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REVIEW ARTICLE

Single-cell sequencing: accurate disease detection

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Abstract
Early and accurate detection of diseases is of great significance for enhancing treatment outcomes and improving patient prognosis. The emergence of single-cell sequencing technology has brought new opportunities for this. This technology breaks through the limitations of traditional sequencing and can analyze the genome, transcriptome, etc. at the single-cell level, clearly demonstrating the heterogeneity between cells. In neurological diseases, single-cell sequencing can reveal the changes in gene expression of nerve cells in the early stage of the disease, facilitating early diagnosis and disease monitoring. Although single-cell sequencing has broad prospects in disease detection, it faces challenges such as complex data processing and high detection costs. With the continuous advancement of technology, single-cell sequencing is expected to become a key means for accurate disease detection, bringing about a revolution in clinical diagnosis.

Keywords Single-cell sequencing · Accurate disease detection · Tumor

Introduction

Research background and significance

In the field of life science research, cells, as the basic units of organisms, are of great significance for understanding life processes through in-depth exploration of their functions and characteristics. Traditional sequencing technologies typically conduct analyses at the multicellular level. The results obtained are the averages of signals from a large number of cells, which largely masks the heterogeneity information among cells. However, an increasing number of studies have shown that this inter-cellular heterogeneity plays a crucial role in the occurrence, development, and treatment response of diseases [1]. For example, in tumor tissues, different tumor cells have distinct gene expression profiles and biological behaviors. Traditional sequencing technologies are unable to accurately reveal these differences, resulting in a lack of precision in tumor diagnosis and treatment [2, 3].

The emergence of single-cell sequencing technology provides a powerful tool to address this issue. This technology can perform high-throughput sequencing analysis of the genome, transcriptome, and epigenome at the single-cell level, thus precisely revealing the heterogeneity and functional differences among cells. This breakthrough enables researchers to gain a deeper understanding of the structure, function, and interactions of cells from a brand-new perspective, bringing revolutionary changes to life science research [4].

Single-cell sequencing(SCS) technology is irreplaceable in the field of disease detection, offering unprecedented opportunities for early disease diagnosis, precision treatment, and in-depth research on pathogenesis [5]. In the aspect of tumor diagnosis, traditional tumor detection methods often rely on tissue biopsies. This approach is not only invasive, but also due to the heterogeneity of tumor tissues, the obtained samples may not fully reflect the real situation of the tumor. Single-cell sequencing technology can sequence and analyze individual cells in tumor tissues, accurately identify tumor cell sub-populations, and discover cell groups with special biological characteristics such as tumor stem cells, providing key information for early tumor diagnosis and personalized treatment [6]. In the research of neurological diseases, single-cell sequencing technology

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can help reveal the diversity and functional differences of neurons, gain an in-depth understanding of the pathogenesis of neurological diseases such as Alzheimer's disease (AD) and Parkinson's disease, and provide a theoretical basis for the development of new treatment methods [7, 8].

The development of SCS technology has greatly promoted the advancement of precision medicine. Precision medicine aims to formulate personalized medical plans based on patients' individual genetic characteristics, environmental factors, and lifestyles to improve treatment efficacy and reduce adverse reactions. SCS technology can provide detailed information at the cellular level of patients, enabling doctors to more accurately understand the pathogenesis and development process of diseases, and thus formulate more precise treatment strategies [9, 10].

The application of single-cell sequencing technology in the field of disease detection has important scientific significance and clinical value, offering new ideas and methods to solve many problems currently faced in the medical field.

Current status and challenges of disease detection

Traditional disease detection methods have played a significant role in the field of medical diagnosis. However, with the advancement of medical research and the increasing clinical demands, their limitations have become increasingly apparent. In tumor detection, tissue biopsy serves as a primary traditional diagnostic approach. While it allows for the acquisition of tumor tissue for pathological analysis, this method is invasive and may subject patients to pain and complications. Furthermore, tumors exhibit high heterogeneity; thus, samples obtained through biopsy often fail to comprehensively represent the characteristics of the entire tumor, leading to potential misdiagnosis or missed diagnoses. For instance, in breast cancer diagnosis, due to variations in cellular composition and molecular features across different regions within a tumor, relying solely on localized biopsy samples may not accurately assess the malignancy grade or biological behavior of the tumor. This can adversely affect subsequent treatment planning [11, 12].

Serological testing assists in disease diagnosis by detecting specific biomarkers in the blood, such as carcinoembryonic antigen(CEA) and alpha-fetoprotein(AFP), which are commonly used for cancer screening and diagnosis. However, the specificity and sensitivity of these biomarkers are limited; they may also be elevated in certain benign conditions or physiological states, leading to false-positive results that impose unnecessary psychological burdens on patients and incur additional costs for further examinations [13]. Moreover, for some early-stage tumors or small lesions, serum markers may not yet be elevated, resulting in false-negative outcomes that can delay the early diagnosis of diseases [14].

Imaging modalities such as X-ray, CT, and MRI can visualize internal anatomical structures and assist clinicians in identifying pathological changes. However, these methods have limited capability in detecting early-stage small lesions and are susceptible to interference based on lesion size, location, and surrounding tissues. In the context of lung cancer screening, conventional imaging examinations may fail to reliably characterize pulmonary nodules smaller than 1 cm in diameter, potentially delaying timely therapeutic interventions for certain patients [15]. More importantly, while these imaging methods primarily provide morphological data, they offer limited insights into the biological characteristics and pathological nature of lesions, thus often requiring integration with complementary diagnostic modalities for comprehensive clinical evaluation.

In the field of neurological disease diagnosis, conventional approaches primarily rely on clinical manifestations, physical signs, and neuroimaging evaluations. For neurodegenerative disorders such as AD and Parkinson's disease, the prodromal phase often presents with non-specific symptoms that frequently lead to misdiagnosis or delayed detection [16, 17]. Current diagnostic modalities face significant challenges in identifying subtle neuronal alterations and molecular-level pathological changes during early disease progression, thereby missing the critical window for therapeutic intervention. Although cerebrospinal fluid (CSF) analysis enables detection of neurological biomarkers, this invasive procedure demonstrates suboptimal patient compliance, while its diagnostic validity remains susceptible to multiple confounding factors, including sample collection techniques and pre-analytical variables.

The emergence of SCS technology offers a transformative approach to address these diagnostic challenges. This cutting-edge methodology enables comprehensive genomic profiling at individual cell resolution, effectively circumventing the limitations of conventional bulk-tissue analysis. In oncological diagnostics, SCS allows precise molecular characterization of malignant cells within tumor ecosystems, including identification of biologically distinct subpopulations such as cancer stem cells, therapy-resistant subclones, and immune-evasive variants. By interrogating cellular transcriptomic signatures, mutational landscapes, and epigenetic modifications at single-cell resolution, researchers can delineate intratumoral heterogeneity with unprecedented precision, thereby establishing critical biomarkers for early cancer detection, prognostic stratification, and personalized therapeutic strategies [18, 19]. Crucially, this technology demonstrates unique clinical value in detecting drug-resistance-associated genetic alterations and immune checkpoint-related molecular signatures. Such molecular insights empower clinicians to optimize treatment regimens by selecting targeted therapies and immunomodulatory agents

based on the dynamic clonal evolution of tumors, ultimately improving therapeutic outcomes [20– 23].

In neuropathological investigations, SCS technology provides unprecedented resolution for deciphering the molecular pathogenesis of neurological disorders. This advanced approach enables systematic characterization of cell-type-specific transcriptional profiles and epigenetic dysregulation in both neurons and glial cells, offering critical insights into disease-driving mechanisms [24]. By leveraging single-cell analysis of brain tissue specimens or CSF-derived cells, researchers can detect preclinical-stage cellular aberrations and identify novel molecular signatures indicative of emerging neurodegeneration, thereby establishing non-invasive diagnostic paradigms for early disease detection [25]. Furthermore, the technology's capacity to map pathogenic signaling networks at single-cell resolution facilitates the discovery of disease-modifying targets. Such a mechanistic understanding of neuroimmune interactions and synaptic pathophysiology provides a robust scientific foundation for developing next-generation therapies with enhanced cellular specificity [26].

SCS technology has emerged as a paradigm-shifting innovation in disease diagnostics, offering transformative potential to overcome the inherent limitations of conventional diagnostic approaches. With continuous refinements in throughput and analytical pipelines, this technology is poised to redefine precision medicine frameworks by enabling spatiotemporal resolution of disease progression at cellular and molecular levels. Future clinical implementation may establish single-cell omics as a cornerstone in next-generation diagnostic platforms, empowering clinicians to decipher complex disease heterogeneity, track dynamic therapeutic responses, and ultimately pioneer preventive healthcare strategies through early-stage molecular aberration detection.

Positioning and novel contributions

Recent reviews have significantly advanced the understanding of SCS applications: Jovic D comprehensively cataloged SCS methodologies but offered limited clinical implementation insights [4]. Zhang Y detailed SCS in oncology research [27], while Su Y emphasized neurodegenerative applications, yet neither addressed cross-disease convergence [28].

Methodes

Search strategy

A comprehensive literature search was conducted on PubMed and Embase to identify studies published up to March 2025. The search strategy integrated Medical Subject Headings (MeSH) terms and key phrases, including"single-cell sequencing,""accuract disease detection"and"tumor"with Boolean operators (AND/OR) employed to optimize precision. Reference lists of included articles and relevant reviews were manually screened to capture studies potentially missed in the initial database search. The retrieval process adhered to the PRISMA 2020 guidelines to ensure transparency and methodological rigor.

Inclusion and exclusion criteria

An initial search retrieved 864 articles, which were filtered to include 101 studies through the following process: first, a time filter retained studies published between January 2015 and May 2025 (prioritizing cutting-edge studies post-2020), then 171 duplicates were removed using bibliographic management software; Subsequently, two researchers (X.Q.W. and B.L.) performed multi-stage screening according to predefined criteria: 264 studies unrelated to single-cell sequencing diagnostic applications (e.g., pure therapeutic mechanism studies or non-clinically translatable animal experiments) were excluded via title and abstract screening; full-text evaluation of the remaining 429 articles excluded those focusing solely on clinical management, epidemiology, or lacking molecular mechanism analysis. The final 101 included studies covered original research, reviews, and meta-analyses in oncology, neurological diseases, genetic disorders, etc. Key data were extracted into a structured database for qualitative synthesis. The screening process was strictly followed by PRISMA 2020 guidelines and was visualized in a flowchart (Fig. 1).

Single-cell sequencing technology method

Single-cell isolation is the first critical step in single-cell sequencing, which aims to obtain a single cell accurately from a complex cell population to provide a pure sample for subsequent nucleic acid amplification and sequencing analysis. At present, the common single cell separation methods include: flow cytometry, microfluidic chip technology, laser capture micro-cutting technology, etc. (Fig. 2). Each of these methods has its own advantages and limitations.

Flow cytometry

Flow Cytometry (FCM) is a technique for the rapid quantitative analysis and sorting of cells or other biological particles arranged in a single file within a fluidic system [29]. FCM demonstrates several remarkable advantages[30]. First and foremost, it combines powerful analytical capabilities with high-precision cell sorting functions, enabling simultaneous identification and isolation of specific cell

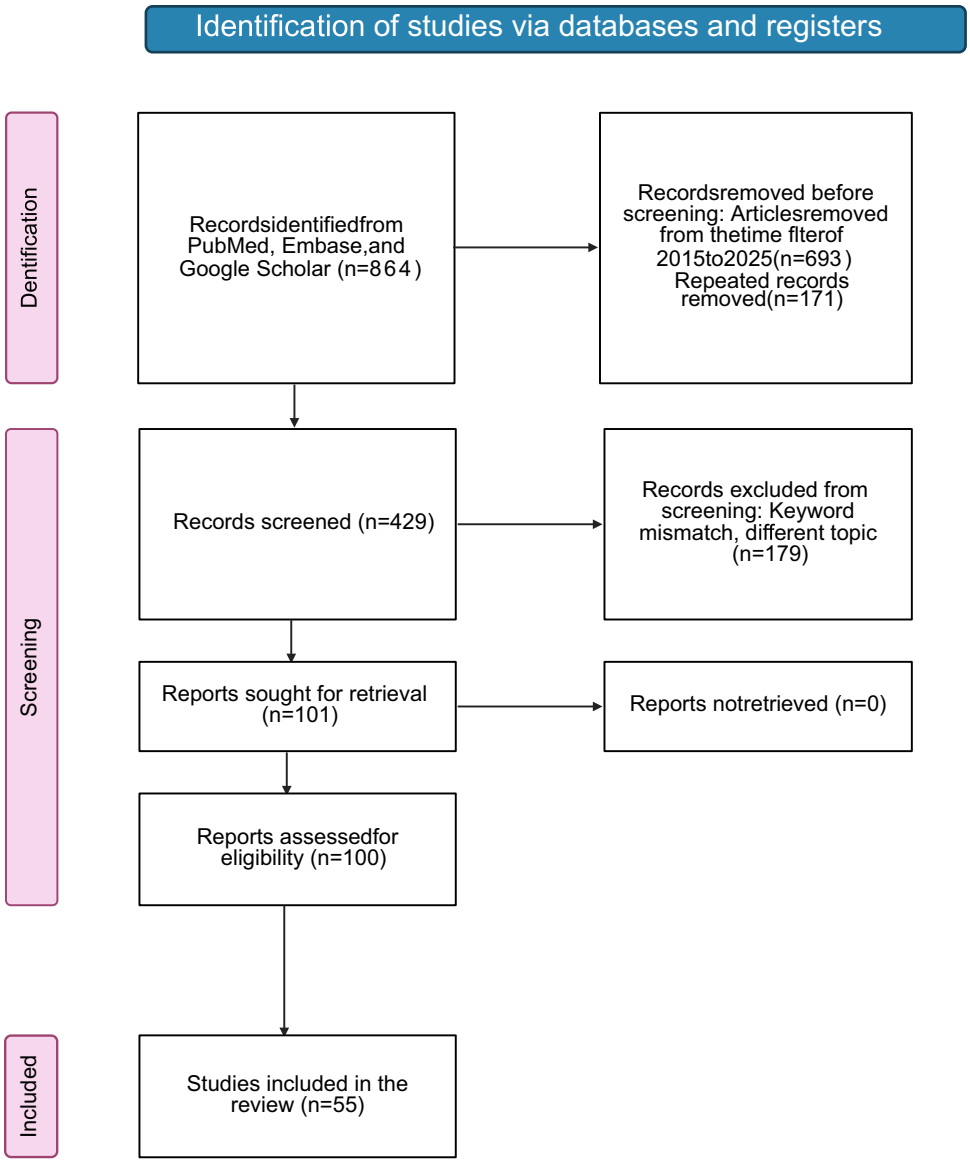

Fig. 1 PRISMA flow diagram for the methodology

populations. The technique achieves exceptional processing speed, capable of analyzing tens of thousands to millions of cells within minutes when sufficient sample quantities are available. Its multiparametric analysis capacity stands out through advanced multiplex fluorescent labeling techniques, where multiple monoclonal antibodies conjugated with distinct fluorophores permit simultaneous detection of various cellular characteristics in a single assay. This multi-parameter approach significantly enhances the resolution of cellular subpopulations, enabling more accurate identification and quantification of rare cell subsets. Furthermore, FCM exhibits remarkable sample versatility, accommodating diverse specimen types including cell culture supernatants, cellular lysates, microbial samples, synthetic microspheres, as well as various biological fluids such as serum and plasma.

FCM, while a powerful analytical tool, presents several inherent limitations. Firstly, the technique mandates stringent sample preparation as single-cell suspensions. During tissue dissociation procedures, mechanical and enzymatic processing may compromise cellular integrity through membrane damage, potentially altering biological characteristics and compromising subsequent analytical accuracy. Secondly, the requirement for high-end instrumentation and specialized personnel for operation and maintenance creates substantial financial barriers, restricting its accessibility in resource-limited settings. From a technical perspective, the methodology demonstrates reduced efficacy in detecting specific intracellular components, particularly cytoplasmic and nuclear markers such as Bcl-2 and Cyclin-D1, where suboptimal detection performance has been documented. Furthermore, unlike pathological specimens that permit

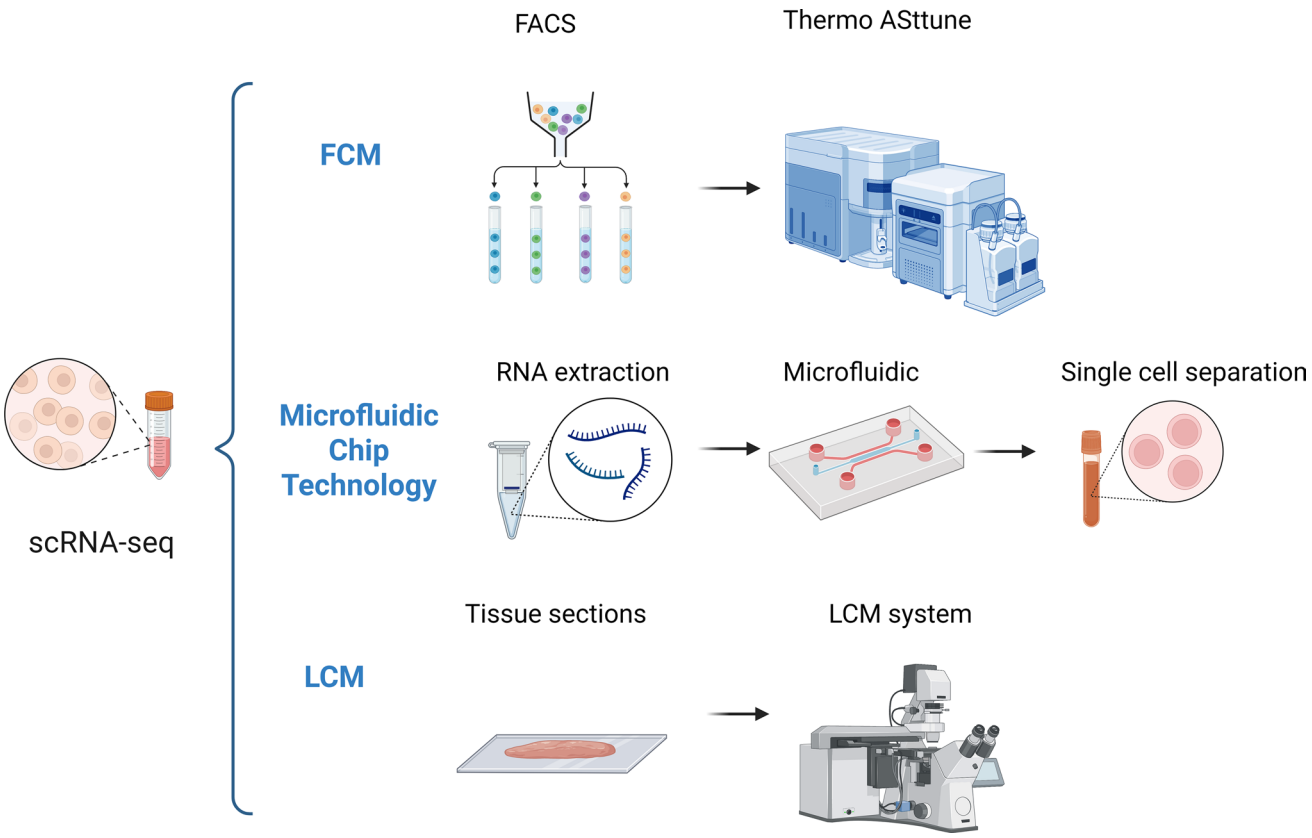

Fig. 2 Single-cell separation technique

long-term archival storage for retrospective analysis, FCM samples lack equivalent preservation capacity. These limitations collectively constrain its diagnostic utility for particular neoplastic conditions, including Hodgkin lymphoma and myeloproliferative neoplasms, as well as in the analysis of specialized cell populations like megakaryocytes.

Microfluidic chip technology

Microfluidic chip technology refers to a technique that integrates the functionalities of an entire analytical laboratory-including sampling, sample pretreatment, reaction, separation, and detection-onto a chip measuring just a few square centimeters. Microfluidic chip technology offers numerous outstanding advantages [31]. First, it integrates miniaturization and a high degree of automation. By cleverly designing the dimensions and curvature of flow channels, microvalves, and cavities, multiple steps in sample detection can be concentrated on a single small chip. This integration significantly reduces the complexity and errors associated with manual operations throughout the entire detection process. Second, it enables high throughput; microfluidic chips can be designed with multi-channel structures that allow simultaneous diversion of samples to multiple reaction units through a network of microchannels. Each reaction unit is isolated from one another to prevent interference, enabling parallel testing of various parameters on the same sample. Compared to conventional sequential testing methods, this approach greatly shortens detection time and enhances efficiency. Third, there is reduced reagent consumption; due to the minuscule size of reaction unit cavities on the chip, although reagent concentrations may need slight adjustments upwards, overall reagent usage is far lower than that required by traditional methods—significantly lowering costs for reagents. Fourth, minimal sample volume is required; only microliter or even nanoliter quantities are needed for analysis, whole blood can also be used directly for testing, which holds significant importance for populations with limited blood volumes or difficulties in venous collection (such as infants, elderly individuals, or disabled persons), making multi-parameter assessments feasible even with rare samples. Fifthly, contamination risks are minimized; the integrated functionality of chips mitigates environmental contamination during manual handling processes—for instance in molecular nucleic acid tests-effectively addressing false-positive issues caused by aerosol diffusion.

However, microfluidic chip technology also faces several challenges [32]. Firstly, there is a lack of standardized

protocols and regulations regarding core technologies. A mature microfluidic product typically requires various components such as reagents, microfluidic chips themselves, driving platforms for these chips, along with optoelectronic detection modules and signal processing systems, including user interface software among others. Due to its nascent stage within technological development cycles, lacking standardization or normalization at present makes achieving component interoperability difficult while hindering collaborative product development between upstream and downstream companies—a situation compounded by stringent technical requirements leading to prolonged development timelines. Second, there exists an acute shortage related talent across disciplines: interdisciplinary professionals, corporate R&D personnel, and specialized market experts remain scarce, particularly within domestic contexts where skilled technicians engaged specifically in product development involving chips are especially hard-to-find. Furthermore, production costs currently remain prohibitively high; most disposable immunoassay-based devices cannot fully leverage potential benefits offered via reusable analytical platforms, resulting in elevated expenses per test conducted under existing manufacturing conditions whereby research-grade standard glass substrates might range anywhere from tens up into hundreds dollars each. Additionally certain platform-specific technical hurdles persist such as antibody immobilization issues which represent critical concerns when dealing non-homogeneous immunoassays performed using these types devices-determining effective means securing antibodies onto channel surfaces remains paramount despite availability diverse methodologies (e.g., direct adsorption onto walls covalent bonding forming active functional groups utilizing techniques like soft lithography) all exhibiting inherent limitations e.g., conformational changes induced upon binding could diminish activity levels alongside necessity ensuring proper sealing around channel surfaces since nonspecific protein interactions coupled denaturation phenomena would severely compromise sensitivity outcomes observed during assays conducted therein. Moreover, integrating external apparatuses (like automated analyzers display equipment etc.) alongside respective fluidics represents yet another key challenge requiring focused attention moving forward.

Laser capture microdissection

Laser Capture Microdissection (LCM) is a technique that allows for the precise extraction of target cells from frozen or paraffin-embedded tissue sections without compromising the structural integrity of the surrounding tissues [33]. The advantages of LCM are significant; it facilitates accurate isolation of specific cell types from complex tissues while preserving cellular morphology and structural integrity [34]. This capability makes LCM particularly suitable for studying specific subpopulations within tissues, which is crucial for advancing our understanding of intercellular interactions, disease mechanisms, and early detection strategies in various diseases. When combined with immunohistochemistry techniques, LCM provides powerful tools for analyzing solid samples at single-cell resolution. Recent advancements have been made utilizing LCM technology in single-cell RT-PCR, short tandem repeat analysis in forensic science, Western blotting techniques, mass spectrometry studies, among others [35].

However, LCM does present certain limitations. The operation is relatively complex and requires skilled personnel as well as specialized laser cutting platforms; thus, placing high technical demands on operators while being time-consuming during execution. Additionally, its throughput is low-making large-scale single-cell isolation challenging, as only a limited number of cells can be isolated per session; this poses difficulties in meeting high-throughput research needs. Furthermore, the equipment associated with this technology tends to be costly, with substantial maintenance and operational expenses that may hinder its widespread adoption across various laboratories. Improper control over laser energy levels or parameters during dissection could potentially damage cellular components affecting nucleic acids' integrity and protein activity within those cells-thereby influencing subsequent analytical results adversely.

Application of single-cell sequencing in tumor disease detection

Analysis of tumor cell heterogeneity

Tumor Cell Heterogeneity and the Role of SCS Tumor cell heterogeneity, a cornerstone of cancer biology, describes molecular diversity among tumor cells across genomic, transcriptional, proteomic, and epigenetic dimensions. This heterogeneity drives divergent behaviors in tumor growth, invasion, metastasis, and therapeutic response, significantly challenging diagnosis and clinical management [36] (Fig. 3). In the research of melanoma, SCS technology has played a crucial role [37]. Melanoma, a highly aggressive cutaneous malignancy, exhibits marked tumor cell heterogeneity. While traditional bulk sequencing fails to resolve this complexity, SCS enables precise dissection of gene expression profiles and mutational landscapes at cellular resolution [38]. Studies applying SCS to melanoma tissues have identified distinct tumor subpopulations with heterogeneous drug-resistance profiles [39]. Certain clones overexpress drug-efflux transporters (e.g., ABCB1), conferring chemotherapy resistance through enhanced drug expulsion, while others harbor mutations (e.g., in MAPK/ERK pathways) that impair targeted therapy response. Notably, SCS has uncovered rare

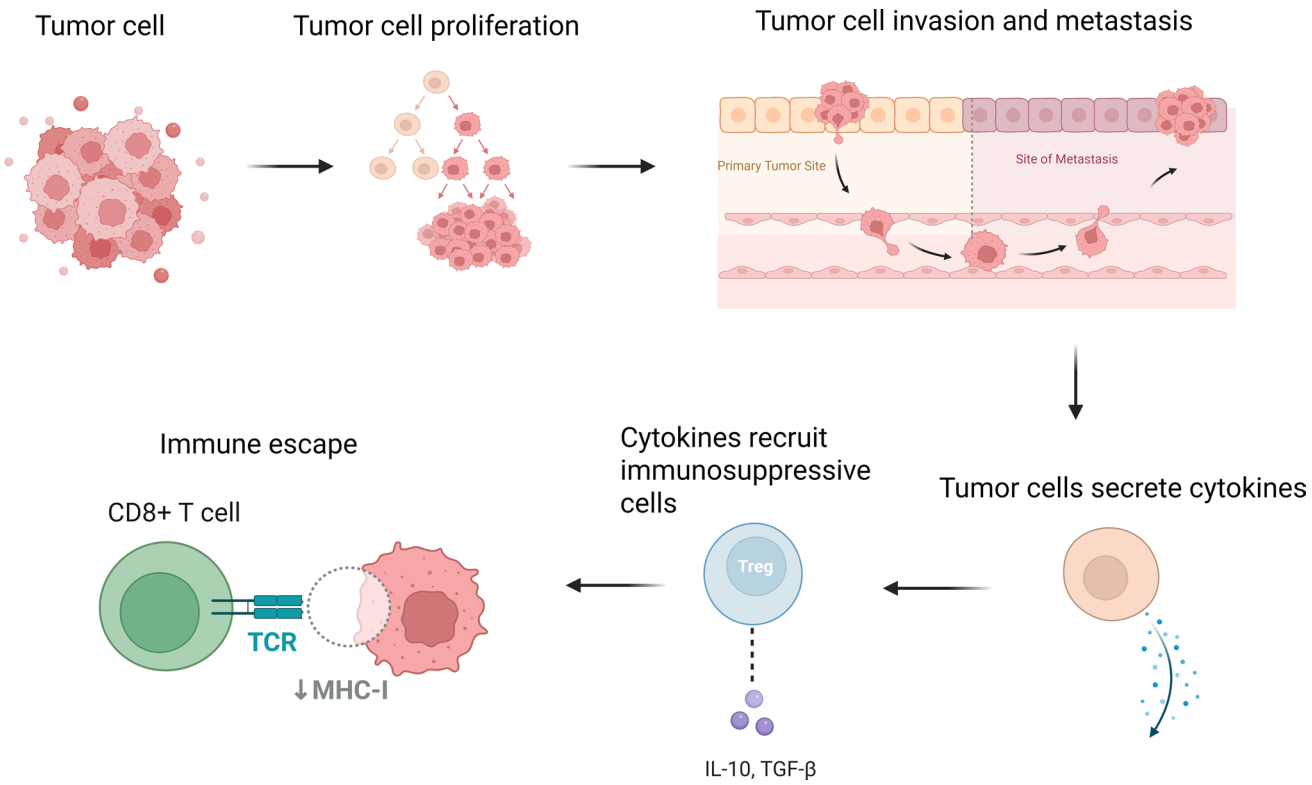

Fig. 3 Tumor heterogeneity analysis and microenvironment

subclones with unique biological properties, despite their low abundance, which may drive tumor recurrence and metastasis through mechanisms, such as immune evasion or niche adaptation [40]. These findings underscore SCS's capacity to reveal functional diversity within tumors, offering critical insights for overcoming therapeutic resistance.

SCS in Breast Cancer Heterogeneity SCS has similarly uncovered profound tumor cell heterogeneity in breast cancer, the most prevalent malignancy among women. Breast cancer encompasses diverse molecular subtypes, including Luminal A, Luminal B, HER2-enriched, and triple-negative breast cancer (TNBC) [41]. SCS enables granular analysis of these subtypes, revealing significant intra-subtype heterogeneity that bulk sequencing obscures [42]. For example, in TNBC, SCS has identified distinct subpopulations with divergent surface markers, gene expression profiles, and functional properties [43, 44]. Specific clusters exhibit heightened proliferative activity, while others demonstrate enhanced invasive and metastatic potential through epithelial-mesenchymal transition (EMT) activation [45]. Furthermore, these subclones interact differentially within the tumor microenvironment (TME), modulating tumor growth and progression via cytokine/chemokine secretion and paracrine signaling. Such insights underscore the clinical relevance of SCS in dissecting cellular hierarchies and informing therapeutic strategies for heterogeneous breast cancers.

Advantages of SCS in Tumor Heterogeneity Analysis. The power of SCS in dissecting tumor heterogeneity lies not only in identifying distinct cellular subpopulations but also in elucidating their functional and molecular characteristics. By analyzing SCS data, researchers obtain multidimensional profiles of individual cells, including gene expression patterns, mutational signatures, and epigenetic modifications, enabling a comprehensive view of tumor biology.

Transcriptomic Insights: Single-cell RNA sequencing (scRNA-seq) resolves gene expression heterogeneity, uncovering critical pathways linked to tumor initiation, progression, and drug resistance [46]. Genomic Precision: Single-cell DNA sequencing detects somatic mutations and copy-number variations, mapping clonal evolution to guide precision oncology strategies [47]. This multi-omics approach bridges cellular diversity to clinical applications, transforming our understanding of tumor dynamics and therapeutic vulnerabilities.

Tumor microenvironment

TME serves as a critical foundation for tumor growth, proliferation, and metastasis. Comprising tumor cells, immune cells, stromal components, and extracellular matrix, these elements engage in intricate cross-talk that collectively governs tumorigenesis, disease progression, and therapeutic

outcomes[48]. Single-cell sequencing has emerged as a transformative approach for dissecting cellular heterogeneity and functional dynamics within the TME, offering unprecedented resolution to characterize tumor-immune-stromal interactions[49].

Single-cell sequencing has revolutionized the investigation of tumor-immune cell interactions. In melanoma, this technology has unveiled tumor cell-secreted factors (e.g., CXCL12) that orchestrate immunosuppressive cell recruitment, including regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs)[50]. Tregs facilitate tumor immune evasion through inhibitory cytokines (IL-10, TGF-β) that suppress effector T cell activity[51, 52], while MDSCs impair T cell function via nutrient deprivation (e.g., arginine depletion) to fuel tumor progression[53]. Furthermore, single-cell analyses elucidate compromised antigen presentation mechanisms: Although tumor antigens are normally processed by APCs to activate T cell responses[54], malignant cells frequently dysregulate MHC-I expression to evade immune detection. This technology enables systematic profiling of antigen presentation-related genes across cellular subsets, providing mechanistic insights into tumor immune escape.

Single-cell sequencing has delineated the complex immune landscape of TNBC, an aggressive subtype with distinct TME features. Notably, TNBC exhibits remarkable macrophage infiltration, with single-cell analyses identifying M1 (anti-tumor) and M2 (pro-tumor) polarization states[55]. M1 macrophages secrete pro-inflammatory cytokines (TNF-α, IL-12) to activate antitumor T cell responses, while M2 counterparts promote tumor progression through immunosuppressive factors (IL-10, TGF-β). This technology precisely maps macrophage spatial distribution and functional specialization within TME. Furthermore, single-cell profiling reveals diverse T cell populations including cytotoxic CD8 + T cells, helper CD4 + T cells, and regulatory T cells, whose compositional dynamics correlate with disease progression and clinical outcomes[56]. Despite cytotoxic potential, CD8 + T cells frequently exhibit functional exhaustion marked by elevated PD-1/CTLA-4 expression-a therapeutic vulnerability illuminated through single-cell transcriptomic mapping. These insights establish a cellular atlas for developing precision immunotherapies targeting TNBC's immunosuppressive niche.

SCS has unraveled the pivotal role of tumor-stromal crosstalk in shaping malignant progression. In colorectal cancer, this technology decodes the dynamic reciprocity between cancer-associated fibroblasts (CAFs) and tumor cells, revealing CAFs heterogeneity across functional subtypes [57]. Mechanistically, CAFs orchestrate tumorigenesis through dual modalities: (1) Secretory activation of growth factors (PDGF) and extracellular matrix remodeling (collagen deposition) to fuel proliferation and invasion; (2) Direct contact-mediated regulation of oncogenic signaling pathways. Single-cell transcriptomic profiling identifies CAF-specific molecular signatures, with SDF-1-enriched subpopulations demonstrating strong correlation with metastatic competence[58]. Therapeutic targeting of these protumoral CAF subsets through pathway inhibition significantly attenuates metastatic dissemination, highlighting the clinical potential of stromal reprogramming strategies.

SCS illuminates endothelial cell heterogeneity driving tumor angiogenesis. Malignant cells secrete vascular endothelial growth factor (VEGF) to activate endothelial proliferation and neovascularization[59]. This technology deciphers tumor-associated endothelial signatures distinct from normal counterparts, revealing pathological features including enhanced vascular permeability and hemodynamic abnormalities that facilitate metastasis[60]. Importantly, single-cell profiling uncovers endothelial-specific biomarkers and druggable targets, advancing precision anti-angiogenic therapeutic development.

Early diagnosis and prognosis evaluation of tumor

Early Cancer Diagnosis and the Role of SCS Early tumor detection is critical to improving patient survival and treatment outcomes. The Li Shao group at Tsinghua University has established a groundbreaking single-cell atlas of human gastric carcinogenesis through sequencing of gastritis and early gastric cancer specimens [61]. Leveraging computational systems biology, this work delineates 17 critical cell types-including pre-malignant gastric epithelial subsets-and constructs the first quantitative interactome mapping gastritis-to-carcinoma transition. Mechanistically, epithelial evolution follows a three-stage transformation trajectory: progressive inflammatory activation, metabolic reprogramming, and proliferative escalation. Crucially, two first-in-field biomarkers were identified: (1) Intestinal metaplasia-associated signatures enabling early detection of pre-cancerous lesions [62]; (2) Molecularly defined pre-malignant cell clusters emerging prior to histopathological confirmation. These subclinical populations, characterized through network analysis of epithelial cell–cell communication, establish a paradigm-shifting framework for precision diagnosis and therapeutic interception in incipient gastric cancer.

SCS in Prognostic Assessment of Tumors SCS demonstrates transformative potential in evaluating tumor prognosis, which is influenced by multifaceted factors such as tumor cell heterogeneity, microenvironment dynamics, and interpatient variability. Patrick Tan's team (Duke-NUS) integrated 2,138 spatial transcriptomic regions and 152,423 single-cell profiles from 226 gastric cancer samples (n = 121 patients), defining two distinct evolutionary trajectories with clinical prognostic relevance [63]. The study delineates: (1) Branched evolution characterized

by progressive somatic copy number alteration (sCNA) accumulation driving subclone diversification; (2) Punctuated evolution featuring early acquisition of divergent sCNAs followed by parallel clonal expansion. Analysis of the TCGA cohort confirmed significantly worse survival in punctuated evolution cases ($p < 0.01$) [64]. These aggressive tumors exhibited elevated intratumoral heterogeneity (ITH) with concurrent upregulation of hypoxia and EMT pathways. TME was enriched with pro-angiogenic endothelial cells, CAFs, and metastasis-associated TAM1 macrophages, collectively establishing a molecular basis for therapeutic resistance and poor prognosis.

SCS technology has important application value in the early diagnosis and prognosis evaluation of tumors. Through the discovery of specific markers in the early stage of tumors, early detection and diagnosis of tumors can be achieved, and more treatment opportunities can be obtained for patients.

Application of single cell sequencing in the detection of nervous system diseases

Neuronal type identification and functional study

SCS Advances in Mammalian Brain Research scRNA-seq has revolutionized our understanding of neuronal diversity in mammalian brains. By profiling individual neurons in mouse models, researchers have systematically resolved novel neuronal subtypes with distinct molecular signatures and functional properties [65](Fig. 4). Case Study: Visual Cortex Neuronal Diversity. In the mouse visual cortex, scRNA-seq identified a novel inhibitory interneuron subtype characterized by high expression of marker genes (e.g., Pvalb, Sst) and unique electrophysiological profiles [66]. Functional validation revealed this subtype modulates signal transmission and integration within visual circuits through: Synaptic specificity: Preferential connectivity with excitatory pyramidal neurons. Dynamic regulation: Activity-dependent modulation of gamma oscillations during visual processing.

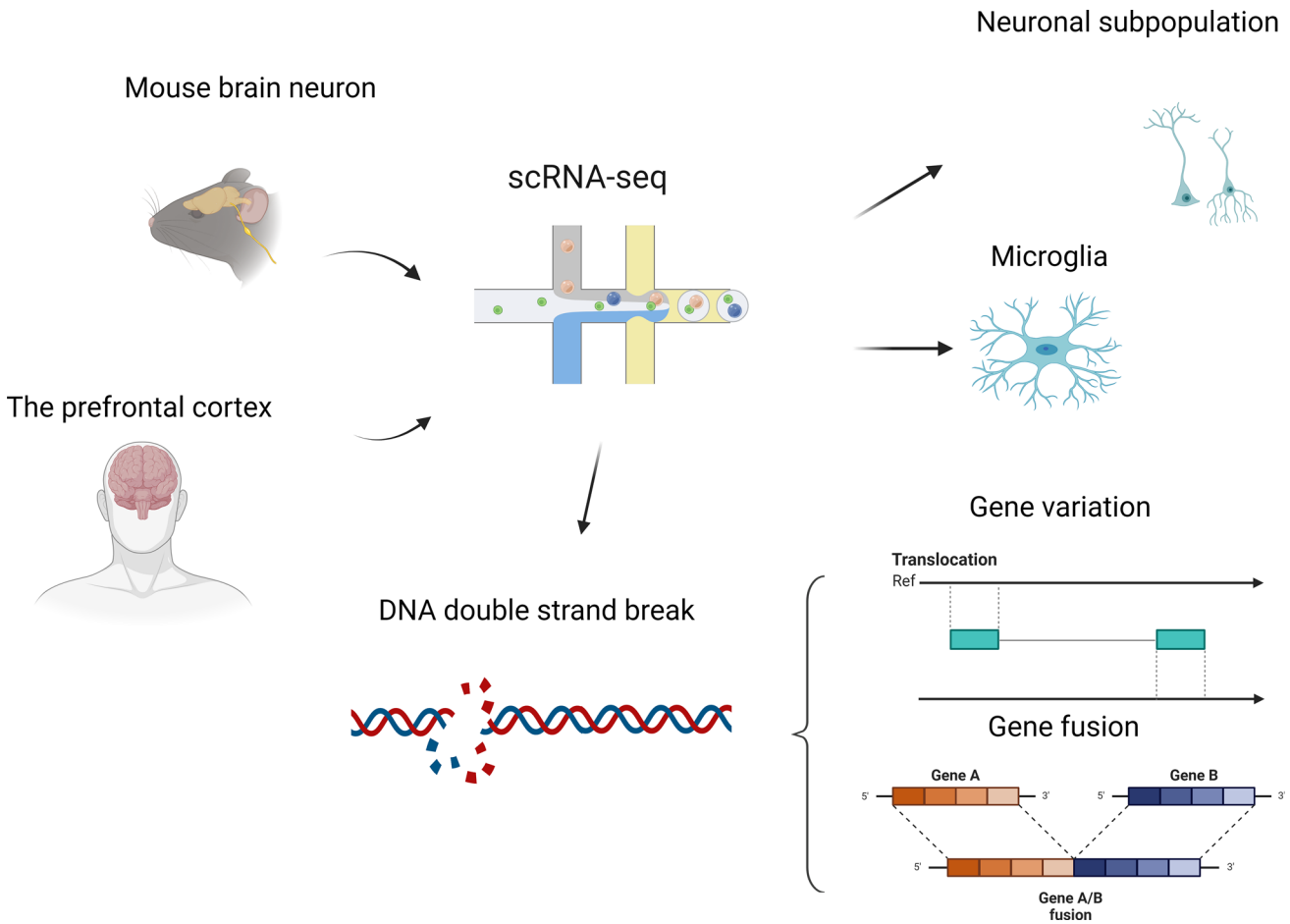

Fig. 4 Single-cell sequencing in neurodegenerative diseases

These findings redefine classical neuronal classifications and provide a molecular framework to investigate circuit-specific roles of neuronal subpopulations in sensory processing and behavior.

In the study of the human brain, SCS technology has also played an important role. SCS of the prefrontal cortex of the human brain identified 21 distinct subpopulations of neurons [67]. These subgroups differ significantly in gene expression, cell morphology, and function, and some of them are closely associated with higher neural functions such as cognition, emotion, and behavior. The in-depth study of these neuron subgroups will help us to better understand the function of the human brain and the pathogenesis of neurological diseases.

SCS in Neuronal Development Research SCS has become a cornerstone for unraveling the molecular dynamics of neuronal development. During neurogenesis, neural stem cells (NSCS) undergo progressive differentiation into diverse mature neuronal subtypes-a process orchestrated by intricate gene regulatory networks. By employing scRNA-seq, researchers can map temporal gene expression trajectories, pinpointing key drivers of neuronal lineage specification.

SCS decodes temporal dynamics of neuronal development by mapping transcriptional trajectories from NSCs to mature neurons. This approach reveals stage-specific regulatory programs governing neurogenesis, with murine models identifying evolutionarily conserved signaling axes (e.g., Notch, Wnt) that orchestrate lineage commitment [68]. Dysregulation of these pathways correlates with neurodevelopmental disorders, pinpointing molecular vulnerabilities in neural differentiation cascades.

In addition to identifying neuron types, SCS technology also enables in-depth studies of neuron function. By analyzing the gene expression profile of neurons, it is possible to infer the biological processes and signaling pathways in which they may be involved, thereby understanding their function in the nervous system. In the study of Parkinson's disease patients, SCS technology was used to analyze dopaminergic neurons in the substantia nigra, and some disease-related gene expression changes were found [69]. These changes can lead to abnormal functioning of dopaminergic neurons, which in turn can trigger symptoms of Parkinson's disease. Further study of the function and mechanism of action of these genes could help develop new treatments for Parkinson's disease.

Study on the mechanism of neurodegenerative diseases

SCS in neurodegenerative Disease Research neurodegenerative diseases, such as Alzheimer's and Parkinson's, pose major threats to global health. Their heterogeneous pathologies involve diverse cell types and molecular pathways. SCS has emerged as a transformative tool to unravel these complexities, providing unprecedented resolution to dissect cell-type-specific molecular alterations linked to disease progression.

SCS in AD Research AD, a prevalent neurodegenerative disorder, is characterized by neuronal loss, extracellular beta-amyloid (Aβ) plaques, and intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein [70]. While traditional studies relied on bulk tissue analyses, SCS now enables precise identification of disease-associated molecular and cellular changes by profiling cell-type-specific gene expression in AD patient brains [71](Fig. 4).

On September 28, 2023, the team of Professor Li Huei Tsai of Massachusetts Institute of Technology published three research papers related to AD in the journal Cell, analyzing the pathogenesis of AD from multiple perspectives from the cellular, genetic and mechanism levels [72–74]. By conducting mononuclear transcriptome and epigenome analysis on 443 human subjects and 194,000 mononuclear microglia with different AD pathological phenotypes, the research team revealed the dynamic changes of microglia state in AD disease progression and the mechanism [72]. The study identified 12 microglial transcriptional states, including homeostasis, inflammation, and lipid processing states of AD dysregulation, and 1,542 AD differentially expressed genes, including specific alterations in microglial state and disease stage. By integrating epigenome, transcriptome, and momoid information, upstream regulators of microglia state, gene regulatory networks, enhancer gene connections, and transcription faction-driven microglia state transitions were inferred, and regulatory networks controlling microglia state transitions during AD progression were described [73]. In human IPSC-derived microglia-like cells, it has been found that predicting ectopic expression of homeostasis activators can induce homeostasis features, and inhibiting inflammatory activators can block the progression of inflammation [74].

In another study [75], CAI Lihui's team used postmortem prefrontal cortex autopsy samples from AD patients and brain tissues from CK-p25 mouse models for mononuclear RNA sequencing (SNNA-SEQ) analysis, respectively, to prove that in the progression of AD disease, Neuronal DNA double strand breaks (DSBs) are a key pathological mechanism that disrupts genomic stability and 3D genomic structure. Using snRNA-seq analysis in postmortem prefrontal cortex samples, gene fusion was found to be abundant in excitatory neurons with DNA damage repair and aging gene characteristics. In the neurodegeneration model of CK-p25 mice, it was also found that DSBs accumulation in neurons would lead to genomic structural variation and gene fusion enrichment, and genes with long transcripts and high transcription levels had a higher frequency of DNA breakage and were more prone to structural variation and gene fusion.

It is suggested that the destruction of genome stability and 3D genome by DSB in neurons is a key pathological factor that cannot be ignored in neurodegeneration.

Parkinson's disease (PD) is another common neurodegenerative disease. SCS technology has also played an important role in the study of PD, helping to reveal the pathogenesis of PD and search for potential therapeutic targets.

Researchers from the Broad Institute developed a novel strategy for enriching dopaminergic neurons from postmortem PD substantia nigra pars compacta (SNpc), combining snRNA-seq with Slide-seq spatial transcriptomics to map 10 distinct subpopulations along the dorsoventral axis[76]. Strikingly, an AGTR1 + neuronal subset localized in the ventral SNpc demonstrated selective vulnerability, exhibiting pronounced TP53/NR2F2 dysregulation. This subpopulation's transcriptional signature showed significant convergence with Parkinson's GWAS risk loci, establishing molecular links between cellular susceptibility and genetic predisposition.

Professor Jiang Qinghua's team at Harbin Institute of Technology integrated single-cell transcriptomics with TCR repertoire sequencing to profile T cell dynamics in PD, analyzing 103,365 T cells from blood and CSF of 8 PD patients and 15 controls (6 fresh + 7 public blood samples; 6 PD/9 control CSF datasets [77]. PD patients exhibited CD4/CD8 ratio inversion ($p < 0.05$) and systemic T cell clonal expansion, particularly within CD8 + subsets across both compartments. Multi-modal analysis of 84,384 dual-profiled cells revealed compartment-specific cytotoxicity signatures in CSF-localized clones, implicating adaptive immune dysregulation in PD pathogenesis.

Single-cell sequencing technology has important application value in the mechanism research of neurodegenerative diseases. Through this technology, we can deeply understand the changes at the cellular level during the disease process, reveal the pathogenesis of the disease, and provide a theoretical basis for the development of new treatments. With the continuous development and improvement of technology, SCS technology will play a more important role in the research and treatment of neurodegenerative diseases and bring new hope for overcoming these difficult diseases.

Diagnosis and therapeutic target discovery of nervous system diseases

SCS technology has shown great potential in the diagnosis of neurological diseases, providing new strategies and methods for the early diagnosis and precise treatment of diseases(Fig. 5). SCS is revolutionizing AD diagnostics by overcoming limitations of conventional methods (clinical assessment, neuroimaging, CSF biomarkers) in early detection sensitivity/specificity. This technology enables high-resolution mapping of disease-associated molecular cascades across neuronal and glial populations (microglia, astrocytes), uncovering cell-type-specific signatures in AD brains[78]. Key advances include: Identification of pre-symptomatic transcriptional biomarkers in vulnerable neuron subtypes. Characterization of microglial activation trajectories showing dynamic correlation with disease progression[79]. Detection of stage-specific gene clusters predictive of pathological severity. These discoveries

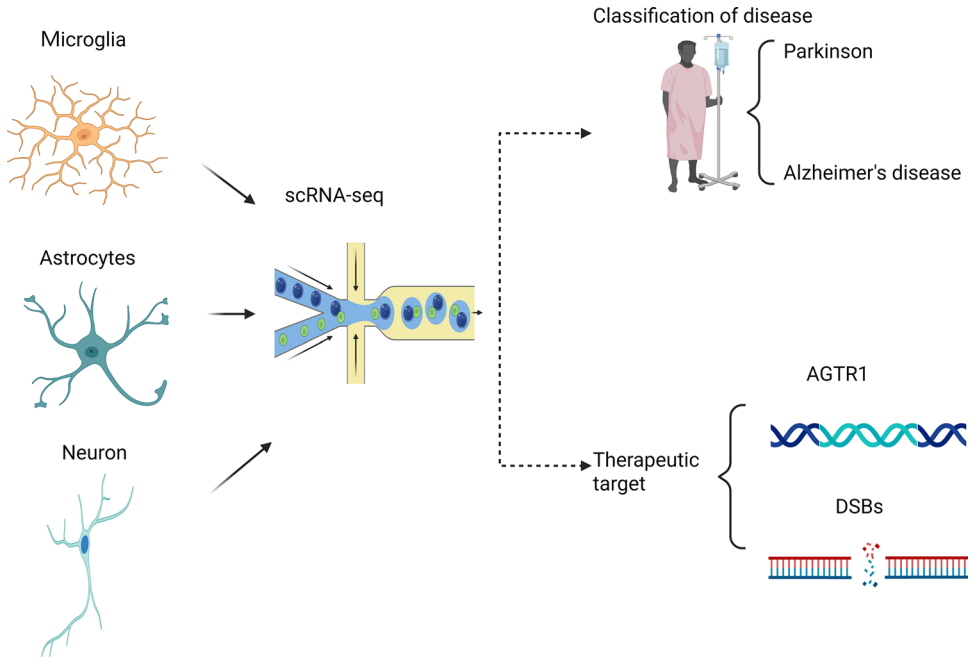

Fig. 5 The study of single-cell sequencing in patients with AD

establish a cellular framework for developing next-generation diagnostic systems based on neural cell-state monitoring.

SCS in PD faces challenges in early detection and molecular subtyping. The hallmark pathologyp-progressive degeneration of midbrain dopaminergic neurons in the substantia nigra, leading to striatal dopamine depletion and motor dysfunction—is traditionally diagnosed via clinical symptoms lacking specific biomarkers. SCS addresses these limitations by profiling dopaminergic neurons in PD patients, revealing vulnerable neuronal subtypes with distinct gene expression signatures (e.g., SNCA, PARK2 dysregulation) [80]. These differentially expressed genes serve as potential biomarkers for early diagnosis and disease monitoring. SCS also dissects immune cell dynamics in blood and CSF, uncovering neuroinflammation pathways (e.g., microglial activation, T cell infiltration) that contribute to PD pathogenesis and offer novel therapeutic targets [81].

SCS in Therapeutic Target Discovery for Neurological Diseases SCS drives therapeutic innovation by identifying disease-specific molecular targets. In AD, SCS studies reveal genes and pathways central to pathogenesis. For example, research [75] identified neuronal DNA double-strand breaks (DSBs) as a key driver of genomic instability and 3D chromatin disorganization, pinpointing DSB repair pathways as therapeutic targets. SCS also highlights microglia's dual role in AD progression-both neuroprotective (Aβ clearance) and pathogenic (neuroinflammation). Targeting microglial activation states (e.g., suppressing pro-inflammatory signaling via TREM2 modulation) emerges as a promising strategy. Current drug development efforts targeting these pathways (e.g., γ-secretase inhibitors, NLRP3 inflammasome blockers) highlight their therapeutic potential. By linking cellular dysfunction to druggable mechanisms, SCS accelerates precision therapy development for neurodegenerative disorders.

SCS has provided pivotal insights into therapeutic target discovery for PD. Profiling substantia nigra dopaminergic neurons revealed AGTR1 + subpopulations with selective vulnerability, marked by TP53/NR2F2 upregulation-key druggable targets for neuroprotection. Simultaneously, this technology uncovered PD-specific immune axis dysregulation, particularly clonal T cell expansion with functional alterations, suggesting immunomodulation as a promising therapeutic avenue. These findings collectively enable precise targeting of both neuronal degeneration pathways and neuroinflammatory mechanisms, paving the way for mechanism-driven therapeutic strategies.

Application of single-cell sequencing in the detection of infectious diseases

Study of pathogen-host cell interaction

SCS in Host–Pathogen Interaction Studies Pathogen infection of host cells is a multifaceted process encompassing invasion, replication, immune evasion, and host defense activation. SCS dissects these interactions with unprecedented resolution, revealing critical insights into infection dynamics by profiling transcriptional responses of individual host and pathogen cells. This approach decodes cell-to-cell variability in immune evasion strategies, viral/bacterial persistence mechanisms, and host defense heterogeneity, offering transformative avenues for understanding infectious disease pathogenesis.

ScRNA-seq has revolutionized HSV-1 infection research by capturing dynamic host-virus interactions at cellular resolution. Analyzing primary neonatal human dermal fibroblasts (NHDFs) at 1/3/5 h post-infection (hpi), researchers identified cell cycle status (S/G2/M phase) and viral replication stages as key heterogeneity drivers of infection susceptibility[82]. Notably, biphasic viral transcriptional dynamics emerged during lytic infection: an initial stochastic phase (1–2 transcripts/cell) followed by coordinated viral gene activation, attributed to US1/UL54-mediated transcriptional switching. These findings illuminate spatiotemporal regulation mechanisms underlying herpesvirus-host interplay.

Single-cell sequencing has unveiled intricate host–pathogen dynamics in Salmonella enterica serovar Typhimurium (S. Typhimurium) infections, as demonstrated by Weizmann Institute researchers. Their analysis of ex vivo infected peripheral blood mononuclear cells (PBMCs) using a novel computational framework revealed cell-type-specific immune response trajectories during bacterial invasion[83]. The study established predictive models linking single-cell molecular phenotypes (e.g., pathogen-sensing pathways, cytokine-signaling cascades) to clinical infection severity, while identifying key immune cell subsets governing bacterial clearance versus systemic spread. These findings provide a mechanistic blueprint for developing diagnostic classifiers and immunomodulatory therapies targeting host vulnerability nodes in bacterial infections.

SCS has emerged as a transformative tool in malaria research, particularly for deciphering Plasmodium parasites' complex life cycle with stage-specific differentiation networks. A landmark study led by the Sanger Institute established a single-cell transcriptomic atlas of > 1,700 Plasmodium berghei parasites across developmental stages in both mosquito vectors and human hosts[84]. This

comprehensive resource captures transcriptional dynamics during host-environment transitions, revealing phase-restricted gene functions and novel regulatory mechanisms governing parasite differentiation. By mapping molecular checkpoints in Plasmodium development, the atlas provides critical insights for antimalarial drug discovery, vaccine development, and transmission-blocking strategies, serving as an essential reference for global malaria control efforts.

Early diagnosis and surveillance of infectious diseases

Infectious diseases are a serious threat to human health. Early and accurate diagnosis and effective monitoring are very important for disease prevention and control. Single-cell sequencing proved instrumental in deciphering COVID-19 pathogenesis and clinical progression through high-resolution immune profiling. Comparative analysis of peripheral blood mononuclear cells (PBMCs) from mild versus severe COVID-19 patients revealed critical immune signatures: Severe cases exhibited lymphopenia with CD8 + T cell functional exhaustion alongside myeloid cell-driven hyperinflammation (IL-6/TNF-α overexpression), directly correlating with cytokine storm severity and impaired viral clearance[85]. Crucially, cell-type-specific early-response genes identified through this approach established predictive biomarkers for presymptomatic diagnosis and disease trajectory forecasting, demonstrating the technology's dual utility in both mechanistic understanding and clinical management of viral infections.

SCS has emerged as a game-changer in influenza management by addressing the limitations of traditional diagnostics (e.g., PCR/antigen tests) in early detection sensitivity. This technology enables single-cell resolution analysis of virus-host interactions, revealing that respiratory epithelial cells rapidly activate innate immune responses through interferon-related gene upregulation upon influenza infection, while the virus simultaneously evades host defenses by hijacking critical signaling pathways[86]. Longitudinal monitoring of immune cell dynamics across disease stages uncovers phase-specific changes: acute infection correlates with CD8 + T cell exhaustion and impaired viral clearance, whereas convalescence features expanded memory T/B cell populations indicative of immune memory formation. These insights not only identify epithelial-derived early-warning biomarkers but also establish immune reconstitution signatures for evaluating therapeutic efficacy, collectively advancing precision strategies for influenza diagnosis, prognosis, and vaccine development.

SCS is revolutionizing tuberculosis (TB) diagnostics and management by overcoming the limitations of conventional methods like sputum smear and tuberculin tests. This technology dissects Mycobacterium tuberculosis-macrophage interactions at cellular resolution, revealing the pathogen's survival strategy through apoptosis suppression (BAX/CASP3 downregulation) and immune response gene silencing in infected macrophages [87]. Crucially, it identifies macrophage-specific metabolic signatures (e.g., IDO1/ARG1 overexpression) as early diagnostic biomarkers while enabling treatment monitoring: therapy responders show restored IFN-γ + CD4 + T cell activity, whereas non-responders exhibit persistent exhausted CD8 + T cell phenotypes with elevated PD-1/TIM-3 expression. These cellular insights advance precision strategies for TB detection, therapeutic optimization, and relapse prediction.

Discovery of new anti-infection targets

Single-cell sequencing technology plays an important role in discovering new anti-infective targets and provides a strong basis for the development of novel anti-infective drugs. In the study of virus infection, single-cell sequencing technology provides a new way to discover new antiviral targets. Taking Hepatitis B virus (HBV) infection as an example [88] (Fig. 6), traditional research methods are difficult to fully reveal the molecular changes at the cellular level after HBV infection. SCS deciphers HBV-hepatocyte interactions with unprecedented resolution, revealing viral-induced transcriptional reprogramming that orchestrates viral replication and immune evasion. This approach pinpoints critical host factors-including proviral dependencies mediating capsid assembly and immune checkpoint regulators-whose therapeutic targeting (via inhibitors/agonists) could disrupt viral persistence. Functional validation of these candidates establishes a translational roadmap for developing precision antiviral strategies against chronic hepatitis B.

Single-cell sequencing is revolutionizing Staphylococcus aureus infection research by decoding host–pathogen interplay at cellular resolution. This technology reveals how immune cells dynamically upregulate defense-related genes (cytokines, chemokines) during early infection, while S. aureus simultaneously deploys toxin-mediated strategies to sabotage host immunity. High-resolution profiling identifies dual therapeutic targets: bacterial virulence regulators (e.g., quorum-sensing systems controlling toxin production) and host factors governing pathogen recognition/clearance. These discoveries enable the development of precision therapies that simultaneously neutralize bacterial virulence and enhance antimicrobial immunity, offering new strategies to combat antibiotic-resistant infections [89].

Parasitic infections are also an important application area of SCS technology. Taking Plasmodium infection as an example, plasmodium has a complex life cycle and a variety of morphological stages, and the regulatory network of cell differentiation is obviously different in different stages of

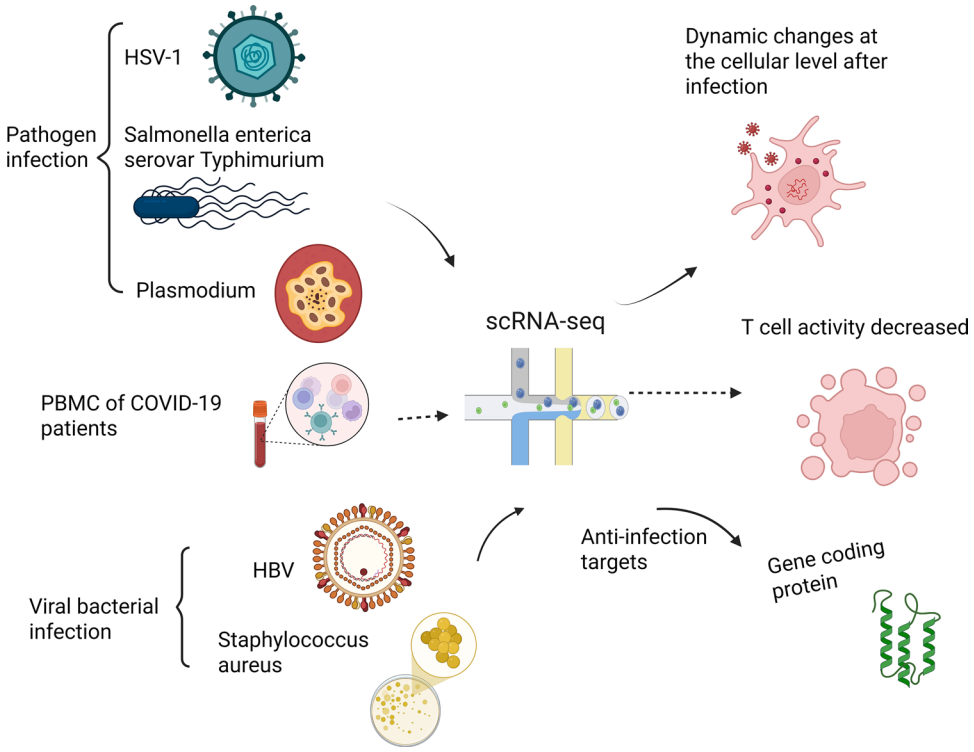

Fig. 6 Application of single cell sequencing in the detection of infectious diseases

its life history. Through the analysis of Plasmodium cells at different developmental stages by single-cell sequencing technology, the regulation mechanism of plasmodium gene expression and pathogenic mechanism can be deeply understood. It was found [90] that the expression of some genes in the erythrocyte phase of Plasmodium is closely related to the invasion, proliferation and escape from the host immune attack. By studying the function of these genes, it is possible to discover new antimalarial targets. For example, the proteins encoded by certain genes may be the key molecules of Plasmodium invasion of red blood cells, or the important factors regulating the metabolism and survival of plasmodium. The development of drugs or vaccines targeting these targets is expected to block the infection and transmission of plasmodium, and provide a new means for malaria prevention and control.

Application of single-cell sequencing in detection of other diseases

Autoimmune disease

Autoimmune diseases are a kind of diseases caused by the body's immune system mistakenly attacking its own tissues and organs. The pathogenesis is complex, involving the abnormal activation and dysfunction of a variety of immune cells(Fig. 7). Rheumatoid Arthritis and Single-Cell Sequencing Insights Rheumatoid arthritis (RA), a chronic autoimmune disorder characterized by joint inflammation and tissue damage, severely compromises patients' quality of life [91]. Traditional research methods struggle to capture the intricate immune cell dynamics in RA pathogenesis, whereas SCS enables precise characterization of immune populations at cellular resolution. Analyses of synovial tissue and peripheral blood mononuclear cells (PBMCs) from RA patients have uncovered disease-specific immune subsets and molecular signatures. Notably, a pro-inflammatory macrophage subset with elevated expression of cytokines (e.g., TNF-α, IL-6) and chemokines was identified in synovial lesions, driving inflammatory responses. Concurrently, T cell profiling revealed expanded Th17 populations secreting IL-17, a key mediator of joint destruction. This approach further revealed novel disease-associated genes and signaling pathways, highlighting potential therapeutic targets for RA treatment.

SCS Advances in Systemic Lupus Erythematosus ResearchSystemic lupus erythematosus (SLE), a highly complex autoimmune disorder characterized by multi-organ involvement and diverse clinical manifestations, has been increasingly studied using single-cell sequencing to decode its pathogenic mechanisms and therapeutic vulnerabilities. Analysis of peripheral blood mononuclear cells (PBMCs) from SLE patients through this approach has revealed multiple immune abnormalities [92]. Notably, abnormally activated B cell subsets exhibiting elevated expression of autoantibody-associated genes were identified, potentially driving autoantibody production and immune complex

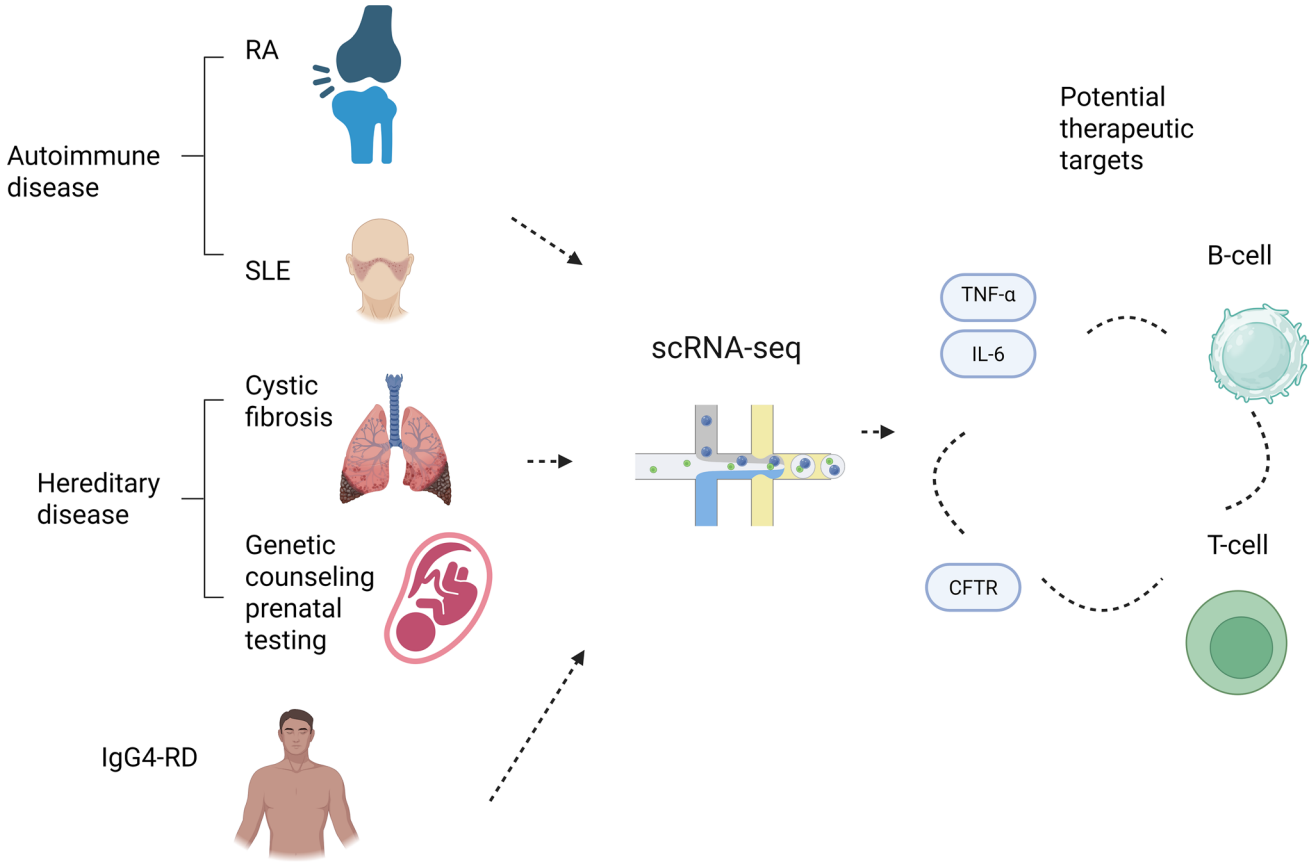

Fig. 7 Application of single cell sequencing in autoimmune diseases, genetic diseases and rare diseases

formation. Concurrently, T cell dysregulation was observed, marked by hyperactivation of Th1 and Th17 cells coupled with impaired regulatory T cell (Treg) function, contributing to immune imbalance and subsequent tissue damage. Additionally, chromatin accessibility profiling uncovered disease-associated regulatory elements and transcription factors, providing novel mechanistic insights into SLE pathogenesis. These findings highlight the utility of single-cell technologies in dissecting SLE immunopathology and guiding precision therapies.

SCS in Other Autoimmune DisordersBeyond RA and SLE, single-cell sequencing has advanced research in autoimmune diseases like Sjögren's syndrome and ankylosing spondylitis. In Sjögren's syndrome, single-cell analysis of salivary gland tissue uncovered immune cell infiltration, functional aberrations in lymphocytes, and stress-responsive epithelial cells with apoptosis-related alterations, illuminating disease mechanisms [93]. Similarly, studies in ankylosing spondylitis identified dysregulated immune subsets in peripheral blood and joint tissues, revealing cellular heterogeneity and functional reprogramming linked to inflammation, while pinpointing candidate biomarkers and therapeutic targets [94]. These findings underscore the broad utility of single-cell approaches in dissecting autoimmune pathogenesis.

Hereditary disease

Genetic diseases are diseases caused by changes in genetic material, and there are many kinds of diseases that seriously affect the health and quality of life of patients(Fig. 6). Single-Cell Sequencing in Rare Genetic DisordersSingle-cell sequencing has become pivotal in studying rare genetic diseases, particularly those with high genetic heterogeneity where conventional methods fail to pinpoint diagnoses or causal variants. By profiling whole genomes or transcriptomes at single-cell resolution, this technology enables comprehensive profiling of genetic variants. For monogenic disorders like cystic fibrosis and Huntington's disease, single-cell sequencing reliably detects pathogenic mutations, facilitating early diagnosis and genetic counseling. In cystic fibrosis, for instance, it identifies CFTR gene mutations causing chloride channel dysfunction [95]. Moreover, this approach uncovers novel pathogenic mutations, expanding the genetic landscape of rare diseases and refining mechanistic understanding.

SCS in Genetic Counseling and Prenatal DiagnosisSingle-cell sequencing holds transformative potential for genetic counseling and prenatal diagnostics, particularly for families with hereditary disease risks. By analyzing individual embryonic or fetal cells, this technology enables early detection of pathogenic variants, empowering informed reproductive decisions. In prenatal diagnostics [96], single-cell approaches detect chromosomal abnormalities (e.g., trisomy 21 in Down syndrome, trisomy 18 in Edwards syndrome) and monogenic disorders through non-invasive profiling of cell-free fetal DNA (cffDNA) in maternal blood. For monogenic diseases, targeted sequencing of fetal cells accurately identifies inherited mutations, guiding clinical interventions such as pregnancy termination or early therapeutic strategies. These applications significantly reduce the incidence of genetic disorders and improve population health outcomes.

SCS in therapeutic development for genetic disorders beyond diagnostics and prenatal testing, SCS drives novel therapeutic strategies for inherited diseases by elucidating molecular mechanisms and actionable targets [97]. Profiling patient-derived cells at single-cell resolution identifies disease-specific signaling pathways and hub genes, enabling targeted drug development. For example, single-cell analyses have uncovered druggable pathways in monogenic disorders, where drugs targeting these pathways may offer therapeutic breakthroughs. Furthermore, the technology facilitates real-time tracking of cellular responses during treatment, monitoring dynamic changes in gene expression profiles and genetic variants to evaluate therapeutic efficacy. This capability allows clinicians to optimize regimens based on molecular evidence, accelerating precision medicine for genetic diseases.

Rare disease

SCS in Rare Disease ResearchRare diseases, characterized by ultra-low prevalence, heterogeneous genetic basis, and diverse clinical presentations, represent a significant unmet medical need. Epidemiologically, over 7,000 rare disorders have been documented globally, collectively affecting approximately 300 million individuals [98]. Conventional approaches struggle to systematically investigate their pathogenesis or identify therapeutic targets due to limited patient cohorts. Single-cell sequencing overcomes these barriers by enabling high-resolution dissection of disease mechanisms at cellular granularity, revealing cell-type-specific molecular aberrations and uncovering novel therapeutic vulnerabilities. This technology has become instrumental in advancing precision diagnostics and targeted therapy development for rare diseases (Fig. 6).

Single-Cell Profiling Elucidates Pathogenesis of IgG4-Related Disease IgG4-related disease (IgG4-RD), a chronic fibroinflammatory disorder characterized by IgG4 + plasma cell infiltration in multiple organs (e.g., salivary glands, pancreas, aorta), leads to fibrotic lesions, irreversible tissue damage, and organ dysfunction. Its pathogenesis remains poorly understood. A collaborative study by Tianjin Medical University researchers employed scRNA-seq and bulk RNA-seq to dissect immune cell heterogeneity in affected tissues and peripheral blood mononuclear cells (PBMCs), uncovering molecular mechanisms underlying IgG4-RD [98]. This study delineates the cellular origins of pathogenic IgG4 + plasma cells and identifies potential therapeutic targets, advancing mechanistic insights into IgG4-RD.

SCS Advances Cystic Fibrosis Research Cystic fibrosis (CF), an autosomal recessive disorder caused by mutations in the CFTR gene, disrupts mucus secretion and ion transport in the respiratory and gastrointestinal systems, leading to severe multisystem complications. Single-cell sequencing of airway epithelial cells from CF patients has revealed distinct transcriptional heterogeneity across cell subsets [99]. Key findings include dysregulated expression of genes governing mucus production and ion transport in specific epithelial subpopulations, directly linking these abnormalities to disease pathology. Furthermore, analyses uncovered inter-patient and intra-patient heterogeneity in cellular composition and gene expression patterns, highlighting the complex molecular landscape of CF. These insights provide a foundation for developing cell subtype-targeted therapies tailored to individual disease variants, advancing precision medicine approaches for CF.

SCS in Functional Profiling of Rare Diseases Single-cell sequencing enables deep functional and phenotypic characterization of disease-associated cells in rare disorders. By analyzing transcriptomic profiles of patient-derived cells [100], this technology identifies aberrant gene expression linked to cellular metabolism, signaling pathways, and functional impairments that drive pathogenesis. For example, single-cell studies in select rare diseases have revealed dysregulated genes contributing to defective cellular processes, offering mechanistic insights for therapeutic intervention. These findings not only elucidate molecular mechanisms but also nominate actionable targets for drug development. As the technology matures, its integration into rare disease research and clinical workflows holds promise for advancing precision diagnostics and tailored therapies, ultimately improving outcomes for patients with these understudied conditions.

Conclusion

Summary of research results

SCS in Disease Diagnostics and Precision Medicine Single-cell sequencing has revolutionized disease research

and clinical practice by enabling cell-level resolution of molecular landscapes across diverse pathologies. In oncology, this technology deciphers tumor heterogeneity, identifying distinct malignant subclones with divergent therapeutic vulnerabilities. It further maps dynamic interactions within the TME, exposing immunosuppressive networks between cancer cells, stromal components, and immune infiltrates. Clinically, it facilitates early cancer detection through rare circulating tumor cell profiling and improves prognostic stratification by correlating TME signatures with treatment response. In neurological disorders, single-cell atlases redefine neuronal and glial taxonomy, uncovering disease-specific subtypes in neurodegeneration and elucidating mechanisms like synaptic dysfunction and neuroinflammation. These insights drive biomarker discovery for preclinical diagnosis and highlight therapeutic targets such as tauopathy regulators. For infectious diseases, the technology dissects host–pathogen interplay, capturing immune evasion strategies and identifying infection-stage-specific immune signatures for rapid diagnostics. It also accelerates antimicrobial target discovery by profiling pathogen adaptation mechanisms. In autoimmune and genetic diseases, single-cell analyses resolve pathogenic immune subsets and their cytokine networks, while pinpointing mosaic mutations in genetically heterogeneous cohorts. For rare diseases, it uncovers cell-type-specific molecular lesions, enabling targeted therapy repurposing. By bridging molecular mechanisms to clinical needs, single-cell sequencing stands as a cornerstone of precision medicine, transforming diagnostics, therapeutic development, and patient stratification across diverse diseases.

Research prospects

Looking ahead, technological refinements promise a broader impact. Cost reductions through microfluidic automation and multiplexed assays will enhance clinical accessibility. Cross-platform standardization and AI-powered analytical frameworks will improve data reproducibility. Integration with multi-omics modalities (spatial transcriptomics, proteomics) and longitudinal sampling will deepen mechanistic insights into disease trajectories. These advances will solidify single-cell sequencing as a cornerstone of precision medicine, enabling dynamic profiling of therapeutic responses and accelerating biomarker-to-therapy translation across diverse pathologies.

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Data availability Not applicable.

Declaration

Conflict of interest The authors declare no conflict.

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