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REVIEW

Advancing Cardiovascular Medicine: Innovative Therapeutic Pathways with Single-Cell Technologies

Jiahui Ji[1,2,3,4] · **Eric L. Lindberg**[1,2,3,4] · **Daniel Reichart**[1,2,3,4]

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Abstract

Purpose of Review Cardiovascular diseases (CVDs) encompass a wide range of conditions affecting the heart and vasculature and remain the leading cause of mortality worldwide. The pathogenesis of CVDs is related to complex molecular, cellular, and systemic interactions, involving dysregulated signaling pathways, inflammatory responses, genetic predispositions, and intercellular communication. Despite significant advancements, the precise mechanisms underlying CVDs remain only partially understood. This review aims to explain how single-cell and single-nucleus transcriptomics facilitate our understanding of CVD pathogenesis. It focuses on their integration with genomic and epigenomic approaches, cellular heterogeneity, intercellular communication, regulatory networks, and genetic associations.

Recent Findings Recent applications of single-cell and single-nucleus transcriptomics in cardiovascular research have already revealed significant alterations in cellular composition and gene expression profiles associated with dilated cardiomyopathy (DCM), arrhythmogenic cardiomyopathy (ACM), and hypertrophic cardiomyopathy (HCM). Furthermore, spatial transcriptomic technologies have provided critical insights into human cardiac development, the conduction system, and region-specific molecular changes in myocardial infarction, advancing our understanding of cardiac structure and function. Integrating single-cell transcriptomics with epigenomics further enhances our understanding of cell type- and state-specific regulatory landscapes, which can be validated through single-cell perturbation technologies. Additionally, combining genomic studies with single-cell technologies helps to recover causal relationships between genetic variants, gene expression patterns, and cellular phenotypes.

Summary Single-cell and single-nucleus transcriptomics technologies have enhanced our understanding of CVD mechanisms, uncovering cardiac cellular diversity and elucidating key regulatory processes in disease states. With larger datasets, more robust multi-omics integration, and advanced computational frameworks, transcriptome studies at single cell level will significantly enhance the ability to explore disease mechanisms and identify therapeutic targets. Integrating individualized transcriptomes into the medical routine will furthermore facilitate more precise and effective interventions in cardiovascular medicine.

Keywords Single-cell sequencing technologies · Cardiovascular diseases · Transcriptomics · Bioinformatics

✉ Daniel Reichart
Daniel.Reichart@med.uni-muenchen.de

Jiahui Ji
Jiahui.Ji@med.uni-muenchen.de

Eric L. Lindberg
Eric.Lindberg@med.uni-muenchen.de

[1] Department of Medicine I, University Hospital Munich, Munich, Germany

[2] Gene Center, University Hospital Munich, Munich, Germany

[3] Interfaculty Center for Endocrine and Cardiovascular Disease Network Modelling and Clinical Transfer (ICONLMU), LMU Munich, Munich, Germany

[4] DZHK (German Center for Cardiovascular Research), Partner Site Munich, Munich, Germany

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Opinion Statement

The application of single-cell/single-nucleus transcriptomics in cardiovascular medicine hold transformative potential to advance the diagnosis, monitoring, and treatment of cardiovascular diseases. Detailed molecular profiling of individual cells, including genes and signaling pathways, generated through single-cell/single-nucleus transcriptomics could allow for more accurate diagnoses, refined disease classification, and early detection of pathological changes.

Another promising avenue is the correlation of tissue-specific signatures with circulating biomarkers found in biofluids such as exosomes and microvesicles, and circulating cell-free nucleic acids. This could facilitate the development of liquid biopsies to diagnosis and monitor diseases with minimal invasion.

Moreover, by characterizing single-cell-specific mutations, therapies can be tailored to individual patients, enhancing treatment precision and efficacy.

Single-cell/single-nucleus sequencing also plays an important role in immunological profiling, enhancing understanding of immune cell dynamics in cardiovascular disease, such as myocarditis and transplant rejection. These advances could facilitate the development of targeted immunotherapies, including engineered regulatory T cells, with improved specificity and reduced systemic toxicity.

In regenerative medicine, single-cell/single-nucleus technologies could guide stem cell differentiation and enable real-time surveillance of transplanted cells integrating into cardiac tissues. These capabilities are essential for advancing myocardial repair and tissue engineering.

Introduction

Heart diseases, collectively termed as cardiovascular disease (CVD), represents a diverse range of conditions that impair the structure and function of the heart and its associated vasculature. CVDs remain the leading cause of mortality worldwide, accounting for approximately 30% of all deaths in 2012 in the United States alone and affecting an estimated 17.5 million people [1, 2]. The mechanisms driving CVDs remain incompletely understood. This is primarily due to the complex interplay of molecular, cellular, and systemic factors that not only occur within the heart but also involve other organs and systems throughout the body.

The human heart is a complex organ composed of four anatomically and functionally distinct chambers, conduction system and valves, and a dynamic microenvironment of diverse cell types [3, 4]. Gene expression and cellular interactions regulate these components, ensuring proper heart function, while a functioning heart, in turn, shapes gene expression and cellular dynamics [5]. Throughout normal development and life, the heart demonstrates remarkable adaptability, responding to physiological and hemodynamic changes by altering structure and function to maintain uninterrupted contraction and blood flow. However, under disease conditions, harmful stimuli, such as ischemic, mechanical, electrical, or chemical injuries, disrupt the balanced processes, altering cellular microenvironments and transcriptional landscapes, ultimately leading to the pathogenesis and progression of CVDs.

CVDs encompass various conditions with specific pathophysiological mechanisms, clinical challenges, and prognostic implications. The most common cardiovascular disease (CVD) is coronary artery disease (CAD), which arises from coronary atherosclerosis [6, 7]. CAD can lead to symptoms such as angina pectoris or dyspnea, as well as more severe outcomes like myocardial infarction and ischemic cardiomyopathy [8, 9]. Another major category of CVDs is non-ischemic cardiomyopathies, which impair the heart muscle's ability to contract effectively and circulate blood adequately [10]. These conditions include (ischemic and non-ischemic) dilated hypertrophic, arrhythmogenic, and restrictive cardiomyopathies. As these diseases progress, they can lead to advanced heart failure (HF), characterized by fatigue, dyspnea, and fluid overload. In end-stage cases, the only definitive treatment options remain heart transplantation or implantation of a mechanical assist device.

Advancements in early diagnosis and medical treatment have significantly improved outcomes for CVDs. However, CVDs remain a major global health challenge, requiring deeper insights into their underlying mechanisms. Recent breakthroughs in single-cell and spatial technologies have revolutionized our understanding of CVDs by characterizing the transcriptional and functional states of individual cardiac cell types [11–18]. These single cell and spatial omics high-resolution approaches have uncovered novel biomarkers, disease-specific transcriptional signatures, and intricate cellular communication networks, offering a transformative, cell-centric perspective on cardiac health and disease progression. As genomics, transcriptomics, and epigenetics continue to evolve, these cutting-edge technologies hold immense promise for improving diagnostic accuracy, refining patient risk classification, and developing targeted therapies. The seamless integration of scientific innovation with clinical research and patient care is poised to drive transformative progress in cardiovascular medicine, ultimately reducing the global burden of heart disease.

Single-Cell Transcriptomics Sequencing

Bulk RNA sequencing has significantly contributed to our understanding of the molecular mechanisms underlying heart disease by providing an averaged gene expression profile across all cardiac cell types [5, 19]. However, this approach masks crucial cell-to-cell variability, which is essential for understanding both normal and diseased hearts. To overcome this limitation, single-cell RNA sequencing (scRNA-seq) and single-nucleus RNA sequencing (snRNA-seq) have emerged as powerful tools, enabling transcriptomics analysis at the level of individual cells or nuclei. These methods enable the identification of distinct cardiac cell populations, uncovering cellular heterogeneity and differential gene expression that would otherwise be masked in bulk analysis [5].

The process of single cell experiments targeting cardiovascular system begins with transcriptomic profiling of heart tissues through scRNA-seq/snRNA-seq. The downstream analysis includes clustering and annotation to identify different cardiac cell populations, compositional analysis to quantify the changes in abundance of different cell types or states, and trajectory analysis along with RNA velocity to infer continuous transitions between cell states. In addition, gene regulatory networks capture transcriptional regulatory interactions, and cell–cell communication analysis reveals intercellular signaling and functional coordination. Taken together, single cell downstream analysis provides comprehensive understanding of the cellular landscape, regulatory mechanisms, and dynamic processes within healthy and diseased heart tissues. Figure 1, retrieved from BioRender scRNA-seq/snRNA-seq experiments begin with the enzymatic digestion of biological tissue samples to dissociate cells for single-cell experiments using fresh tissues or the isolation of nuclei from cells for single-nucleus experiments using frozen tissues. This is followed by reverse transcription of mRNA into complementary DNA (cDNA), cDNA amplification, library construction, and high-throughput sequencing. scRNA-seq/snRNA-seq protocols include plate-based and droplet-based methods. Plate-based protocols, such as SMART-seq, isolate single cells into wells of multi-well plates, allowing for full-length transcript analysis [20]. High-throughput droplet-based methods, including platforms like Chromium (10X Genomics), ddSEQ (Bio-Rad/Illumina), Nadia (Dolomite), and inDrop (1CellBio), use microfluidic droplets to encapsulate cells or nuclei with barcoded beads, enabling scalable single-cell or single-nucleus transcriptomics capture [21–24]. For example, the 10X Genomics Chromium platform employs high-throughput droplet-based encapsulation to isolate single cells or nuclei, lyse them within droplets to release mRNAs, and construct cDNA libraries from 3' or 5' poly-A tails [5].

scRNA-seq/snRNA-seq is able to generate the transcriptomics landscape of thousands to millions of cardiac single cells or nuclei. Importantly, since cardiomyocytes, the most functionally important cardiac cell type, are too large for microfluidics-based single-cell methods, snRNA-seq is particularly advantageous, allowing for studying cardiomyocytes' transcriptional profiles through isolated nuclei.

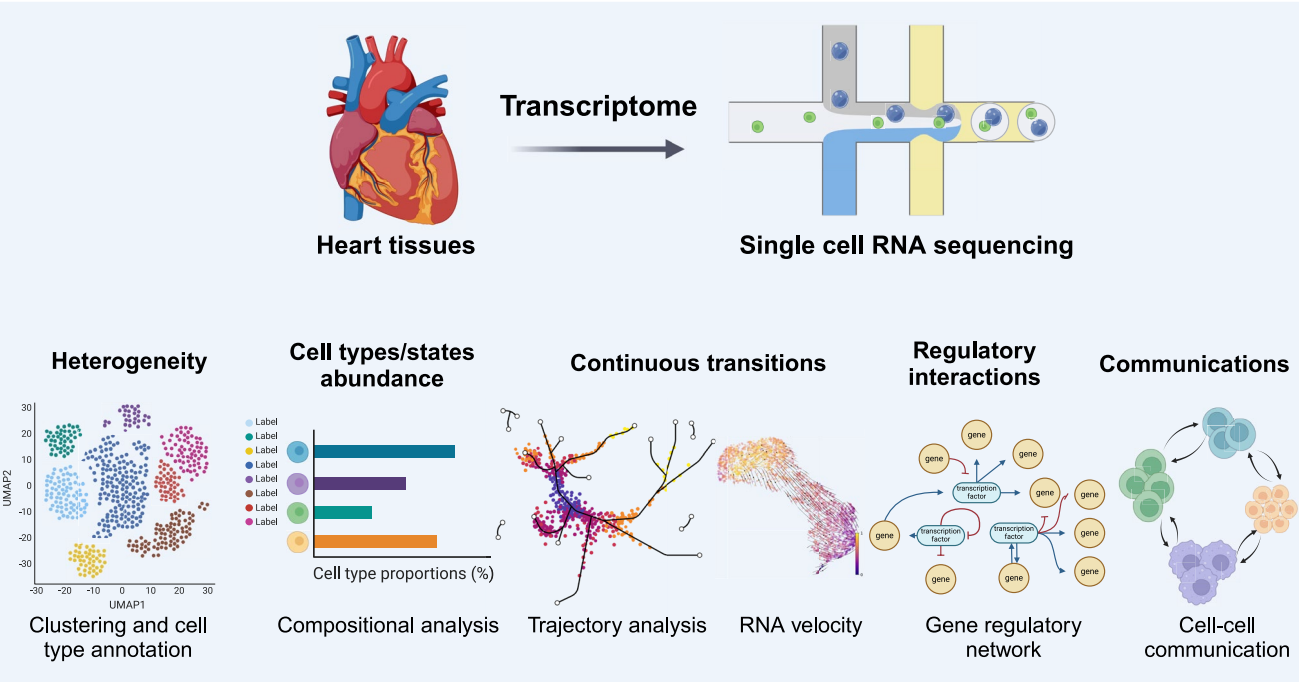

Fig. 1 Comprehensive methods for downstream analysis of scRNA-seq/snRNA-seq data

scRNA-seq/snRNA-seq enable various downstream computational analysis to understand cellular heterogeneity, rare cell types, and dynamic biological processes, such as cardiac development, disease progression, and therapeutic response. The downstream analysis following single cell experiments include cell type annotation, compositional analysis, trajectory analysis, RNA velocity, gene regulatory network inference, and cell–cell communication modeling (Fig. 1). Each of these techniques provides unique perspectives on cellular behavior and interaction, which together drive progress in the development of targeted treatments, and prediction of drug responses.

Clustering Cells based on Single-Cell Transcriptomics Profiles and Composition Changes in Cell Types and States of Clustered Cells

scRNA-seq/snRNA-seq have advanced the study of gene activity in individual cells. This tool allows researchers to examine complex heart tissues in detail, revealing the different types of cells and their various states [25, 26]. A fundamental step of understanding tissue diversity is identifying unique groups of cells, such as specific cell types and their different conditions. This step is accomplished by clustering individual cells based on their gene expression profiles, thereby grouping cells with similar transcriptional profiles [27].

Cell clustering is performed using k-nearest neighbor (KNN) graph based community detection algorithms, such as Louvain algorithm and Leiden algorithm [28–31]. Further cell type annotation can be performed through manual or automated approaches. Manual annotation relies on cluster-specific gene signatures, referred to as marker genes [32]. Canonical marker genes, which are well-established and commonly used for identifying major cardiac cell types, are summarized in Table 1. Automated cell-type annotation uses classifier-based methods like CellTypist and Clustifyr, which rely on pre-trained models from previous datasets [33, 34]. Automated cell-type annotation also includes reference mapping techniques like scArches, Symphony, and Azimuth, which match new data to annotated references using label transfer algorithms [35, 36].

Table 1 Canonical marker genes of cardiac cell types

Cell type	Marker genes
Cardiomyocytes	*RYR2*, *TTN*, *MYBPC3*, *TNNT2*, *PLN*, *SLC8A1*, *MHRT*, *MYH6*
Endothelial cells	*VWF*, *PECAM1*, *CDH5*, *CCDC85A*, *BTNL9*
Fibroblasts	*DCN*, *GSN*, *PDGFRA*, *PCDH9*, *BMPER*
Smooth muscle cells	*MYH11*, *ACAT2*, *CDH6*
Neurons	*NRXN1*
Macrophages	*FCGR1*, *F13A1*, *ADGRE1*
Adipocytes	*ADIPOQ*, *TSHR*, *PLIN1*
Pericytes	*PDGFRB*, *TRPC3*, *VTN*
Endocardial cells	*PECAM1*, *NPR3*, *TMEM108*, *PLVAP*
Epicardial cells	*MSLN*, *PCDH15*, *MUC16*
Schwann cells	*PLP1*, *GFRA3*, *PCDH9*
B cells	*PAX5*, *LY6D*
T cells	*NKG7*, *THEMIS*, *CD3E*, *ITK*

Another important aspect of single-cell/single-nucleus analysis is the identification of distinct cell states (cell subtypes) within a given cell type, indicating further heterogeneity of one cell type. For example, in cardiomyocytes, single-cell/single-nucleus transcriptomics can reveal different subpopulations, stress-responsive CMs (with marker genes of *MYH9*, *NEXN* and *CNN1*) or metabolic-active CMs (with marker genes of *NDUFB11*, *NDUFA4*, *COX7C* and *COX5B*) [11], based on specific gene expression patterns.

Furthermore, changes in the relative abundance of different cell types or states, referred to as compositional changes, are indicators of cell types/states involved in biological processes and pathological conditions. Univariate statistical models, such as Poisson regression or Wilcoxon rank-sum tests, analyze the changes in abundance for each cell type individually [37]. Univariate statistical models can mistakenly interpret changes in cell populations as significant due to compositional bias, which occurs because the data represents proportions rather than absolute values. This bias can increase false positives and lead to incorrect conclusions. Tools like the Centered LogRatio (CLR) transformation and scCODA enhance cell compositional analysis. CLR normalizes data by converting raw counts into log-ratios relative to the geometric mean, while scCODA, a Bayesian framework, accounts for interdependence among cell types, enabling robust modeling of their relationships [15, 38].

Continuous Transitions Between Discrete Cell States

scRNA-seq/snRNA-seq techniques provide static views of cell states. However, in biological systems, cells transition smoothly between states [39]. This transition occurs through gradual changes in gene activity, specifically in the process of transcription [40]. Computational trajectory inference methods, including Monocle, Slingshot, RaceID/StemID, and PAGA, have been developed to reconstruct the continuous progression of cell states [41–44]. These trajectory inference methods could infer complex structures, including linear progressions, cyclic patterns and intricate branching structures that denote divergent cellular differentiation fates [41, 42]. Beyond generating a lineage structure representing cellular progression, mathematical models also enable the identification of key regulatory genes that drive and define lineage progression [41, 45].

RNA velocity analysis enhances these trajectory inferences by introducing directional information based on

splicing kinetics, using tools like scVelo and velocyto [46, 47]. RNA velocity predicts a cell's future transcriptional state by analyzing the ratio of unspliced to spliced RNA reads. Since unspliced RNA represents newly transcribed molecules, an excess of unspliced RNA suggests gene upregulation, while a decline indicates downregulation, revealing dynamic changes in gene activity over time [46]. This approach offers dynamic insights into cell fate transitions [46]. Under conditions where RNA kinetics are variable or multiple transcriptional dynamics coexist, lineage-specific modeling approaches can further improve the accuracy of trajectory inference [48–50]. It is worth noting that applying RNA velocity on snRNA-seq data is challenging, due to the technology characteristics of focusing on nuclear RNA [5]. Compared to scRNA-seq/snRNA-seq, snRNA-seq is biased toward capturing unspliced pre-mRNA, as mature mRNA is predominantly located in the cytoplasm, increasing the complexity of the analysis [48].

Gene Regulatory Networks

Transcriptomics data enables the inference of regulatory interactions between genes by analyzing co-expression patterns, and transcription factor activity. Gene regulatory networks (GRNs) serve as comprehensive frameworks to study the interactions between gene and gene expression regulators, such as transcription factors (TFs), regulatory RNAs, and RNA-binding proteins (RBPs), and their target genes. scRNA-seq/snRNA-seq further facilitates the construction of GRNs specific to distinct cell types or states, particularly in disease contexts, providing deeper insights into cell-type-specific regulatory mechanisms and their alterations between healthy and diseased conditions.

Many network inference methods, which were developed for bulk RNA sequencing, such as GENIE3 and ARACNE [51, 52], have been applied to scRNA-seq/snRNA-seq datasets. GENIE3 uses random forest models to predict regulatory genes for a target gene by assessing how well regulatory gene expression can predict the target's expression [51]. ARACNE, an information-theoretic method, infers regulatory networks based on Mutual Information (MI), which measures the dependency between two variables [52]. In addition to bulk RNA sequencing, single-cell-specific approaches, such as Partial Information Decomposition and Context (PIDC) and Single-cell rEgulatory Network Inference and Clustering (SCENIC) have been developed [53, 54]. PIDC leverages multivariate information to quantify dependencies among variables, decomposing them into redundant, unique, and synergistic components [53]. SCENIC combines GENIE3-based network inference with downstream pruning to identify active regulatory networks and corresponding cell states [54].

Cellular Communications

In multicellular organisms, cells work together within and across tissue niches to maintain homeostasis and respond to external and internal perturbations [55]. This coordination is achieved through cell-to-cell signaling, which in turn affects intracellular activities, such as gene regulatory processes within each cell [55]. Cell–cell communication (CCC) refers to a subset of cell–cell interactions (CCIs) that involve biochemical signals exchanged between or within cells, which further generate intracellular effects [55]. CCC research mainly focuses on protein-mediated interactions, such as ligand-receptor, extracellular matrix-receptor interactions, and receptor-receptor [55]. CCC inference involves analyzing gene expression in sender and receiver cells, with communication quantitatively defined by the expression of ligands and their corresponding receptors [56, 57]. Tools such as CellChat, CellPhoneDB, and ICELLNET are widely used to infer CCC between cell clusters by assigning communication scores to ligand-receptor pairs and evaluating their statistical significance [56–58]. Notably, platforms like CellChat and CellPhoneDB consider the role of multisubunit protein complexes in ligand-receptor interactions [56, 58]. Furthermore, tools such as Nichenet and Cytotalk complement CCC analysis by providing additional insights, such as induced gene expression changes, thereby increasing confidence in predicted interactions.

Advances in Spatial Transcriptomics

Advancements in genomic technologies have enabled spatially resolved transcriptomics profiling, allowing for the simultaneous assessment of gene expression while maintaining cellular location information within tissues [59]. Integrating transcriptomics data with spatial localization, spatially resolved transcriptomics (SRT) provides crucial insights into cell-type-specific and region-specific gene expression patterns, intercellular interactions, and the influence of the tissue microenvironment on cellular function [60].

The spatially resolved transcriptomics workflow involves carrier design, tissue treatment and RNA capture, reverse transcription and cDNA amplification, library construction and followed sequencing to generate data [61, 62]. The carrier design, integrated with spatial probes, has advanced to enhance resolution, progressing from multicell to single-cell/single-nucleus and even subcellular levels [63]. Tissue treatment is an important step in the workflow, ensuring optimal RNA extraction and hybridization, with fresh-frozen and formalin-fixed, paraffin-embedded (FFPE) tissues being the two common preparation methods. Both methods rely on enzymatic permeabilization, using

proteases like proteinase K for fresh-frozen tissues and a combination of heat-induced antigen retrieval (HIAR) and enzymatic treatment for FFPE tissues—to break down cell membranes and cross-links [64, 65]. A wide range of spatial transcriptomics technologies has been developed. For example, Slide-seqV2 and DBiT-seq improve upon this by reaching a nearly single-cell resolution of 10 μm, while Visium HD provides a resolution of 2–8 μm, being suitable for analyzing tissue-level gene expression patterns [66, 67]. For applications requiring subcellular resolution, technologies such as Pixel-seq, Seq-Scope, and Stereo-seq push the boundaries by achieving spatial resolutions as fine as 0.5 μm, allowing researchers to study gene expression at the level of individual organelles and cellular compartments [61, 68].

The downstream analysis of spatially resolved transcriptomics data mainly involves spatial matrix generation, image registration, cell segmentation, deconvolution, gene imputation, and cell–cell communication analysis [69]. Sequencing-based and imaging-based SRT methods require accurate spatial barcode assignment and fluorescence signal processing [70]. Lower-resolution SRT relies on deconvolution algorithms, such as Robust cell type decomposition (RCTD) and Tangram to infer single-cell gene expression, with challenges remaining in resolving rare cell types [71, 72]. Moreover, SRT enables direct spatially constrained cell–cell interaction analysis using tools like CellChat and NICHES [56, 73]. Despite the advancements in offering an extra layer of position information, downstream analysis of SRT data is still challenging, particularly in resolution, data integration, and computational scalability. Addressing these limitations requires innovations in deep learning and probabilistic modeling to enhance spatial transcriptomics analysis and advance its applications in disease research.

Single Cell Transcriptomics Profiles of Cardiovascular Systems

In recent years, scRNA-seq/snRNA-seq technologies have emerged as essential tools in cardiovascular research, offering unprecedented resolution in studying the cellular and molecular mechanisms driving the pathogenesis and progression of CVDs [74, 75].

These technologies have been applied across a wide range of cardiac disease models, including cell-based models, such as cardiac cell lines and human embryonic stem cell (hESC)-derived cardiac cells, patient-specific models, where induced pluripotent stem cells (iPSCs) are used to generate cardiac cells for personalized studies, animal models, including mice, rats, zebrafish, and pigs, providing insights into disease mechanisms in vivo [13, 14, 76, 77], or patient biofluids, such as blood samples from individuals with coronary syndromes and circulating CD31 + cells from heart failure patients [78, 79].

Large-scale scRNA-seq/snRNA-seq has been instrumental in profiling myocardial tissues, providing insights into both healthy human hearts and a broad spectrum of cardiac diseases including dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), arrhythmogenic cardiomyopathy (ACM), ischemic cardiomyopathy (ICM), cardiac hypertrophy and heart failure, heart failure in patients with left ventricular assist devices (LVADs), or cardiac complications associated with COVID-19 (Table 2).

A notable study by Reichart et al. (2022) investigated genotype-specific mechanisms underlying DCM and ACM, focusing on pathogenic variants in LMNA, RMB20, TTN, and PKP2 [15]. Their analysis identified 10 major cardiac

Table 2 Single cell transcriptomics data of human heart conditions

Dataset	Condition	Sample size	Reference
Healthy human hearts	Healthy hearts	7 healthy human hearts	[32]
Human heart atlas	Healthy hearts	14 healthy human hearts	[11]
DCM/ACM	Genotyped DCM and ACM patients	61 failing, non-ischemic human hearts and 18 controls	[15]
DCM/HCM	DCM or HCM compared with non-failing donors	12 DCM, 16 HCM, 16 controls	[80]
DCM	DCM compared with nonfailing donors	17 DCM and 28 controls	[74]
ICM	Non-infarct region of ICM compared to non-failing controls	7 ICM and 8 controls	[81]
Pressure-induced hypertrophic heart	Hypertrophic cardiac tissues compared with regionmatched healthy cardiac tissue data from human heart atlas	5 aortic stenosis samples	[82]
End stage heart failure	Patients with advanced HF with LVADs implantation	13 HF with LVAD, 13 HF without LVAD and 14 controls	[83]
Inflammatory cardiomyopathies after COVID	Patients with myocarditis related and unrelated to COVID-19	8 Non-COVID-19 related myocarditis and 10 COVID-19 related myocarditis	[84]
Long COVID effects	Blood draws to generate iPSCs for iPSC-derived endothelial cells and cardiomyocytes	4 mild long COVID, 4 severe long COVID and 5 controls	[85]

DCM dilated cardiomyopathy; *HCM*: hypertrophic cardiomyopathy; *ACM* arrhythmogenic cardiomyopathy: *ICM* ischemic cardiomyopathy; *LVAD* left ventricular assist devices

cell types and 71 distinct transcriptional states, revealing key alterations in cellular composition and gene expression associated with heart failure. Key findings included a significant depletion of cardiomyocytes and a notable increase of immune cell populations, extensive extracellular matrix remodeling, driven by fibroblast activation, and a genotype-specific alterations in intercellular signaling, such as enhanced endothelin signaling in LMNA-variant hearts and dysregulated TNF signaling in PKP2-associated cardiomyopathy.

Similarly, Chaffin et al. (2022) performed trajectory analysis on fibroblasts from DCM and HCM patients, uncovering a continuous transition from quiescent to activated fibroblast states. Their findings highlighted dynamic transcriptional changes, with upregulation of LC44A5, COL22A1, POSTN, AEBP1, and THBS4, and downregulation of PDGFRA, NEGR1, and COL4A4 along the fibroblast activation trajectory [80].

Beyond single-cell sequencing, spatial transcriptomics has been increasingly applied to map cardiac structures, providing insights into heart development, conduction systems, and infarct tissue remodeling [86–88]. Kanemaru et al. integrated single-cell transcriptomics, epigenomics, and spatial transcriptomics to create a spatially resolved multiomic atlas of the human heart, highlighting FOXP2 as a key regulator in pacemaker cells and detailing the compartmentalization of the sinoatrial node [87]. In addition, Kuppe et al. (2022) utilized spatial transcriptomics to study tissue organization during infarct healing, identifying molecular pathways that regulate fibrotic and regenerative processes [88].

These groundbreaking technologies continue to refine our understanding of cardiac biology, disease progression, and potential therapeutic targets, paving the way for more precise diagnostics and treatment strategies in cardiovascular medicine.

Single Cell Multi-Omics Integrating Transcriptomics and Open Chromatin Accessibility

Beyond the transcriptomics information, the complexity of cellular phenotypes also arises from intricate regulatory mechanisms [89]. Epigenetic mechanisms such as DNA methylation, histone modifications, and chromatin accessibility orchestrate gene regulation, influencing processes ranging from development and differentiation to disease pathogenesis [90]. Chromatin accessibility profiling, using methods like single-cell Assay for Transposase-Accessible Chromatin (scATAC-seq), identifies active regulatory elements driving cell-type-specific gene expression [91].

The additional integration of transcriptome profiling helps to further understand the regulation of genes. Transcriptome profiling reveals gene expression patterns, while chromatin accessibility offers insights into the regulatory elements controlling them [92]. These processes are interconnected, as chromatin accessibility governs transcription factor and chromatin remodeler access to DNA, driving cis-regulatory activities and cell-type-specific gene expression [92–94]. Gene expression and chromatin accessibility profiles can be obtained by performing separate scRNA-seq/snRNA-seq and scATAC-seq experiments on split portions of the sample or by using e.g. the advanced 10X Genomics EpiMultiome platform, which enables simultaneous profiling from the same cell [95].

Transcriptomics and chromatin accessibility data could facilitate the recovery of regulatory interactions between genes as GRNs [96]. Through single cell transcriptomics data, TF genes are identified from external databases to distinguish their regulatory genes, and TF–gene interactions are inferred by modeling gene expression as a function of TF abundance [54]. Chromatin accessibility data are processed to identify accessible peaks, creating a peak accessibility matrix that encodes the openness of CREs [93]. CREs are associated with nearby genes based on genomic proximity, and TF binding to CREs is predicted using motif databases and algorithms [97]. This results in TF–CRE–gene triplets, which are subsequently simplified into TF–gene interactions and can be further aggregated into GRNs.

Genomics and its Combination with Single Cell Technology

Combination of Genomics with Transcriptome Data

Genome-Wide Association Studies (GWAS) are large-scale analyses examining genetic variants across the whole genome to identify associations with specific traits or diseases, linking genotypes to phenotypes [98]. GWAS studies focus on single nucleotide polymorphisms (SNPs), which are single-base variations in DNA that can influence biological functions and disease susceptibility [98].

The major limitation of GWAS studies is their difficulty to determine the biological function of causal variants, as over 90% of genome-wide significant single nucleotide polymorphisms (SNPs) lie in noncoding regions, often within regulatory elements that might influence distant genes [98]. This makes identifying causal genes and disease mechanisms particularly challenging. To address this, post-GWAS approaches integrate in silico analyses with experimental validation to link variants to molecular phenotypes. Molecular quantitative trait loci (QTL) analyses, including

expression QTLs (eQTLs), protein QTLs (pQTLs), and splicing QTLs (sQTLs), provide insights into how genetic variation influences gene regulation [99, 100].

A key post-GWAS strategy is the study of gene expression. Genetic determinants and their relationship between gene expression can be systematically examined through expression quantitative trait loci (eQTL) analysis [101]. eQTLs are specific genomic regions where genetic variants, such as SNPs, are statistically associated with variations in gene expression levels [102–104]. By integrating single-cell RNA sequencing with genotype data, single-cell expression quantitative trait loci (sc-eQTL) analysis enables the precise mapping of genetic regulatory effects within distinct cellular contexts, revealing genetic regulation that operates in specific cellular states or conditions that may be obscured in bulk analyses [105]. A significant breakthrough in the field of conducting single-cell expression quantitative trait loci (sc-eQTL) analysis was achieved by Cuomo et al. (2020), who conducted the study to investigate how genetic variants influence gene expression dynamics during the differentiation of induced pluripotent stem cells (iPSCs) [106]. By integrating scRNA-seq with genotype data, their study revealed context-dependent genetic regulatory effects that vary across developmental states, highlighting the dynamic nature of eQTL influences on gene expression. Their study discovered that certain eQTL effects were activated or repressed at specific differentiation stages, shaping cellular identity and function in a stage-specific manner [106].

Combination of Genomics with Transcriptome Data with Single Cell Functional Genomics

Recent advancements in genetic engineering and molecular biology, especially with the development of CRISPR technology, have enhanced the field of functional genomics [107, 108]. Single-cell CRISPR screening technologies, by combining high-throughput genetic perturbation with single-cell resolution phenotypic analysis, allow simultaneous capture of genetic alterations and their corresponding high-dimensional phenotypes [109]. Early iterations of these approaches, such as Perturb-seq and CROP-seq, focused primarily on transcriptomics phenotypes [110–113]. Subsequent advances have extended their applicability to epigenetic features, imaging-based phenotypes, and multimodal datasets [109, 114, 115]. The combination of single-cell technologies and perturbation modeling enables a deeper understanding of how external factors, such as genetic modifications, disease progression, or environmental stimuli, affect cellular physiology and molecular pathways.

One of the applications of single cell perturbation of combining single-cell CRISPR screening with GWAS enables the functional investigation of genetic variants by perturbing GWAS-identified target genes. STING-seq (Systematic Targeting and Inhibition of Noncoding GWAS Loci with Single-Cell Sequencing) integrates large-scale GWAS data, CRISPR screens, and single-cell sequencing to identify causal variants, map target genes in cis and trans regions, and uncover regulatory networks influencing disease risk [116].

Application of Genomics and Integrated Single Cell Functional Genomics in Cardiovascular Disease Research

Building on the advances of GWAS studies, Weng et al. (2025) conducted large-scale meta-analyses of GWAS involving more than 1.3 million individuals, including 30,000 cases from ten studies, with robust phenotypic definitions of sinus node dysfunction (SND), distal conduction disease (DCD), and pacemaker implantation (PM) based on diagnostic codes, procedural data, and electrocardiograms [117]. Rare-variant association tests performed on exome-sequencing data from 460,000 participants, combined with Mendelian randomization and cell-type enrichment analyses, identified 13 loci for SND, 31 for DCD, and 21 for PM [117]. Jurgens et al. (2024) conducted large-scale GWAS and multitrait analysis of dilated cardiomyopathy with 9,365 cases and 946,368 controls, identifying 70 significant loci mapped to 63 prioritized genes [118]. Enrichment analyses highlighted the central role of cardiomyocytes and the contractile apparatus in DCM pathogenesis, while polygenic risk scores (PRS) predicted DCM risk across diverse ancestries and genetic backgrounds [118].

The V2G2P framework was specifically applied to CAD, revealing that 43 CAD-associated GWAS signals converge on the CCM signaling pathway, highlighting its role in CAD risk [119]. The V2G2P framework comprises five steps [119]. Through this framework, 306 CAD-associated GWAS signals were mapped to their potential target genes within enhancers, coding regions, and splice sites. To functionally validate these associations, Perturb-seq was applied to knock down candidate genes located within ± 500 kb of the 306 GWAS signals. The perturbed cellular effects were analyzed through scRNA-seq/snRNA-seq, followed by unsupervised machine learning to identify gene programs. CAD loci were found to converge onto five gene programs related to the cerebral cavernous malformations

(CCM) signaling pathway, which regulates vascular development [119]0.41 genes were identified as potential mediators of CAD risk through endothelial cell function. Notably, TLNRD1 and CCM2 knockdown mimicked atheroprotective laminar flow, and TLNRD1 was identified as a novel regulator of the CCM pathway.

Integrating Multi-Omics Single-Cell Profiles to Unravel Cardiovascular Disease Mechanisms

Large-cohort single-cell profiling of genomics, transcriptomics, epigenomics, proteomics, and metabolomics will provide a more comprehensive molecular landscape of CVD pathogenesis and progression. Firstly, integrating multi-omics data by combining genomics with single-cell transcriptomics provides a more comprehensive understanding of gene regulation in CVDs. Especially, sc-eQTL studies in the cardiovascular field offer insights into how genetic variants influence gene expression at the cellular level within the heart [101]. By mapping these genetic variants to specific cell types, sc-eQTL analyses can uncover cell type-specific regulatory mechanisms that contribute to disease pathogenesis [106]. Further integrating sc-eQTL data with Mendelian randomization, where gene expression levels serve as exposures, enables the establishment of causal relationships between genetic variants, gene expression, and CVD phenotypes [120]. Secondly, the integration of single-cell transcriptomics and epigenomics will help reconstruct cell-type-specific regulatory landscapes, providing a mechanistic understanding of gene regulation in distinct cardiac cell types/states [92]. These regulatory interactions can be further validated through functional genomics approaches [121]. Additionally, integrating spatial transcriptomics and spatial multi-omics sequencing provides a detailed view of the spatial organization of cells within cardiac tissue, capturing the precise localization of distinct cell types and their molecular states to reveal how cellular heterogeneity, signaling networks, and microenvironmental interactions contribute to heart diseases [60].

Future Direction of Application of Single Cell Technologies

The future of single cell technologies in cardiovascular medicine lies in the advancements of disease understanding, diagnosis, and treatment. By dissecting cellular and molecular complexities, this technology enables the identification of cell-specific mechanisms, biomarkers, and therapeutic targets. These perspectives drive precision medicine, regenerative therapies, and next-generation diagnostics, revolutionizing cardiovascular care (Fig. 2).

Single-cell technologies facilitate the identification of distinct cell types, cellular states, and rare or disease-driving cell populations. By analyzing individual cells, this technology enables more precise diagnoses, improves disease classification, and allows for early detection of health conditions. scRNA-seq/snRNA-seq provides detailed molecular profiles of each cell, helping researchers discover cell-specific biomarkers, including genes, proteins, and signaling pathways, that reflect disease states. Furthermore, considering the correlation between tissue-specific signatures and biofluids, scRNA-seq/snRNA-seq studies can improve non-invasive diagnostic and monitoring techniques. By analyzing circulating cells and extracellular vesicles, such as exosomes and microvesicles, liquid biopsies offer a promising approach for disease detection and progression tracking [122].

Single-cell technologies are transforming personalized medicine by generating patient-specific cellular profiles, enabling tailored treatments based on e.g. sc-SNPs. These technologies facilitate targeted therapies for specific cell types, enhance treatment response prediction, and improve therapeutic precision.

In regenerative medicine, single-cell technologies plays an important role in guiding stem cell differentiation for heart repair and tissue engineering. It also enables real-time monitoring of transplanted stem-cell-derived cells, ensuring proper development, function, and integration into cardiac tissue [123].

In addition, single-cell sequencing holds great promise for immune system analysis in cardiovascular diseases. By distinguishing immune cell subtypes and rare cell populations, it provides a deeper understanding of immune dynamics in disease progression and therapeutic responses. This technology aids in detecting drug-resistant immune phenotypes, offering insights into immune evasion and therapy resistance in conditions like myocarditis, atherosclerosis, and transplant rejection [124]. It can also drive the development of personalized immunotherapies, such as engineered regulatory T cells (CAR-Tregs), for treating cardiovascular and inflammatory diseases [125].

The integration of large language AI models with single-cell technologies enhances data analysis, enabling efficient multi-omics integration and deeper biological insights. AI-driven predictive modeling further supports the identification of biomarkers for disease diagnosis, patient monitoring, and therapy response prediction.

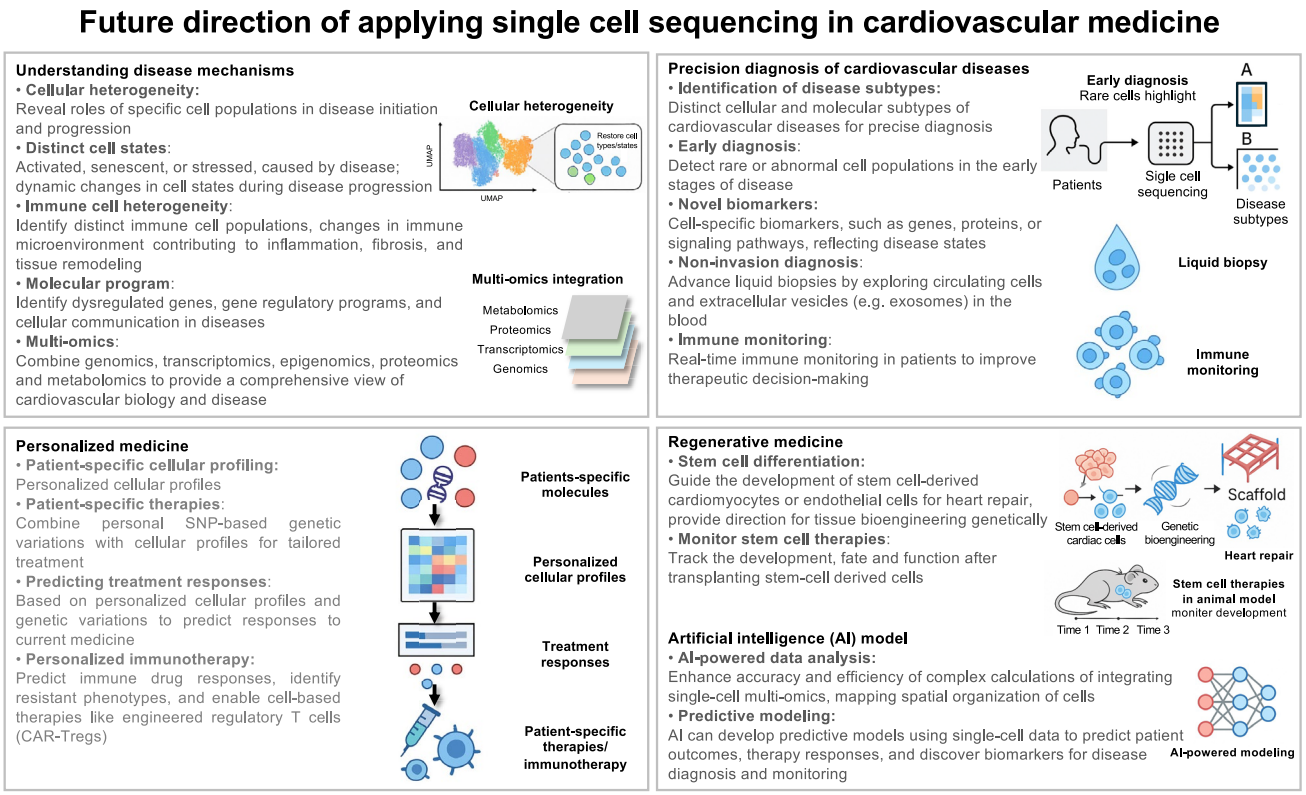

Fig. 2 Future direction of single cell technologies application in cardiovascular medicine. This figure outlines the potential applications of single-cell technologies in advancing cardiovascular medicine

These innovations collectively pave the way for more precise, personalized, and effective treatments in cardiovascular medicine.

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  Findings from this study genotype-specific mechanisms and cell type/cell state proportion changes of dilated cardiomyopathy and arrhythmogenic cardiomyopathy.

- Litviňuková M, Talavera-López C, Maatz H, Reichart D, Worth CL, Lindberg EL, Kanda M, Polanski K, Heinig M, Lee M, Nadelmann ER, Roberts K, Tuck L, Fasouli ES, DeLaughter DM, McDonough B, Wakimoto H, Gorham JM, Samari S, Mahbubani KT, Saeb-Parsy K, Patone G, Boyle JJ, Zhang H, Zhang H, Viveiros A, Oudit GY, Bayraktar OA, Seidman JG, Seidman CE, Noseda M, Hubner N, Teichmann SA. Cells of the adult human heart. *Nature*, 588(7838):466–472, 2020.

  This study generated human heart atlas at single-cell resolution to generate comprehensive map of cell types, cell states, and cellular communication interactions of hearts to enhance our understanding of cardiac biology.

Author Contributions Jiahui Ji conducted the literature review, drafted the manuscript, and created the figures and tables. Eric L. Lindberg contributed to manuscript editing and content refinement. Daniel Reichart contributed to manuscript revisions, provided critical feedback, and supervised the overall work.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

Conflict of interest The authors declare no competing interests.

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