN), and so on [168].

Tang et al. used scRNA-seq to map the transcriptome of IgAN to elucidate the molecular mechanisms of renal injury in this disease [169]. Zambrano et al. also made use of scRNA-seq analysis of glomerulosa-associated cells in IgAN mice in animal experiments, revealing the role of ECs in the early pathogenesis of IgAN [170]. Zeng et al. found that NK cell numbers and toxicity were reduced in patients with IgAN by scRNA-seq of peripheral blood single nucleated cells. They also found that a distinct subpopulation of B cells inhibiting NF-κB signaling and a subpopulation of monocytes expressing interferon-inducible genes were positively associated with disease progression. This study successfully revealed early transcriptomic changes in IgAN immune cells, providing new perspectives for the identification of new biomarkers and treatment of glomerulonephritis [171]. Zheng et al. also used single-cell transcriptomics to reveal the immune mechanisms of IgAN, providing an advanced idea about the treatment of the disease [172]. In addition, Chen et al. found a positive correlation between CD163 + dendritic cells (DC3s) and the severity of LN by scRNA-seq, making renal DC3 counts a possible indicator for guiding therapeutic decisions for patients with LN in the clinic [173]. Fava et al. analyzed renal biopsies from LN patients by combining single-cell transcriptomics and proteomics. They identified a role for IL-16 in the pathogenesis of LN, providing a potential therapeutic target and biomarker for the disease [174]. Fava et al. also integrated urine proteomics with renal single-cell transcriptomics to define the IFN-γ response gradient in LN, which facilitates the identification of immune mechanisms and pathways of the disease thereby enabling diagnosis and personalized treatment [175]. Tang et al. analyzed the local immune response in LN kidneys by single-cell sequencing and spatial transcriptomic analysis and identified a role for APOE monocytes, providing a new therapeutic target [176]. What's more, Shi et al. applied scRNA-seq to idiopathic membranous nephropathy (IMN) to reveal the characteristics of immune cells in patients' renal tissues to explore the molecular mechanisms of IMN pathogenesis [177]. Xu et al. also made use of scRNA-seq to reveal interactions between renal cells in IMN. They characterized the transcriptional profile of the disease and identified novel therapeutic targets based on an enhanced understanding of the pathogenesis of the disease [178]. These studies revealed the role of ECs, NK cells, DCs, monocytes, and some inflammatory mediators in immune-mediated kidney disease. Cell atlas of different subtypes of immune-mediated kidney disease needs to be further compared and integrated.

Others

ScRNA-seq analysis of BM cells from T1D model mice by Zhong et al. revealed some differences in the diversity of immune cells in different populations. They also found that the BM neutrophil/B lymphocyte ratio was negatively correlated with osteoporosis. This study used scRNA-seq to reveal, for the first time, the characteristics and heterogeneity of BM immune cells in streptozotocin-induced T1D mice. It provided a reference for the treatment of T1D and the prevention of T1D-related osteoporosis [179]. To study the

transcriptome of individual cells in patients with myasthenia gravis (MG), Jin et al. used single-cell sequencing to find that B cells, CD4 + T cells, and monocytes showed more heterogeneity in patients with MG. They also identified a subpopulation associated with the disease, CD180- B cells, which suggests a higher IgG composition and is associated with disease activity and anti-AChR antibodies. This study further elucidates the cellular heterogeneity of MG and provides several specific cellular markers for subsequent studies [180]. Pan et al. made use of scRNA-seq to study the differences and associations between papillary thyroid cancer and Hashimoto's thyroiditis (HT). They found a unique molecular signature of predicted copy number changes in epithelial and mesenchymal cells in cancer cells, as well as an association of tumor-infiltrating B lymphocytes with a concomitant HT origin. It facilitates a deeper understanding of the two diseases and their differential diagnosis [181]. Lu et al. found elevated expression of phosphoglycerate kinase 1 (PGK1) in CD4 + T cells of myocarditis model mice by single-cell sequencing. They speculated that PGK1 may be a key regulator of CD4 + T cell metabolism. And they proposed that autoimmune myocarditis can be suppressed by inhibiting PGK1 to reprogramme the metabolism of CD4 T cells [182].

In summary, single-cell sequencing has been widely applied to a variety of AIDS. Some representative studies are summarized in Table 3.

Limitations and Prospect

Since the first breakthrough in scRNA-seq methods in 2009 [186], single-cell sequencing has seen tremendous developments in technology, algorithms, and clinical applications. As previously described, single-cell sequencing has evolved from a single perspective of the genome, epigenome, transcriptome, and so on to more comprehensive joint multi-omics analyses and has progressively merged with AI, organoid research, third-generation sequencing, and so on, providing a powerful tool for life science research. Based on the technology's feature of high resolution at the cellular level, it stands out in the field of heterogeneity research. It contributes to the construction of cellular maps, the understanding of pathogenesis, and the diagnosis, treatment, and prognosis of diseases, greatly advancing the progress of medical research including AIDs.

Although single-cell sequencing is relatively well established and has been widely used in the field of AIDs, there are still some limitations and challenges. We propose some future directions for research to overcome the ongoing challenges, to explore the greater potential of the technology in AIDs in the following areas.

Limitations

The cost of performing RNA-seq is determined by the type and depth of sequencing, the type of library used, sample size and quality, and other factors. Currently, the cost of scRNA-seq ranges from €1,000 to €9,000 per sample, and these costs do not include bioinformatics services [187]. Single-cell sequencing does have higher accuracy and reliability compared to traditional sequencing, but it is also extremely costly due to the complexity of dealing with individual cells and the high depth of sequencing. What's more, in experiments using single-cell sequencing, there is often a high noise level in the data due to differences in capture times, operators, reagent batches, or equipment and technology platforms [188]. Such batch effect can lead to false-positive or false-negative results, rendering experimental results inaccurate. For example, direct integration analyses of the same tissues using two different techniques yielded significant differences. However, these differences could be attributed to differences in techniques rather than the biological differences desired by researchers. Data generated by single-cell sequencing are sparse and noisy, yet rich in information. Processing, analyzing, representing, and interpreting these data are extremely challenging. Complicated experimental operations and data analysis require a high level of operator expertise, which limits the widespread use of single-cell sequencing. The amount of nucleic acid in a single cell is also a limiting factor. A single cell contains only about 4–6 picograms of DNA, whereas the second-generation sequencing usually requires a hundred nanograms of DNA to start with. It is too small to be directly detected by sequencing [189, 190].

Enhancing Economic Efficiency

Although the cost of performing individual cells using scRNA-seq technology has been significantly reduced, the total cost for each sample remains high, which is a major limiting factor for the application of the technology. Therefore, approaches to improve the economics of single-cell sequencing would greatly expand its application. Several low-cost alternatives through the use of specific libraries and extensively optimized sequencing technologies have now emerged. Other researchers have suggested that targeted RNA-seq is also beneficial in reducing costs if the specific transcripts of interest are known in advance [191, 192]. In the future, we still need to explore more ways to enhance the economic efficiency of single-cell sequencing to address biological reproducibility in research and expand its application in the field of AIDs.

Table 3 Some experiments in AIDs based on single-cell sequencing

Disease	Researches	
	Contents	Significance
SLE	Resolving cellular chromatin accessibility at single-cell resolution [17]	Uncovering key TFs in PBMCs
	Mapping T cell and NK cell populations at single-cell resolution [116]	Identifying new biomarkers of disease activity
	Analyzing the role of anergic autoreactive B cells in SLE [118]	Demonstration of the role of IL-4 and discovery of new biomarkers
	Analyzing shared genes for SLE and MetS [124]	Helping develop a new combined treatment approach
	Comparative study of immune cell dysregulation of SLE patients [125]	Constructing a DNB model to aid in disease diagnosis and prognosis
	Mapping SLE heterogeneity at the level of single cells [130]	Further clarification of the origin of transcriptional signatures and heterogeneity
	Analyzing immune cells and TCR/BCR repertoire at single-cell resolution [131]	Revealing the transcriptomic profile of immune cells from SLE patients and their immune functions
	Investigating the relationship between SLE variants and cell-specific molecules [132]	Exploring cellular composition, transcriptional profiles, and genetic variation associated with SLE
	Study of the development of B cells in the BM of SLE patients [133]	Revealing immunological features of abnormal differentiation of BM B cells in SLE
	Integrated analysis of the pathogenesis of SLE in different cell populations [134]	Revealing cellular subpopulations associated with transcriptional and epigenetic regulation in SLE
RA	Analysis of synovial tissue from RA patients at single-cell resolution [103]	Facilitating the elucidation of RA pathogenesis
	Study of ACPA and RA heterogeneity [110]	Highlighting the need for different treatments based on ACPA status
	Investigating the role of Notch signaling in RA pathogenesis [137]	Identifying the critical role of NOTCH3 signaling
	Assessment of cellular changes in lymph node sinuses and their association with RA development [140]	Identification of a mechanism for cellular interactions during RA development
	Analyzing the role of synovial tissue macrophages in RA [183]	Identifying phenotypic changes over time and a potential therapeutic strategy for RA
	Analyzing the origin and role of macrophages in RA [184]	Revealing the functional diversity of synovial macrophages
SSc	Study of markers and pathways associated with SSc damage [142]	Identifying two markers of EC in SSc
	Study of T cell-mediated immune responses in SSc [143]	Discovering a unique T cell population and providing new insights into the mechanisms and therapeutics
	Analysis of myofibroblast and fibroblast heterogeneity in SSc-ILD [144]	Identifying the critical role of myofibroblasts in SSc-ILD fibrosis
	Multi-omics analysis of the effect of autoantibodies on SSc [185]	Demonstrating that autoantibodies can affect certain subtypes
SV	Mapping immune cell populations of GCA patients [146]	Determining the cellular characteristics of GCA and associated treatments
	Investigating the role of CD4 + T cells in the pathogenesis of GCA [147]	Uncovering the role of CD4 + T cells and suggesting a novel treatment
	Determining the pathogenic process of vertebrobasilar aneurysms in patients with PAN [148]	Identifying relevant EC subpopulations
	Exploring gene expression and immune response in acute KD [149]	Providing insights into the pathogenesis and treatment of KD
	Exploring the immune mechanisms of BD pathogenesis [150]	Discovery of C1q as a therapeutic target and clinical assessment for BD
SS	Analysis of SMG in the pSS mouse model [153]	Revealing the diversity of immune cell dysregulation in SS
	Analyzing immune cell subsets and gene expression in pSS patients [154]	Revealing specific immune cell subpopulations and providing new targets for pSS

Table 3 (continued)

Disease	Researches	
	Contents	Significance
IBD	Characterization of immune cell populations in IBD patients at single-cell resolution [156]	Helping in developing therapies for patients with different types of IBD
	Exploring the role of GSDMB in IBD [159]	Revealing the role of GSDMB in repairing epithelium and eliminating inflammation
DM	Comparative study of DM and CLE [160]	Highlighting the role of IL-18 and implications for differentiating DM from CLE lesions
	Identification of cells associated with disease activity in JDM [161]	Providing new insights for probing immune dysregulation in JDM
IgG4-RD	Assessment of specific cell subpopulations and pathways for IgG4-RD [165]	Enhancing understanding of the cellular heterogeneity and transcriptional features involved in the pathogenesis
	Investigating cellular drivers of class switching in disease [166]	Revealing a new subpopulation of Tfh cells and the process leading to B cell class switching
	Characterization of transcription in affected tissues and cells at the single-cell level [167]	Providing valuable insights into disease etiology and therapeutic targets
IgAN	Analyzing kidney transcription at single-cell resolution [169]	Mapping the transcriptome of IgAN
	Exploring the mechanisms of IgAN glomerular injury [170]	Revealing the role of ECs in the pathogenesis
	Exploring transcriptomic changes in IgAN immune cells [171]	Providing new perspectives for the identification of new biomarkers and treatment of glomerulonephritis
	Analyzing immune events in the development of IgAN [172]	Revealing the immune mechanisms in the development of IgAN
LN	Single-cell analysis of the single-cell landscape of the kidney in LN [173]	Providing evidence that renal DC3 counts can be an indicator to guide LN treatment
	Identifying an IFN-γ response gradient and implicating IL-16 in LN [174]	Identifying the roles of IL-16 as well as IFN-γ in LN
	Exploring the cellular composition and the cells associated with the autoimmune response [176]	Identifying a role for APOE monocytes in LN
MN	Showing the immune cell landscape in the kidneys of patients with IMN [177]	Revealing characterization of immune cells in renal tissues of IMN patients
	Analyzing the transcriptomic landscape of IMN at single-cell resolution [178]	Revealing interactions between renal cells in IMN
Others	Revealing the relationship of BM and osteopenia in STZ-induced T1D mice [179]	Providing a reference for the treatment of T1D and the prevention of T1D-induced osteoporosis
	Study of cell populations in patients with MG at single-cell resolution [180]	Elucidating the cellular heterogeneity of MG and providing specific cellular markers
	Mapping the transcriptome of papillary thyroid cancer and HT [181]	Revealing differences and associations between papillary thyroid cancer and HT
	Studying the role of PGK1 in CD4 + T cells and autoimmune myocarditis [182]	Providing a promising strategy for the treatment of autoimmune myocarditis

Supplementing Disease-Related Cell Atlas and Pathogenic Mechanisms

As mentioned earlier, single-cell sequencing has a high resolution up to the single-cell level, which can be applied to constructing a cellular atlas and explaining disease-causing mechanisms. Although the cellular maps of some diseases have been basically mapped, there are still some diseases, especially AIDs with heterogeneity as one of their main characteristics, that have yet to be further explored. We hope to utilize single-cell sequencing and other technologies to continue to explore the cellular heterogeneity of AIDs that have not yet been studied or to conduct more in-depth studies on AIDs that have already been studied like mapping different subtypes or stages of the same disease. In recent years, the study of cell atlas across tissues has been gradually carried out [193]. Cell atlas related to AIDs can also be carried out in this direction. Supplementing the disease-related cell atlas will help to better explain the pathogenic mechanisms of AIDs. For these purposes, improving the data analysis capabilities of the technology is an important step. Several methods have been developed to remove the batch effect [194]. What's more, with the concerted efforts of scientists, many

methods of data analysis are now available, including principal component analysis, neighbor graphs, and clustering cells. As mentioned by Kharchenko in his review, different methods have their own advantages and disadvantages [2]. The direction of future studies could be to integrate the advantages of each method and develop user-friendly platforms.

Integrating Single-Cell Sequencing into Routine Clinical Diagnostics and Personalized Medicine

With the development of single-cell technology, the cost and the ability to analyze data have improved considerably. And with the advent of Multiple Annealing and Looping-based Amplification Cycles, a technological breakthrough in single-cell amplification has also been achieved [25]. Single-cell sequencing has been sufficient for some scientific and clinical applications. However, the challenges have not yet been completely overcome. For example, the ideal single-cell whole-genome amplification is one in which every locus on the genome is amplified to an equal magnitude and with zero allele drop-out rate, but there is no technology that can do this yet [25, 27, 79–81]. Lower costs, stronger data analysis tools, and more advanced gene amplification technologies are key to integrating single-cell sequencing into routine clinical diagnostics and personalized medicine for AIDs.

Discovering New Targets and Developing the Corresponding Drugs

The ultimate goal of all technological developments is to expand their application in the treatment of clinical diseases. Utilizing the extremely high resolution of single-cell sequencing technology, many AID-specific targets, including key cells, mediators, and conduction pathways, have been identified, and corresponding therapeutic drugs have been or are being developed, as mentioned in the previous section. To discover more targets and develop corresponding drugs, we can develop tools that replace cell culture or mouse models, thereby reducing time and economic costs for better experimental research.

Performing New Technical Innovations and Multi-omics Combined Analysis

The innovations already available in single-cell sequencing have been described, but there are still many aspects that can be developed and can be combined with other technologies to enable multi-omics co-analysis. For example, during single-cell sequencing analysis, phenotypic parameters of the cell such as the spatial organization or the microenvironment are mostly lost [195], whereas the newly developed single-cell spatial transcriptome sequencing measures gene expression while preserving the spatial information. We can integrate more cellular phenotypic parameters with the transcriptome to reconfigure the spatial structure of the cell. Jovic et al. in their review mention that combining scRNA-seq and other genetic screening tools can further expand the application of this technology [196], and examples of Perturb-seq that can assess the transcriptional effects of knocking out multiple genes with CRISPR are also given [197]. We believe that more similar combinatorial technologies will emerge to take single-cell sequencing to the next level of application and further dissecting diseases including AIDs.

Conclusions

Based on the high-resolution feature of single-cell sequencing at the cellular level, it stands out in the study of AIDs characterized by immune cell heterogeneity. The technology has evolved from a single view of the genome, epigenome, and transcriptome to more comprehensive joint multi-omics analyses. Moreover, it is gradually integrating with AI, organoid research, third-generation sequencing, and so on, contributing to the construction of cellular maps and the understanding of pathogenesis, diagnosis, treatment, and prognosis of diseases, which has greatly contributed to the advancement of medical research, including that on AIDS. If we can continue to promote the integration of single-cell sequencing with other technologies to simplify the process and reduce the cost, we will be able to further expand the application of this technology for the benefit of patients suffering from diseases like AIDs.

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Declarations

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